NANOTECHNOLOGY: A NEW APPROACH FOR OCULAR DRUG DELIVERY SYSTEM

MEETALI MUDGIL[1], NIDHI GUPTA[1], MANJU NAGPAL[1], PRAVIN PAWAR[1*]

Chitkara College of Pharmacy, Chitkara University, Chandigarh-Patiala National Highway, Rajpura 140401, Patiala, Punjab, India. Email: pkpawar80@yahoo.com, pravin.pawar@chitkara.edu.in

ABSTRACT

In current drug delivery scenario, despite numerous scientific efforts, efficient ocular drug delivery remains a challenge for pharmaceutical scientists. In ocular drug delivery system, ocular infections are treated by various topical drug applications in the form of solutions, suspensions and ointment. These conventional dosage forms suffer from the problems of poor ocular bioavailability due to minimum ocular residence time, because of various anatomical and pathophysiological barriers prevailing in the eye. This review provides an insight into the various constraints associated with ocular drug delivery, summarizes recent findings and applications of various nanoparticulate systems like nanosuspensions and nanoparticles in the field of ocular drug delivery and review also summarizes the various method of preparation of nanocarriers for ophthalmic drug delivery.

Keywords: Ocular drug delivery, Nanocarriers.

INTRODUCTION

The eye is a unique organ, both anatomically and physiologically, containing several widely varied structures with independent physiological functions that render the organ highly impervious to foreign substances. For example, the cornea and the crystalline lens are the only tissues in the body besides cartilage that have no blood supply. This complexity of the eye provides unique challenges to drug delivery strategies in front of the pharmaceutical scientist.[1]

Ocular bioavailability of drug is depending upon some physiological properties of drug and physiological factors. There are physiologic factors, which can affect a drug's ocular bioavailability including protein binding, drug metabolism and lacrimal drainage. In addition to physiologic factors affecting ocular bioavailability, other factors as the physicochemical characteristics of the drug substance, and product formulation are important. Because the cornea is a membrane-barrier containing both hydrophilic and lipophilic layers, drug substances having both hydrophilic and lipophilic characteristics permeate it most effectively. It is advantageous for corneal penetration to adjust the pH of solution to increase the fraction of unionized drug in the instilled dose. Drugs, which are highly water-soluble, do not readily permeate the cornea. Ophthalmic suspensions ophthalmic ointments mix with lacrimal fluids less readily than solutions, and thus, remain in the cul-de-sac for longer period of time, enhancing the bioavailability of the drug substance.

An exciting challenge for developing suitable drug delivery systems targeted for ocular diseases is one of the major focuses of pharmaceutical scientists. There are several new ophthalmic drug delivery systems under investigation such as: hydrogels [2], microparticles [3] nanoparticles [4], liposomes [5], collagen shields [6], ocular inserts/discs [7], dendrimers [8], and transcorneal iontophoresis [9]. Polymeric nanoparticles are also able to target diseases in the posterior segment of the eye such as age-related macular degeneration, cytomegalovirus retinitis, diabetic retinopathy, posterior uveitis and retinitis pigmentosa [10]. Colloidal particles (nanoparticles) can be applied in the liquid form just like eye drops and reduce discomfort caused by application of semisolid ointments. They are patient friendly due to less frequent application, extended duration of retention in the extraocular portion without blurring vision.

SLNs are submicron-sized carriers composed of a lipid solid matrix stabilized by a surfactant. Such systems have some advantages like low toxicity, high drug payload, capability of including lipophilic and hydrophilic drugs, drug targeting, controlled release (fast or sustained), and occlusive properties.[11]

Commercial ophthalmic suspension formulations were formulated with many problems such as non homogeneity of dosage form, cake formation, settling of particles, aggregation of suspended particles. To overcome these problems, attempts have been made to prepare nanosuspensions for successful drug delivery. Nanosuspension not only improves the saturation solubility of drug in media but it is also ideal approach for ophthalmic delivery of hydrophobic drugs in eye. Nanosuspensions can also used to achieve sustained release of the drug by incorporating or formulating with suitable hydrogel or mucoadhesive base or in ocular inserts.[12] Nanosuspensions are submicron colloidal dispersions of nanosized drug particles stabilized by suitable surfactants. Nanosuspensions are prepared using poorly water soluble drugs without any matrix material suspended in dispersion. These can be used to enhance the solubility of drugs that are poorly soluble in water as well as lipid media. It also improves drug stability as well as bioavailability of poorly soluble drug. [13-20]

Recently nanotechnology can be used in drug delivery and gene therapy by applying novel self-assembled materials and devices of nanoscale size. For instance polyplexes (DNA/polycation complexes), block copolymer micelles and nanogels. Among these, polymer micelles have been evaluated for the delivery of anti-cancer agents in human trials. The above trials shows promising results suggested the practical use of nanomaterial in medicine. [21]

Nanosuspension is favoured for compounds with high partition coefficient (log P) value, which are insoluble in water, but soluble in oil, high doses and high melting point. Nanaosuspensions can also be formulated for drugs which are insoluble in both water and organic solvents. Hydrophobic drugs can also be formulated as nanosuspensions such as naproxen, clofazomine, nimesulide, mitotane, amphotericin B, omeprazole and spironolactone. [22]

The average particle size of the nanosuspensions ranges between 200 and 600 nm which is usually less than one micron. The compounds with high log P value, high melting point and high dose are most suitable for nanosuspension formulation approach. The uniform particle size is desired for the stability of the particles obtained in the nanosuspension. Nanosuspensions can be given through various routes of administration like oral, parenteral, topical, ocular and pulmonary routes.[23] Nanoparticles can easily adsorb, bind and carry drugs or proteins as they have a large surface area.[24]

Increase in the dissolution rate and bioavailability is observed on reduction of drug particles into the sub-micron range. Vapour pressure effect leads to increase in the solution velocity and saturation solubility of the nanosized particles. Also an increased concentration gradient is observed due to decreased diffusional distance on the surface of drug nanoparticles, thereby increase in saturation solubility is also observed.[25]Various physiochemical parameters such as, ionic strength, pH, monomer concentration, particle size and molecular weight as well as surface charge are important for drug delivery.[26]

Nanosuspensions had a quicker onset of action and enhanced dose proportionality.[27] Nanosuspensions also alter the pharmacokinetic parameters, improves the safety and efficacy of the drugs. Formulation of nanosuspension includes stabilizers like poloxamers, lecithins, povidones, polysorbates etc. Solvents used in formulation includes water miscible solvents like butyl acetate, benzyl alcohol, ethyl acetate and other pharmaceutically acceptable and less hazardous solvents. Tweens and spans are widely used as surfactants which are added the dispersion by reducing interfacial tension. Surfactants act as wetting or deflocculating agents. Ethanol, glycofurol, isopropanol etc can be used as co- surfactants, buffer salts, osmogent, cryoprotectants, polyols are used as additives in nanosuspension formulation.[28]

Nanosuspensions offers many advantages: firstly, the physical and chemical stability of drugs in the nanosuspension can be increased as they are actually in the solid state.; secondly, dose and toxicity can be reduced and the high drug loading can be achieved.; thirdly, It is valuable for those molecules which are insoluble in oils.; finally, nanosuspensions can be used for the passive targeting.[29]

Nanocarriers such as nanoparticles have wide applications in ophthalmology as they have capacity to deliver ocular drugs to specific target sites. Different polymers are used in nanoparticle formulations.[30]Nanoparticles are prepared from different biodegradable polymers like poly(lactic acid).[31]poly (alkyl cyanoacrylate) (PACA),[32]poly(lactic-co-glycolic acid)(PLGA),[33]poly(epsilon-caprolactone) (PCL),[34]as well as different natural polymers like chitosan,[35]gelatine,[36]sodium alginate, and albumin,[37]can be used effectively for drug delivery to ocular tissues.

Nanosuspensions can be valuable for the drugs which have poor solubility in lachrymal fluids. The nanoparticulate nature of the drug shows sustained release effect by increasing its residence time in the cul-de-sac. The nanoparticles protect the drug against agents which cause degradation.[38]The choice of matrix constituents can readily modulate the controlled release and particle degradation characteristics.[39]To enhance the drug therapeutic efficacy along with reduction in side effects, nanoparticles sustain release of the drug at the site of localization, modifying drug's organ distribution followed by clearance of the drug. Development of a particular drug's pharmacokinetic release profile can be done by controlling both the architecture and particle size of nanoparticles. To achieve a constant therapeutic concentration at the site of delivery, zero-order kinetic drug release profile is required. Finally the present study is to design nanosuspension (NS) as a novel controlled dosage form that could release the drug in a controlled manner at the site to have better therapeutic efficiency at a much lower dose.

On the basis of above information, this article focuses on various method used in preparation of nanoparticles for improvement of bioavailability.

SOLVENT DISPLACEMENT METHOD

It is also called as nanoprecipitation method and has been widely used to prepare nanoparticles. The method is based on the precipitation of preformed polymer following displacement of a semi polar solvent miscible with water in the presence or absence of surfactant. The basic principle of this technique is similar to spontaneous emulsification of the organic phase containing drug and polymer into the external aqueous phase. In this method the polymer and drug are dissolved in a water miscible organic solvent of intermediate polarity (e.g. acetone and ethanol). The resulting organic phase is injected into a stirred aqueous phase containing a surfactant as stabilizer. The nanoparticles are formed instantaneously during the rapid diffusion of the organic phase into the aqueous phase.[40]
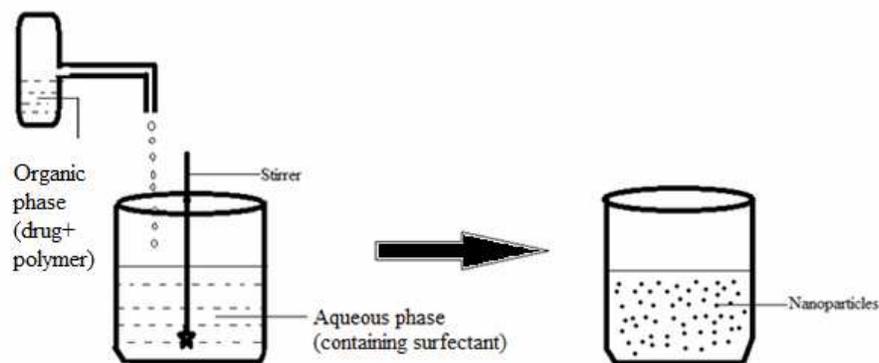

Fig. 1: Solvent displacement method[64]

Mandal *et al.* used this technique to formulate sulfacetamide loaded Eudragit RL-100 nanosuspension for ophthalmic delivery. Eudragit RL-100 nanosuspensions containing sulfacetamide were prepared using 1% w/v pluronic F-109 as a surfactant and acetone. Different ratios of drug to polymer were selected as formulation variable. The results indicated that nanosuspension could be utilised as potential delivery system for treating ocular bacterial infections.[40]

Yadav *et al.* reported the study of carvedilol loaded Eudragit E-100 nanoparticles by nanoprecipitation technique using poloxamer F-407 as polymeric stabilizer and suggested the feasibility of formulated nanoparticles for treatment of hypertension. The prepared nanosuspension particle size ranged from 190nm-270nm. The nanoparticle size was found to be directly depended on Eudragit E-100 amount.[41]

This method can also used to encapsulate some fluoroquinolone like sparfloxacin using PLGA for sustained ocular drug delivery system.[42]Gupta *et al.* investigated sparfloxacin- loaded PLGA nanoparticles for sustained ocular drug delivery using nanoprecipitation technique. The developed PLGA nanoparticle was demonstrated to improve precorneal residence time and ocular penetration and was found to be stable for longer duration of time then conventional marketed formulations with a good shelf life.

Another same study comprises the encapsulation of fluoroquinolone like levofloxacin nanoparticles have been reported.[43] Nanosuspension of levofloxacin using PLGA as biodegradable polymer was prepared by solvent evaporation method by varying drug and polymer ratio. The study focuses on particle size distribution, i.e. the polydisperse index of all formulation were found to be less than 0.1, which is monodisperse system. Transcorneal permeation study concluded that the levofloxacin loaded PLGA nanoparticles shows a significantly higher permeation capability as compared to marketed eye drops of levofloxacin and was further concluded that the formulated levofloxacin encapsulated PLGA nanosuspension gives prolonged retention with better tolerability and extended release at corneal site.

Kerur *et al.* reported the release of acyclovir from nanosuspension prepared by using the combination of Eudragit RS-100 as sustained polymer and tween 80 as a surfactant. The study concluded that polymeric ophthalmic nanosuspension of acyclovir was capable of

releasing the drug for a prolonged period of time and increased bioavailability. [44]

Pignatello *et al.* identified the stability of cloricromene in ophthalmic formulation and enhanced bioavailability at ocular level by utilizing the solvent evaporation method. The nanosuspensions obtained showed positive surface charge and mean particle sizes which make them suitable for ophthalmic applications. High polydispersity index was also noted which indicates a high degree of heterogeneity in particle size. Higher chemical stability of ester drug was observed when nanoparticles were formed in saline in absence of tween or with lowest amount of tween.[45]

Aksungur *et al.* reported nanoparticles containing cyclosporine (CsA) using poly lactide co-glycolic acid (PLGA) and Eudragit RL-100 in combination or PLGA coated with carbopol for the treatment of severe dry eye syndrome.[46]The smallest nanoparticles were obtained with Eudragit RL. The release profile of CsA from nanoparticles followed Weibull model. It was found that nanoparticles size was decreased with increasing in Eudragit-RL concentration due to physicochemical properties of polymer. The study suggested the freeze dried cyclosporine nanoparticles slight increased in particle size.

Adibkia *et al.* prepared Eudragit RS100 loaded piroxicam nanoparticles using similar method for control the inflammatory symptoms in rabbits with endotoxin-induced uveitis (EIU). The study suggested the non-invasive implementation of the piroxicam-Eudragit RS-100 nanosuspensions as a safer controlled ocular delivery of anti-inflamation agents for inhibition of the uveitis symptoms.[47]

Another study investigated the potential of amphotericin B nanoparticles prepared by solvent evaporation method for the improvement of the delivery of drugs to the ocular mucosa. The study includes the release of amphotericin B from nanoparticles was evaluated using diffusion cells using dialysis membrane with a molecular weight cut off of 12,000-14,000 Da. Drug to polymer ratio and organic phase- aqueous phase ratio had a great effect on particle size and particle size distribution. The positive zeta potential of amphotericin B nanoparticles and fine particle size helps to prolong the corneal contact time.[48]

Ahuja *et al.* prepared nanoususpension of diclofenac loaded Eudragit S100 for ophthalmic delivery using same method. Mannitol (5%w/v) was added as cryoprotectant. The average particle size and entrapment efficiency of the formulation containing Eudragit S100 (lyophilized with cryoprotectant) was found to be 172nm and 95.77% respectively compared to the formulation containing Eudragit S100 (lyophilized without cryoprotectant) was found to be 2720nm and 92.56% respectively. The average particle size and polydispersibility index of the nanosuspension is important characteristics in governing saturation solubility, physical stability, dissolution rate and biological performance of nanosuspensions. The larger average particle size and smaller value of zeta potential in the formulation containing Eudragit S100 lyophilized without mannitol compared to formulation containing Eudragit S100 lyophilized with mannitol is due to the aggregation of suspended particles during lyophilisation stage. The study also state, the higher entrapment efficiency, sustained *in vitro* release indicated that Eudragit S100 nanosuspension is an effective ocular delivery system for diclofenac.[49]

Agnihotri *et al.* investigated diclofenac loaded biopolymeric nanosuspension for ophthalmic delivery prepared by emulsion and solvent evaporation method using two different polymers poly[Lac(Glc-Leu)] (PLDA) and poly(lactide-co-glycolide) (PLGA). The study demonstrated the nanosuspensions enhanced corneal adhesion and stability during storage, especially at low temperature. The results indicated that nanosuspension could be utilised as potential delivery system for topical treatment of inflammatory conditions of eye.[50]

Some steroids like hydrocortisone can be encapsulated using microfluidic reactors using hydroxypropyl methyl cellulose (HPMC) and sodium lauryl sulphate (SLS) as surfactant by using nanoprecipitation technique.[51] Results revealed that nanosuspensions made of spherical paricles with mean particle size of 500±64nm, zeta potential of -18±2.84mV, polydispersity index of 0.21±.026 was found. It was also concluded that it was feasible to prepare the hydrocortisone in the form of nanosuspension for ophthalmic drug delivery.

Yoncheva *et al.* reported solvent evaporation method for preparation and coating of pilocarpine loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles. Various mucoadhesive polymers like chitosan, sodium alginate and poloxamers were used for coating. Only the surface charge of chitosan-coated particles was positive and rest all other particles have negative surface charge. Their surface charge was changed from negative (−22.8 mV) to positive (+61.0 mV). The turbidimetric measurements of nanoparticles were performed by Hitachi U-1500 to obtain the mucoadhesive properties of prepared nanoparticles. It was concluded that after local ocular application, the coating with chitosan could be a useful approach in aiming to provide longer residence time of the nanoparticles.[52, 53]

In study, the Ibuprofen, NSAIDs was able to formulate polymeric nanoparticle suspensions from inert polymer resins (Eudragit RS100) for the ophthalmic controlled delivery with the aim of improving the availability of ibuprofen sodium (IBU) at the intraocular level.[54]The nanosuspensions were prepared by a modification of the quasi-emulsion solvent diffusion technique. *In vivo* efficacy was assessed on the rabbit eye after induction of an ocular trauma (paracentesis). The study also revealed that there was no toxicity of IBU-loaded nanosuspension on ocular tissues and was concluded that even at lower concentration of the free drug in the conjunctival sac from nanoparticulate formulation, an inhibition of the miotic response to the surgical trauma was achieved when compared to a control aqueous eye-drop formulation.

In another same study comprises that, Flurbiprofen-loaded acrylate polymer nanosuspensions using quasi-emulsion solvent diffusion technique have been reported. The aim of study is to improve the availability of the drug at an intra-ocular level for the prevention of the myosis induced during extracapsular cataract surgery. The rabbit eye after induction of an ocular trauma was used for *in vivo* anti-inflammatory studies. Also FLU-loaded nanosuspensions did not show any toxicity on ocular tissues. It was concluded that, when compared to a control eye-drop formulation, an inhibition of the miotic response to the surgical trauma was obtained, even though an actual lower concentration of free drug in the conjunctival sac was achieved from the nanoparticle system. After application of the nanosuspensions, a higher drug levels in the aqueous humour were also observed.[55]

Ganciclovir (GCV) as antiviral drug loaded nanoformulations were prepared using reverse-phase evaporation technique for cytomegalovirus retinitis treatment. This study was done with to investigate the comparative potential of different mucoadhesive nanoformulations for the topical ocular delivery of ganciclovir. Various nano-formulations which were prepared for the studies include GCV mucoadhesive nanoemulsions (GCV-NEs), chitosan nanoparticles (GCV-NPs) and GCV mucoadhesive niosomal dispersion (GCV-NDs). It was revealed from the results that the developed formulations were nonirritant and nontoxic in nature. It was further concluded that GCV nanoformulatios could be utilised as potential delivery system for treatment of ocular infections by topical instillation.[56]

Mohammadi *et al.* carried out nanoprecipitation technique for prepration of clarithromycin (CLR) loaded biodegradable nanoparticles for colloidal drug delivery system were using PLGA as a biodegradable polymer. *Staphylococcus aureus* was used for the study of the antimicrobial activity of clarithromycin nanosuspension. The intact drug CLR was found to be less effective than nanoparticle formulation against *S. aureus* as that the nanoparticle formulation showed equal antibacterial effect at 1/8 concentration of the intact drug. The study demonstrated that the prepared CLR nanoparticles were more potent against S. *aureus* with improved minimum inhibitory concentrations (MICs) .[57]

The brimonidine tartrate nanoparticles were prepared using Eudragit by double emulsion-solvent evaporation technique for the treatment of open-angle glaucoma has been reported. The prepared brimonidine tartrate nanoparticles were found to have improved drug loading and narrow particle size range. The *in vivo* ocular irritability and tolerability tests results revealed that the nanoparticle formulations were found to be well tolerated with no signs of irritation. The nanosuspensions produce prolong reduction on intraocular pressure (IOP) and drug release *in vivo* study.[58]

Javadzadeh *et al.* formulated nanoparticles of naproxen with poly (lactic-co-glycolic acid) (PLGA) using single emulsion technique. Drug/polymer ratio, aqueous phase volume and speed of homogenization were considered as process parameters to achieve optimal preparation conditions. The study suggested the feasibility of formulating nanoparticles of PLGA with satisfactory physicochemical characteristics increases the anti-inflammatory effects of drug following its ocular or intra-joint administration.[59]

Jimenez *et al.* reported methyl trypsin loaded poly (D,L-lactide-coglycolide) nanoparticles for contact lens care formulated by double emulsion-solvent evaporation technique. A factorial design was performed to select the optimum lactic acid proportion in the copolymer and conditions of the second sonication. It was observed that, with increasing lactic acid proportion, the higher particle size nanoparticles were formed and the increase entrapment efficiency when the time of second sonication was decreased. When compared to PLGA 75:25 NPs, PLGA 50:50 NPs were chosen for further development since PLGA 50:50 NPs settled fast with different particle size in the sediment in contrast to former nanoparticles which led to form aggregates. The addition of Tetronic 1304 promoted the fast release of enzyme initially and decreased the zeta potential (zeta) up to neutral values after gamma irradiation while the addition of glycerol to the nanoparticles provided the highest entrapment efficiency of Methyl trypsin (>90%). It was observed that after *in vitro* HET-CAM test and *in vivo* Draize test, nanoparticle formulations showed an acceptable irritation ocular tolerance. The nanoparticles were found to be effective as a lens care cleaner after 3 days or even longer with a very low quantity of enzyme released.[60]

HOMOGENIZATION

This method is also used for preparation of nanosuspension. The process can be summarized into three steps: firstly, the pre-suspension is formed by dispersing the drug powders in a stabilizer solution; then pre-suspension formed was then homogenized by the high-pressure homogenizer at a low pressure for several times. It is also called as premilling, and finally the pre-milled suspension was homogenized at a high pressure for 10-25 cycles until the nanosuspensions with desired particle size were prepared.[61]

Using high pressure homogenization method, nanosuspensions for ophthalmic delivery of practically insoluble glucocorticoid drugs like hydrocortisone, prednisolone and dexamethasone was prepared. Pluronic F68 (1%w/v) was used as surfactant. The effect of sub-micron and nanosized particles as well as effect of viscosity of the nanosuspension on ocular bioavailability was studied by measuring the intraocular pressure using Shioetz tonometer. From the mean particle diameter and particle size distribution of above glucocorticoid drugs analysed by laser diffractometer (LD) and photon correlation spectroscopy (PCS) were distinguished as micron size range and nano size range. After instillation of drug nanosuspension of different mean particle diameter, a mean percentage increase in intraocular pressure was calculated. It was concluded that, nanosuspensions of glucocorticoid drugs enhance the intensity of drug actions as well as rate and extent of ocular drug absorption. It was also observed that viscosity of nanosuspension increases that result, the duration of drug action was increased.[62]

Pardeike et al. studied the newly secreted phospholipase A2 inhibitors PX-18 and PX-13 as drug nanosuspension prepared by high pressure homogenization. The formulated nanosuspension had been classified as safe for dermal and ocular application. PX-18 and PX-13 are not soluble in aqueous media. The dermal application of PX-18 and PX-13 had found to be a good approach for psoriasis treatment due to its increased expression in psoriatic epidermis and dermis. On PX-18 or PX-13 bulk material and PX-18 or PX-13 (5% w/w) nanosuspensions, pre-clinical skin and eye irritation tests were carried out using the EPISKIN and the HET-CAM test to improve the design of safe and efficient human studies. It was observed that nanosuspensions with an active content of 5% (w/w) prepared by high pressure homogenisation technique produces particles in the nanometer size range. The results concluded that EPISKIN test and the HET-CAM test permits the classification of new secreted phospholipase A2 inhibitors that confirms PX-18 or PX-13 bulk material and their nanosuspensions were non irritant to the skin and eye. [63]

IONIC GELATION

In the ionic gelation method, the positive or negative charge of the hydrophilic polymer is complexed with a multivalent cationic (e.g. calcium chloride) or polyanionic (e.g. sodium tripolyphosphate) to form highly viscous gel particles with a size in the range of a nanometer. Ionic gelation method was developed by Calvo and Co-workers for the preparation of chitosan nanoparticles. In this method polymer solutions and polyanion solutions are mixed to form nanoparticles. The basic mechanism involved in the formation of nanoparticles is the electrostatic interactions between positively charged amino groups present in polymer and negatively charged anion. In other words it can be seen that in the ionic gelation method, due to interaction the material undergoes transition from liquid to gel phase. The obtained chitosan nanoparticles generally are of small size in the range of 200-500nm.[64]
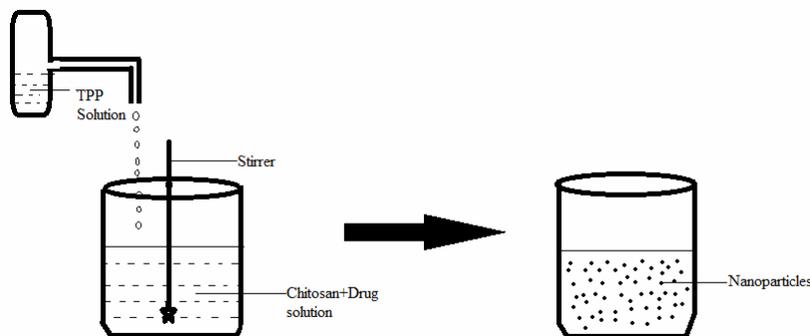

Fig. 2: Ionic Gelation Technique.[64]

The Ionic gelation method is used to prepare gum cordia loaded fluconazole nanoparticles. The study design was done using the

design expert software for the statistical data presentation for analysing the effect of % encapsulation efficiency, particle size and

zeta potential. The permeation study concluded that the optimized formulation of fluconazole showed higher permeation (%) compared to marketed formulation of fluconazole.[65]

The comparative study of ophthalmic soya lecithin loaded Mycophenolate mofetil (MMF) suspension and chitosan loaded MMF nanosuspensions were investigated for ocular drug delivery. The combinations of soya lecithin and chitosan loaded nanosuspension technology increased preocular drug retention time. The modified chitosan nanosuspension was showed higher value of zeta potential results, more stability of formulation. The study concluded that chitosan loaded nanosuspension uses showed higher corneal mucoadhesion and pharmacokinetics in lachrymal fluid and aqueous humour.[66]

To improve the bioavailability and to prolong the residence time, Brimonidine tartrate (BT) loaded chitosan (CS) nanoparticles have been prepared by Singh *et al.* by ionic gelation technique. The formulated polymeric nanoparticles were evaluated by particle size, polydispersity index (PI), entrapment efficiency, DSC, SEM, TEM, which gave an insight of physicochemical interaction that influenced the CS nanoparticle formation. *In vitro* studies reveal the sustained release effect of BT nanoparticle over the period of 4 hrs in saline phosphate buffer pH 7.4. The structural interactions between BT, TPP and CS matrix were demonstrated by DSC. The results concluded that dosage frequency was reduced by sustained drug release from CS Nanoparticles in the treatment of glaucoma.[67]

Konat *et al.* reviewed the expression of MUC5AC in ocular surface epithelial cells using cationized gelatin nanoparticles containing polyanions (chondroitin sulfate or dextran sulfate). Dry eye syndrome was caused by the decreased production of the mucin MUC5AC in the eye. The formulated nanoparticles was found to be of small size (<200 nm), high plasmid association efficiency (>95%) and positive zeta potential (+20/+30 mV). *In vitro* transfection of the nanoparticles reveals the detection of MUC5AC mRNA and protein in conjunctival cells. Higher MUC5AC expression in the conjunctiva was found after *in vivo* administration of the nanoparticles compared to untreated control and naked plasmid. These results concluded that the nanoparticles showed a promising carrier for restoring the MUC5AC concentration in the ocular surface and for gene therapy.[68]

Mahmoud *et al.* reported Econazole nitrate (ECO) loaded chitosan nanoparticles developed using sulfobutylether-β-cyclodextrin sodium as polyanionic cross-linker to achieve sustained therapeutic effect for ocular drug delivery systems. *In vitro* release of drug from nanoparticles was able to controlle 50% of the original amount released from nanoparticles upto 8 hrs. The *in vivo* studies suggest that, the chitosan loaded Econazole nitrate nanoparticles provide better antifungal activity as compared with Econazole nitrate solution. On the bases of results chitosan/sulfobutylether-β-CD nanoparticles were proved an effective candidate for ophthalmic delivery.[69]

In another study hybrid nanoparticle based on cationized gelatine, polyanions dextran sulphate (DS) and chondroitin sulphate (CS) were prepared by Zorzi *et al.* for ocular gene therapy. It was reported that these systems can be used as a carrier for plasmid DNA by avoiding the DNAse I degradation. It was observed that the *in vitro* toxicity of the nanoparticles to human corneal cells can be reduced by the introduction of CS or DS in the formulation without compromising the transfection efficiency. These systems are prospective carriers for the development of safer and more valuable nanomedicines for ophthalmic delivery.[70]

Nagarwal *et al.* formulated 5-fluorouracil (5-FU) loaded chitosan nanoparticles (CH-DNPs) using sodium tripolyphosphate (TPP) as polyanion for ocular delivery by ionic gelation technique. The particles size morphology and encapsulation efficiency was altered by modifying the CH/TPP mass ratio. The spherical shape of the nanoparticles was investigated by atomic force microscope (AFM) and scanning electron microscopy (SEM). The drug release kinetics of all formulation followed diffusion controlled release kinetics.[71]

In another study Jain *et al.* evaluated the PLGA-chitosan Rhodamine loaded nanoplexes for their interaction with ocular mucosa, *ex vivo*

study and *in vivo* study to judge their feasibility as an ocular delivery system. Ionotropic gelation method was used for preparation of fluorescent Rhodamine Nanoplexes (Rd-Nanoplexes). Spectrofluorimetry and Confocal microscopy were used for analysis of corneal retention, uptake and penetration of Nanoplexes. The paracellular and transcellular uptake of the Nanoplexes were observed by Confocal microscopy of the corneas. Corneas from rabbits treated with Rd-Nanoplexes were evaluated for the ocular tolerance and *in vivo* uptake. The adsorptive-mediated endocytosis and opening of the tight junctions between epithelial cells was considered as an uptake mechanism. No alteration was microscopically observed after ocular surface exposure to Nanoplexes.[72]

Wadhwa *et al.* studied Hyaluronic acid (HA) or modified chitosan (CS) nanoparticles loaded with dorzolamide hydrochloride (DH) or timolol maleate (TM) for the treatment of glaucoma. The synergistic effect of Hyaluronic acid (HA) for mucoadhesion in association with chitosan provides sustained and local delivery of drugs to the ocular sites. It was revealed that CS-HA nanoparticles show higher reduction in intraocular pressure level as compared to drug solution. These results suggest that HA potentially enhance the mucoadhesiveness and efficiency of CS nanoparticles and may be promising carrier for ocular drug delivery.[73]

The chitosan loaded indomethacin nanoparticle and nanoemulsions were developed for improve its ocular bioavailability.[74] Ionic gelation technique was used for the preparation of chitosan nanoparticles while emulsification technique for nanoemulsion. *In vivo* studies demonstrated that clearer healing of corneal chemical ulcer with moderate effective inhibition of polymorph nuclear leukocytic infiltration (PMNLs) was observed in the rabbits eyes treated with nanoemulsion as compared to nanoparticles preparation. The high indomethacin level in inner ocular structure, aqueous humour in rabbit eyes was achieved upon topical instillation of chitosan nanoemulsion as compared with indomethacin drug solution. The chitosan nanoparticle prepared were capable to make contact intimately with the cornea thus giving a slow continuing drug release with long-term drug intensity, thus enhancing delivery to both internal and external ophthalmic tissues.

Chitosan loaded Cyclosporine A (CsA) nanoparticles of were formulated by Campos *et al.* by ionic gelation method for improvement of delivery of drugs to ocular surface. The polydispersity index of CsA loaded chitosan nanoparticles was found to be 0.34. *In vitro* release studies revealed that during the first hour a fast release was observed followed by a more gradual drug release during a 24 hrs period when performed under sink conditions. *In vivo* experiments revealed that, it was possible to achieve therapeutic concentrations in cornea and conjunctiva during at least 48 hrs on topical instillation of nanoparticles to rabbits. The nanoparticles are proved to be an excellent vehicle in enhancing the therapeutic index of clinically challenging drugs with potential application at extraocular level.[75]

MILLING METHOD

High-shear media mills or pearl mills are used to prepare nanosuspensions. The media mill consists of three parts- a milling chamber, a milling shaft and a recirculation chamber. As a result of impaction of the milling media with the drug, high energy and shear forces are generated which provide the necessary energy to disintegrate the microparticulates drug into nanosized particles. The balls or milling media are ceramic-sintered aluminium oxide or zirconium oxide or highly cross-linked polystyrene resin and have high abrasion resistance. The size below 0.1 µm is achieved by planetary ball mills. In the media milling process, the milling chamber is charged with the milling media, water or suitable buffer, drug and stabilizer. Then milling media or pearls are rotated at a very high shear rate.[76]

In this study cyclosporin A (CsA)-loaded nanosuspensions was prepared using a top-down media milling method. The effect on the particle size of the nanosuspension was investigated by studying the effect of bead material, polymer, milling time and milling speed. The nanosuspensions prepared with polyvinyl alcohol (PVA) were found

to be stable as it showed no creaming or sedimentation phenomena and provide the smallest particles of about 530 nm. Nanosuspension having finer particles was obtained with zirconia beads of as compared to those with polystyrene beads. This nanosuspension was physically and chemically stable for at least two months. Draize test and Schirmer tear test were performed to study ocular irritation. In Draize test, very slight ocular irritation was observed with both nanosuspension and commercial product. However in Schirmer tear test commercial product cause more ocular irritation the rabbits' eyes as compared to nanosuspension. The above prepared CsA-loaded nanosuspension were found to be effective candidate for causing less ocular irritation.[77]

SUPERCRITICAL FLUID METHOD

Supercritical fluids are environmentally safe, so this technology have been investigated for the preparation of biodegradable micro and nanoparticles. Supercritical anti-solvent (SAS), rapid expansion of supercritical solution (RESS) and precipitation with compressed anti-solvent process (PCS) are commonly used methods using supercritical fluids. Supercritical anti-solvent process involves one liquid solvent and other is supercritical fluid. Both are entirely miscible with each other. As the solute is not soluble in the supercritical fluid, the formation of nanoparticles takes place as a result of the instantaneous precipitation of the solute formed by the extract of the liquid solvent by supercritical fluid. The SAS method can be used to prepare dexamethasone phosphate drug nanoparticles[78] (for microencapsulation) and griseofulvin nanoparticles. In RESS process, a supercritical fluid dissolves the solute and then the solvent power of supercritical fluid considerably falls and solute precipitates due to the quick extension of the solute through a tiny nozzle into a region lower pressure. This is the major point by which RESS differs from the SAS process.[79]

Table 1: Summary of methods used for preparation of polymeric nanoparticles for ocular delivery.[40-77]

Method	Drug	Category	Polymer	Stablizer	References
Solvent displacement method	Sulfacetamide	Antibiotic	Eudragit RL100	Pluronic F109	40
	Carvedilol	Non-selective beta blocker	Eudragit E100	Poloxamer 407	41
	Sparfloxacin	Fluroquinolone Antibiotic	PLGA	PVA	42
	Levofloxacin	Antibiotic	PLGA	PVA	43
	Cloricromene		Eudragit RS100 Eudragit RL100	Tween 80	45
	Cyclosporine A	Immunosuppressant	PLGA Eudragit RL100	PVA	46
	Piroxicam	NSAID	Eudragit RS100	PVA	47
	Amphotericin B	Antifungal	Eudragit RS100	PVA	48
	Diclofenac	NSAID	Eudragit S100	Poloxamer 188	49
	Diclofenac	NSAID	PLGA PLDL	Tween 80	50
	Hydrocortisone	Glucocorticoid	HPMC	SLS	51
	Pilocarpine	Para-sympathomimetic alkaloid	PLGA Chitosan	-	52,53
	Ibuprofen	NSAID	Eudragit RS100	-	54
	Flurbiprofen	NSAID	Eudragit RS100R Eudragit RL100R	-	55
	Ganciclovir	Antiviral	Chitosan	-	56
	Clarithromycin	Macrolide Antibiotic	PLGA	-	57
	Brimonidine	Non-selective beta blocker	Eudragit	TPP	58
	Naproxen	NSAID	PLGA	-	59
Homogenization	Prednisolone Dexamethasone	Antiinflamatory	Hydroxy ethyl cellulose	PLuronic F68	62
Ionic Gelation	Fluconazole	Triazole antifungal	Gum cordial	Di octyl sodium sulfosuccinate	65
	Mycophenolate mofetil	Immunosupperssant	Chitosan	PLuronic F68	66
	Brimonidine tratrate	Non-selective beta blocker	Chitosan		67
	Econazole nitrate	Antifungal	Chitosan		69
	5- Fluro Uracil	Anticancer	Chitosan		71
	Indomethacin	NSAID	Chitosan		74
Milling	Cyclosporine A	Immunosuppressant		PVA	77

CONCLUSION

Ocular drug delivery systems provide local as well as systemic delivery of the drugs. The novel advanced delivery systems offer more protective and effective means of the therapy for the nearly inaccessible diseases or syndromes of eyes. Nanosuspension technique opens new vista for agents having poor bioavailability and instability that are related with the delivery of hydrophobic drugs, including those that are poorly soluble in aqueous as well as organic media. In this review we provide the details of the method used for the preparation of nanoparticles or nanosuspension. Special properties of this nanosuspension technology can be considered as a new beginning in formulation technologies in the imminent years.

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