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REVIEW ARTICLE

NANOPRECIPITATION AS EMERGING TECHNIQUE FOR NANOSUSPENSION IN OCULAR DRUG DELIVERY

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ABSTRACT

The ocular conventional formulation shows poor ocular bioavailability due to the precorneal elimination of drugs that includes rapid tear turnover, poor permealibility of drugs into the corneal membrane, naso lacrimal drainage and less residence time in the conjuctival sac. This poor ocular bioavailability need to be improved by frequent instillation of dose to achieve the therapeutic effect. Nanotechnology is one of the better approaches to overcome challenges of conventional ocular drug delivery. The nanotechnology related ocular products available in the market are limited and nanotechnology is still in its earlier stage in drug delivery. Hence polymeric ocular nanosuspension is one of the strategies currently used to improve drug absorption across biological membranes. Nanoprecipitation technique (NPTN) is easy, less energy consuming, and widely applicable without any additives for the manufacture of desirable size nanoparticles. The Nanosuspension formed by diffusion of drug and polymer containing solvent into the anti solvent, the polymer droplets immediately precipitate or adsorbed with drugs. The rapid nanoparticle formation is described by marangoni effect, which is subjected to interfacial turbulences that occur at the interface of the solvent and the anti solvent. Nanosuspension can be further sterilized and subsequently evaluate for various parameters like particle size, drug release, zeta potential character. This review article mainly focused on conventional nanoprecipitation, modified nanoprecipitation technology and effect variables in nanosuspension preparation.

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INTRODUCTION

Design of safe and effective drug delivery systems that can transport and drug release in to the specific site of action is a big task of a pharmaceutical research scientist (Sanjeeb, 2008). The drug delivery to the eye can be broadly classified based on anterior and posterior part of the eye. Anterior portion of eye is a common route for administration are conventional ophthalmic preparation whereas posterior-segment of the eye is common routes for, periocular and intravitreal injections, and sub conjunctival injection (Ripla Gaudana, 2009). The Conventional ophthalmic preparations like eye drops, suspensions and ointments cannot be considered an effective treatment of vision threatening ophthalmic diseases such as conjunctivitis, blepharitis, keratitis and sicca. However, more than 90% of the commercial ophthalmic preparations have existed in the form of eye drops.

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These formulations mainly target the anterior segment eye diseases and easily eliminated from the eye by various factors such as nasolacrimal driange, blinking of an eye, tear dilution and tear turnover, which result in low ocular bioavailability of drugs due to precorneal elimination. Hence the conjunctival sac can accommodate approximately 20 µl of added fluid only. Various approaches have been developed to enhance the ocular bioavailability of drugs and provide the sustained release by minimizing precorneal drug loss (Swarnali Das, 2010). Though the eye consist one of the most selective barriers to foreign molecules for the eye, transcorneal penetration of topically administered ophthalmic medicines for the posterior segment is a major concern. There are four types of barrier which influence the drug deliver in to the anterior part of the eye 1.Tear film barrier 2. Corneal barrier 3.Conjuctival barrier 4.Scleral barrier (Maria de la, 2010). The targeted drug delivery system was introduced by "Paul erhlich" 100 years ago (Strebhardt, 2008) in terms of "Magic Bullet" concept (Chandy, 2002) which have great affinity and specificity to the cells, tissue and organs in the human body. The term nanotechnology were derived from the greek word nano

meaning dwarf, applies the principles of engineering, electronics, physical and material science and manufacturing at a molecule or submicron level (Gopal, 2008). Nanotherapeutics is a rapidly progressing area in the field of nanomedicine, which is being utilized to overcome several problems of conventional preparations including poor aqueous solubility, lack of site specific target. Which can be achieved by using an nanocarriers such as solid-lipid nanoparticles (SLN), nanoemulsion, nanosuspension (NS), Gold nanoparticles, silver nanoparticles, mesoporous silica nanoparticles, nanocrystals, magnetic nanoparticles, carbon nanotubes, nanosponges, albumin nanoparticles, fullerene nanoparticles and polymeric nanoparticles. In 1970 the nanoparticles were first developed and used as carriers for vaccine and anticancer drugs (Majeti, 2000). Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm.In which drug is adsorbed, entrapped, encapsulated or attached to a carrier. Depending upon the method of preparation, nanospheres or nanocapsules can be obtained. Nanocapsules are systems in which the drug is enclosed in a cavity surrounded by a polymer membrane, while nanospheres are matrix systems in which the drug and polymers were homogenously dispersed. Nanospheres are mostly prepared by emulsion polymerization. Whereas nanocapsules are obtained by interfacial polymerization performed in emulsion (Nagavarma, 2012).

Figure 1. Nanosphere Nanocapsule

Nanoparticles can be prepared by using a variety of materials such as proteins, polysaccharides and synthetic polymers. The formulation of nanoparticles can be implemented to all drug compounds belonging to biopharmaceutical classification system (BCS) classes II and IV to increase their solubility. Nanosuspension technology can also be used for drugs which are insoluble or poorly soluble in water (Das, 2011). Nanosuspensions (NS) is defined as colloidal dispersions of nano size drug particles that are produced by a suitable method and may or may not stabilized by a suitable stabilizer. Reduction of drug particles to nano size range leads to an enhanced dissolution rate because of increased surface area and also because of saturation solubility (Suzanne, 2011). Depending on the properties of the polymers and the drug used. The nanoparticles can be prepared through simple techniques namely solvent evaporation method, salting-out method, nanoprecipitation method, emulsion diffusion method, quasi emulsion diffusion method (QESD), dialysis method, double emulsification method, nano spray drying method, layer by layer method, desolvation method, top down(Ball mill, high pressure homogenization), bottom up (Supercritical fluid technology) and ionic gelation method, and particle replication in non-wetting templates (Hugo Almediaa, 2014). Particle size, particle size distribution, and stability of nanosuspension are major issue considered by pharmaceutical scientists when formulating dispersed systems, especially those intended for parenteral or ocular administration. Very small particles such as nanoparticles are well tolerated and possess mucoadhesive properties, which could prolong the residence time of the drug in the conjuctival sac, prevent tear washout, and increase ocular bioavailability (Harikumar, 2011)

Ideal characters for polymeric nanoparticle

- It should be non toxic.
- It should be non antigenicity.
- It should be biodegradable and biocompatible.
- It should protect the drug against environmental factors.
- It should not release the drug to reach the sites of action.
- It should be degraded or eliminated from the body after the release of the drug (Ghosh, 2000).

Advantages of polymeric nanoparticle

- It can ease to alter particle size and surface characteristics of nanoparticles.
- It can be modified drug release character either sustained release or controlled release.
- It can enclose a variety of drugs without any chemical reaction.
- It provides site-specific action by attaching targeting ligands to surface of particles.
- Small sized nanoparticles can penetrate through smaller capillaries, which could allow efficient drug accumulation at the target sites (Meenakshi, 2014).
- It can be used for various routes of administration including oral, nasal, parenteral, intra-ocular etc.
- They have longer shelf stability.
- It enhances the drug solubility.
- It reduces the frequency of dose and systemic toxicity of drug.
- The positive charge of nanoparticle can strongly interact with negative charge of mucin in the conjuctival sac, thus allowing for long residence time of drug release in the eye.

Disadvantage of Polymeric Nanoparticle

- The small size of nanoparticulate suspension can lead to particle-particle aggregation (Singh Davinder, 2013).
- It is difficult to handle in liquid and dry forms.
- It can entrap least amount of drug with burst release.
- They may trigger an immune response and allergic reaction.
- It may involve the use of harsh toxic solvents in the preparation process.

Conventional Nanoprecipitation (NPTN)

This technique has been widely used for the encapsulation of mainly, hydrophobic drugs in either nanocapsules or nanospheres in aqueous system (Budhian, 2007). Nanoprecipitation is also called as solvent displacement (Uma maheswari, 2013) or anti solvent precipitation or interfacial deposition or precipitation (Sovan lal, 2011) or drop by drop addition method (Archana, 2015) or modified solvent evaporation (Serveh Ghaderi 2015) or solvent shifting (Frederik, 2014), solvent extraction method (Aude Munin, 2011). It is considered as one of the first developed techniques used for the encapsulation of drug molecules. In 1989 nanoprecipitation technique was introduced by fessi *et al* for encapsulating indomethacin as a model drug (Fessi, 1989).

The basic principle of this technique is similar to spontaneous emulsification of the organic phase (Solvent stream) into the aqueous phase /Anti solvent stream (Manikandan, 2015). It is describes as "a process for the preparation of dispersible colloidal systems of a substance in the form of spherical particles of the matrix type and of a size less than 500 nm". The method is based on the single step precipitation of preformed polymer following displacement of a semi polar solvent miscible with water in the presence or absence of surfactant. In organic phase consist polymer and drug that are dissolved in a water miscible organic solvent. The resulting organic phase is injected as drop by drop into a stirred aqueous phase containing a surfactant as a stabilizer. The nanoparticles are formed due to the rapid diffusion of the organic phase into the aqueous phase and turned the aqueous phase slightly milky opalescence. Since sonication process was used to help the size reduction and to evaporate residual organic solvent from the nanoformulation (Mandal, 2010).

interfacial turbulence and thermal inequalities in the system. This leads to the continuous formation of vortices of solvent at the interface of both liquids. The organic solvent diffuses from regions of low surface tension which causes gradual precipitation of the polymer and forms nanocapsules **(**Mora, 2011). The nanoprecipitation (NPTN) technique can be used for preparation of nanocapsules for lipophilic (oil) drugs with high loading capacity (Amit, 2011). The formation of nanocapsule are depend on the organic phase injection rate, aqueous phase stirring rate, the method of organic phase addition and the organic phase and aqueous phase ratio. Similarly, the drug release of nanocapsule maybe influenced by the nature and concentration of their components. According to sugimoto's theory on polymer precipitation indicated that the process of particle formation in the nanoprecipitation method. Which include three stages a) nucleation b) growth c) aggregation. The rate of each step determines the particle size and the driving force of these

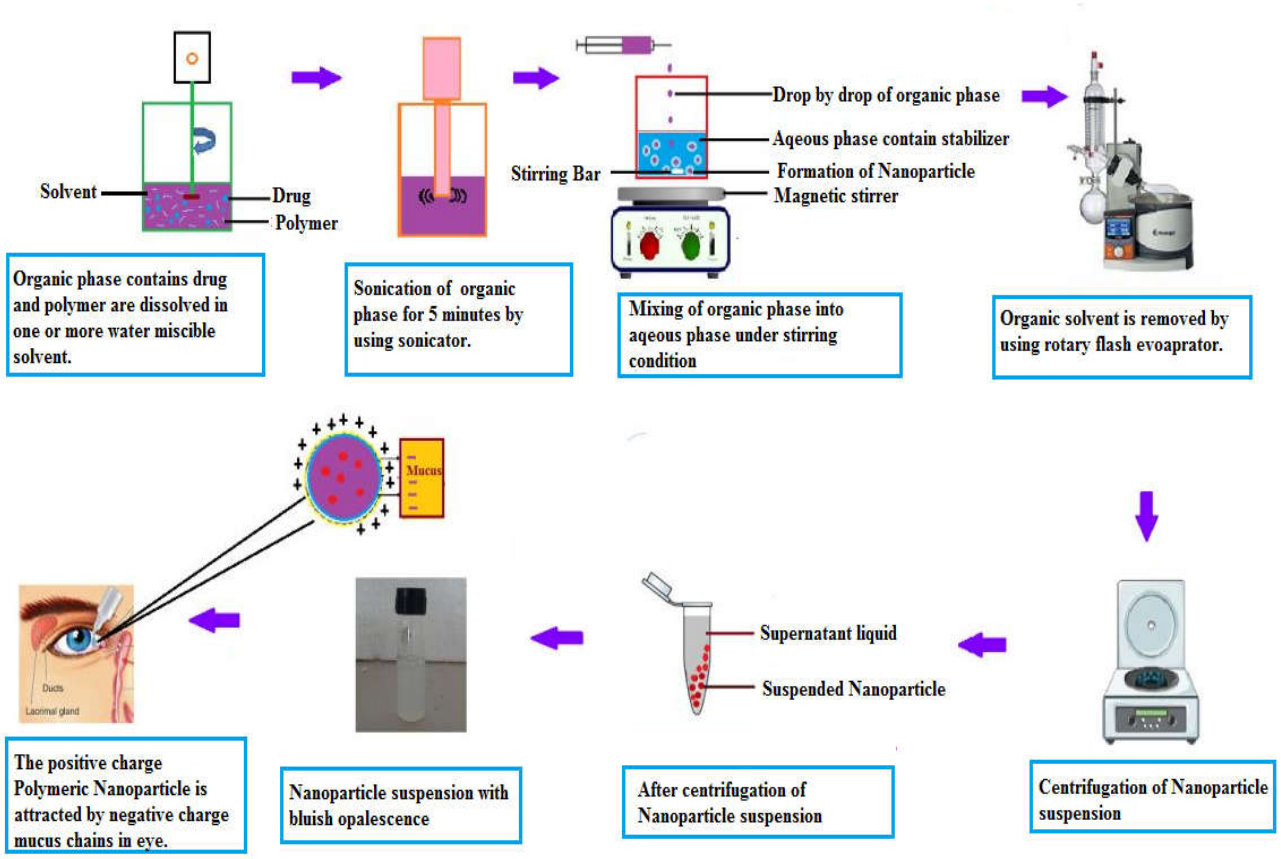

Figure 2. Various steps involved in modified nanoprecipitation techniques

The formation of a colloidal nanoparticle dispersion can be visualized by the bluish opalescence colour (Pignatello, 2000). This phenomenon is known as the Tyndall effect which results from a scattering of light caused by the dispersed colloidal particle (Mecklenburg, 1915). Therefore it is described minute differences between solvent displacement and interfacial deposition, explaining as first forms the nanospheres or nanocapsule while the latter is restricted only to nanocapsule (Catarina, 2006). Nevertheless, both are based on the spontaneous emulsification of the organic phase into the aqueous phase. When a liquid with a high surface tension (Aqueous phase) pulls stronger on the surrounding liquid than one with a low surface tension (Organic phase solvent). This difference between surface tensions of the aqueous and the organic phase causes phenomena is supersaturation (Mora Huertas, 2010). It is defined as the ratio of polymer concentration over the solubility of the polymer in the solvent mixture. There are two types of nanoprecipitation techniques a) Single step nanoprecipitation b) Two step process of modified nanoprecipitation.

Merits of Nanoprecipitation

- It is simple and requires lower energy consumption.
- It does not need high-shear or high-pressure mixing devices.
- They require water miscible organic solvents to dissolve the polymer.

- Good mixing conditions give birth to high nucleation rates, i.e., The larger population of smaller nanoparticles (Jasdeep, 2015; Edel Sah, 2015)

Demerits of Nanoprecipitation

- This causes the nucleation of very small aggregates of polymeric molecules. Therefore, a large amount of water should be added into a polymeric dispersed phase.
- The incomplete removal of residual solvents from nanoparticles brings about severe aggregation.
- Poor mixing of organic and aqueous phase produce few big nanoparticles.
- The major drawback is that the success of preparing nanoparticles is restricted only to a narrow region of the polymer/solvent/anti-solvent composition map, the so called Ouzo region (Sah, 2015; Lince, 2008)

Modified Nanoprecipitation Method-(MNPTN)

The polymer was dissolved in water miscible organic solvent, which was diluted with distilled water under the influence of sonication. Prepared organic phase was loaded in to a syringe equipped with needle (inner diameter of 0.30 x 8 mm). The loaded organic phase was injected at the rate of 2 ml per minute by inserting the needle (submerged position) in to aqueous phase containing stabilizers under the influence of sonication. Subsequently, nanoparticles were formed and turned the aqueous phase slightly milky with bluish opalescence (Moorthi Chidambaram, 2013). However, sonication process was continued up to 60 minutes to reduce particle size and to evaporate residual solvent present in the nanoformulation. The manufacturing of polymeric nanoparticulate suspension via modified nanoprecipitation technique, as illustrated in Figure 2. When hydrophobic solutes (Oil) is dissolved in a water-miscible organic solvent and mixed with large amount of water, causes spontaneous emulsification to make a kinetically stable nanodroplet. This process is called as ouzo effect (Ganachaud, 2005) The appearance of slight precipitate in the solution was considered as the end point. The organic solvent was then allowed to evaporate with continuous stirring on a magnetic stirrer (Shakeri, 2015) or removed by using rotary flash evaporator (Archana, 2012). The nanosuspension was then centrifuged at 15,000 rpm for 30 min at 4^{o}C using a ultracentrifugation (Weiwei Zou, 2009) to collect the nanoparticle and after its centrifuge supernatant was taken for further evaluation like drug entrapment efficiency. In modified nanoprecipitation method co solvent can be used to either increase the entrapment efficiency of the drug in nanoparticles or to reduce the mean particle size of the nanoparticles (Archna, 2017). The mechanism behind the reduction of particle size by sonication is due to creation of bubbles (Cavitation) followed by collapse which releases shock waves along with temperature and pressure changes for nucleation. Ultrasonic waves were found to cause i) Faster and more uniform nucleation through the sonicate volume, leading to smaller and more uniform-sized particles and ii) Reduction of agglomeration by reducing contact between particles and controlling the number of nuclei (Abhijit, 2013).

Sterilization of Nanosuspension

The selection of sterilization method for Nanosuspension which is based upon drug characters. It can be sterilized by filtration through 0.22-µm bacteria proof membrane filter and filled in presterilized glass vials (Sagar, 2009). Alternatively, Nanosuspension can also be sterilized by autoclaving or gamma irradiation method (Suman, 2009).

Novel Approaches in Nanoprecipitation Flash Nanoprecipitation-(FNPTN)

The flash nanoprecipitation process involves high velocity jets mixing, which provides micromixing of the copolymer and drug, leading to mixing timescales that are shorter than the timescale for nucleation and growth of particles, and allowing for the formation of nanoparticles with size distributions, and drug loading efficiencies not provided by other technologies (Prud home, 2006). In the flash nanoprecipitation technique a highly hydrophobic drug is dissolved along with a block copolymer (BCP) in a water miscible organic solvent. This solution is injected into a small chamber at a high velocity along with water (Zhengxi, 2014). The high velocity generates turbulent mixing, causing the hydrophobic drug and polymer to co precipitate very rapidly, Forming nanoscaled particles. This technique was first described by "Johnson and Prud homme" to produce nanoparticles encapsulating hydrophobic drugs. The nanoparticles are obtained via a rapid precipitation process and offers also high loading capacity and the ability to encapsulate drugs (Christine, 2016). Several successful applications of the flash nanoprecipitation technique have been reported for encapsulation of various hydrophobic drugs, peptides, imaging agents, or a combination of both therapeutics and inorganic colloids.

Micro fluidics nanoprecipitation (MF-NPTN)

Nanoprecipitation is usually performed via one-pot pouring of the polymer solution into the nonsolvent, or by drop wise addition of one phase into the other. Micro fluidic processes, using a hydrodynamic flow focusing setup or a confined impinging jet reactor have emerged to improve the mixing of the two phases. It is a continuous-flow nanoprecipitation process in which, a diluted polymer solution and water were separately pumped and nanoprecipitation occurred within the micromixer. The latter consisted either of either a T-junction or a High Pressure Interdigital multilamination micromixer. The obtained suspension of nanoparticles could be collected at the outlet of the micromixer (Hany, 2011).

Formulation of Nanosuspension

The organic and aqueous phase contains five basic components such as drug, polymer, solvent, antisolvent and surfactant. The various components are involved in the preparation of the nanoparticle. Which is shown in Table 1.

Drugs

Nanoprecipitation (NPTN) is an essentially technique used to encapsulate hydrophobic molecules and encapsulation of water soluble compounds by this method is still providing challenges to the pharmaceutics person (Nagavarma, 2012). Most of the drug encapsulation studies focused either on poorly water-soluble or amphiphilic compounds that are highly soluble in water. When the drug has a low affinity for the polymer, it tends to diffuse from the organic phase to the external aqueous medium during the nanosphere formation process, leading to a low drug loading capacities (Maribel, 2005). The physical

chemical properties of the incorporated drugs might significantly affect the resulting release patterns and the degradation of the polymer matrix, especially at high initial drug loadings. For example, high content of freely water soluble drugs can facilitate water penetration and lead to the creation of highly porous polymer networks upon drug leaching (Klosea, 2008). In contrast lipophilic drugs can hinder water diffusion into the system, slowing down polymer degradation.

Oil Phase or Organic phase

The organic phase (Serveh, 2015) comprised hydrophobic drug (Yichao, 2016) polymers and water miscible solvent/ driving solvent suchas, ethanol or acetone (Leena, 2002). In nanoprecipitation (NPTN) technique the most commonly used organic solvent is acetone, ethanol (Couvreur, 2002).The selecting the best solvent are high miscibility of the polymer and solvent should ease to evaporation and removal. Common solvents for nanoprecipitation are acetone, dichloro methane (DCM) (Jerome, 2007) acetonitrile, tetrahydrofuran (THF) (Moritz, 2010), dimethyl formamide (DMF), dimethylacetamide (DMA), dimethylsulfoxide (DMSO) (Sara, 2017).

Water Phase or Anti solvent

The aqueous phase is usually contained distilled water and hydrophilic surfactants could be added to avoid particle aggregation. The hydrophilic drugs could be dissolved in the aqueous phase (Ethiraj, 2013).

Polymers

The most commonly used natural polymers are chitosan, gelatin, sodium alginate, albumin and biodegradable polyesters such as PCL, PLA, and eudragit, PLGA (Soosan, 2012) or poly (lactic-co-glycolic acid) is a synthetic biodegradable and biocompatible polymer that received FDA approval in the pharmaceutical industry for human applications.

Stabilizers or surfactants

Stabilizers are amphiphilic molecules that possess both hydrophilic head and hydrophobic tail parts. The most commonly used stabilizers of polymer particles include Polyvinyl alcohol (PVA), Polyvinyl Pyrrolidone (PVP), Tween 80 (Zainab), Fluonic-127 (Poloxamer 407), Fluonic-68 (Poloxamer188), didodecyl dimethyl ammonium bromide (DMAB). However, polymer stabilizer may prevent aggregation of nanoparticles, they are difficult to remove even through washing (Archana Mehrothral, 2015).

Preservatives

The most commonly used preservative in polymeric Nanosuspension is benzalkonium chloride (BKC) with the concentration of 0.01% (w/v) for effective against bacterial contamination (Azza, 2012).

Influence of variables in nanosuspension preparation

The following parameters which influences the Nanosuspension preparation, which includes

Effect of Particle size

In the development of a drug delivery system for topical use in the eye, the particle size is an important consideration in irritation and comfort. If the particle induces tears, a rapid drainage of the instilled dose would occur and could reduce bioavailability, decreasing the residence time of drug in the conjunctival sac. Particle size for ophthalmic application should not exceed 10 μm. The United States Pharmacopoeia (USP) specifies that ophthalmic solutions should contain no more than 50 particles with a diameter more than 10 μm, 5 particles with a diameter of not greater than 25 μm, and 2 particles with a diameter of not greater than 50 μm per ml of solution when using the microscopic particle count method. The particle size depends on the interplay between several variables, like the organic (Polymer) phase viscosity, stabilizers, solvent properties and the polymer properties in nanoprecipitation method (Hyvonen, 2005).

Effect of polymer concentration

The increasing concentration of dissolved polymer resulted in increasing organic phase viscosity and reduction of the efficiency of stirring which caused the formation of bigger emulsion droplets. A higher polymer concentration might also result in increasing viscosity of the organic phase which might decrease the diffusion rate and might lower the rate of Ostwald ripening (Zambaux,1998) for the more viscous solutions so smaller particles were produced.

Effect of molecular weight of the polymer

Polymer molecular weight, being an important determinant of mechanical strength, is also a key factor in determining the degradation rate of biodegradable polymers. Low-molecular-weight polymers degrade faster than high-molecular-weight polymers thereby losing their structural integrity more quickly. As chain scission occurs over time, the small polymer chains that result become more soluble in the aqueous environment of the body. This introduces “holes” into the polymer matrix. Consequently, lower molecular weight polymers release drug molecules more quickly .This can be used to further engineer a system to control the release rate. A combination of molecular weights might be used to tailor a system to meet the demands of specific release profiles (Yury, 2006).

Effect of stabilizer

The stabilizer added to the formulation which can influence the properties of the polymeric nanoparticulate suspension and stabilizer can also be incorporated on the particle surface, modifying nanoparticles characters such as particle size, zeta potential, and mucoadhesion. The most commonly used stabilizing agent is poly vinyl alcohol (PVA) with two molecular weights, low molecular weight (LMW) 31000 g/mole and high molecular weight (HMW) 89000 g/mole, Poly vinyl alcohol (PVA) is a swellable, hydrophilic macromolecule, but the addition of stabilizer helps to preserve the nanoparticle suspensions from agglomeration over long storage periods (Swarnali Das, 2011).

Effect of Stirring rate

In nanoprecipitation, the most commonly used stirring method is magnetic stirring. An increase of the stirring rate generally

results in a decrease in the particles size. This is explained by more efficient shear mixing and thus, more rapid diffusion of the organic solvent to the water phase.

Effect of organic and aqueous phase ratio

The aqueous phase consists of an nonsolvent for the polymer. This phase causes polymer precipitation to form nanoparticles. It was found that size of nanoparticles was inversely proportional to increasing the ratio of organic to the aqueous phase, as nanoparticle size decrease with increasing the ratio of organic phase of the polymer to aqueous phase of surfactant. The increase in aqueous phase volume results in decreasing the particle size due to increase of diffusion rate of the water-soluble organic solvent (acetone) in the aqueous phase (Ahmed, 2015). The addition of organic phase into aqueous one during the procedure was completed by using two kinds of syringes to apply two different flow rates; 5cc syringe (2ml/min) and 1cc syringe (1ml/min).

value is close to 1, the size range is wide. Generally, a value closer to 0 is desired (Lucia, 2015). It could be said that a greater amount of polymer would promote the formation of much more homogenous nanoparticle samples.

Effect of Zeta Potential (ζ)

The electrostatic potential also called as zeta potential .which offers important information about the stability of colloidal dispersions. It is created by the electric charge present on the nanoparticles' surface. Nanoparticles having a zeta potential ranging from -10 mV to +10 mV are considered fairly neutral. On the other hand, a zeta potential lower than -30 mV or higher than +30 mV is an indicator of a very stable dispersion (Karen, 2012).

Table 1. Various example of drugs and polymers used in nanopreciptation technique

Drugs	Dose (mg)	Polymers	Organic solvent	Aqueous solvent	Stabilizer	Reference
Rivastigmine tartrate	10	PLGA	Acetone	Water	Pluronic F-127	(Natarajan, 2014)
Rivastigmine Hydrogen tartrate	35	Eudragit RL 100	Acetone	Water	Poloxamer 407	(Sara , 2017)
Pramipexole dihydrochloride	10	MPEG-PCL	Acetone	Phosphate buffer pH 9.0	Pluronic F-68 (1%)	(Somasundaram, 2016)
Camptothecin	10	Eudragit S100	Dimethyl sulfoxide	Water	Poloxamer 188(0.1%)	(Manikandan, 2015)
Budesonide	10	Eudragit S100	Acetone	Water	Pluronic F- 68	(Sanjay, 2016)
Quercetin	25	Eudragit E 100	Ethanol	Water	Poloxamer 188	(Moorthi, 2013)
Simvastatin	50	PLGA	Acetone.	Distilled water	Pluronic F-68	(Vikram, 2010)
Amphotericin B	100	Poly lactic acid	Acetone, Methanol	Water	Pluronic F-68,	(Hany, 2012)
Flurbiprofen	10	Eudragit RL 100	Acetone, Methanol	Water	PVA	(Bhavani, 2015)
Nisoldipine	NM	Eudragit S100	Ethanol	Water	Tween 80.(0.02ml)	(Nepolean, 2012)
Zidovudine	50	Chitosan	Chloroform	Water	Propylene glycol	(Arunprasath, 2015)
Acyclovir	25	Eudragit RLPO	Alcohol	Water	Pluronic F-68	(Bhosale, 2011)
Risperidone	10	PDLLA 100	Acetone.	Water	Pluronic F-68	(Muthul, 2009)
Prednisolone	NM	PLGA	Methanol Acetone Acetonitrile	Water	Poloxamer,PVA	(Rameswari,2014)
Simvastatin	10	PVP- K30	Acetone, Acetonitrile	Water	SLS,Tween-80,PVA	(Vikram, 2010)
Hesperidin-Diazepam	100	PLGA	Acetone, DMSO	Water	Poloxamer 407	(Deepak, 2015)
Abacavir	NM	Eudragit RS100	Acetone, Ethanol	Water	Propylene glycol	(Dheivanani, 2012)
Flurbiprofen	1.5mg/ml	PLGA	Acetone	Water	Poloxamer 188 Pluronic F-108	(Estefania, 2012)
Moxifloxacin	100	PLGA	Acetone	Water	Tween 80(0.02%)	(Meetali, 2013)
Moxifloxacin	10	Eudragit RL 100	Acetone, Methanol	Water	PVA 1%	(Srinivas,2012)
Moxifloxacin	100	PLGA	Acetone	Water	Tween 80(0.2%)	(Gadad,2012)
Flurbiprofen	37.5	PLGA	Acetone	Water	Poloxamer 188	(Vega,2006)
Curcumin	5	PCL	Acetone	Water	Poloxamer 188	(Leticia, 2012)
Ibuprofen	NM	Ethyl Cellulose	Ethanol, Acetone	Water	Tween-20 (0.1%)	(Swathi, 2104)
Valsartan	NM	Eudragit L 100	Ethanol, Acetone	Water	Tween-20 (0.1%)	(Bharathia, 2012)
Piroxicam	10	PLGA	Acetone, DCM	Water	Pluronic F 68	(Lynda, 2013)
Doxorubicin	NM	PHB	Ethanol	Water	Tween 80	(Sasikumar, 2015)
Sulfacetamide	10	Eudragit RL100	Acetone	Water	Pluronic F-108	(Bivash, 2010)
Levofloxacin	10	PLGA	Acetone	Water	PVA 1.5%	(Himanshu, 2013)
Saprofloxacin	10	PLGA	Acetone	Water	PVA 1.5%	(Himanshu, 2010)
Diclofenac sodium	NM	Eudragit RS100	Ethanol	Water	PVA	(Khosro, 2012)
Paclitaxel	1 mg/ml	PLGA	Acetone	Water	Poloxamer 188	(Cristina, 2002)

Note : NM(Not mentioned),PLGA (Poly(D,L-lactide-co-glycolide), PHB(Poly hydroxyl butyric acid), PVP K 30(Poly vinyl pyrrolidone), PVA(Poly vinyl alcohol), MPEG -PCL (Methoxypoly (ethylene glycol) Poly(caprolactone), PDLLA 100 (Poly (D, L-Lactide), SLS(Sodium lauryl sulphate)

Poly dispersity index (PDI)

Polydispersity indicates the uniformity of droplet size within the formulation. Most frequently, the size of a population of particles follows a multimodal distribution. When the PDI

At higher zeta potential values, the repulsive forces between similarly charged particles prevent their aggregation and increase their stability.

Effect of drug loading efficiency

The drug loading efficiency may be increased by increasing polymer ratio, so that a sufficient quantity of polymer will be available to entrap the drug present in the solution and low drug loading efficiency due to poor diffuse ability of organic phase into the aqueous phase (Kharia,2012).

Effect of using Cryoprotectant

Freeze dried nanoparticles without cryoprotectants appeared as off-white fluffy and sheet-like materials. Using sucrose as the cryoprotectant resulted in the formation of a white, brittle, crystalline material with perforated structure. Mannitol formed white spongy, cotton like material upon lyophilization. The freeze dried sample without cryoprotectants did not redisperse in water after manual hand shaking (Pankaj, 2016)

Effect of drug release

The drug release is also affected by particle size of the nanoparticle and smaller particles size has a larger surface area therefore, most of the nanoparticles are leads to faster drug release. In contrast, larger particles which allow more drugs to be encapsulated per particle and give slower release (Roli Jain, 2015). The release rate of nanoparticles depends upon i)Desorption of the surface-bound/adsorbed drug ii) Diffusion through the nanoparticle matrix iii) Diffusion through the polymer wall iv) Nanoparticle matrix erosion v) a combined erosion/diffusion process. Thus, diffusion and biodegradation govern the process of drug release.

Conclusion

The nanoprecipitation technique (NPTN) is one of the easiest technique for preparation of nanosuspension for ocular drug delivery by using a biocompatible and biodegradable polymers. In this technique organic phase is injected into aqueous phase containing stabilizer under agitation condition of magnetic stirrer or homogenizer. The diffusion of organic phase in to aqueous phase causes slight precipitation leading to milky bluish opalescence due to light scattering effect. These nanosuspensions can be dropped into the conjuctival sac of the eye and the positive charge of drug coated nanoparticle may be bind with negative charge mucin in the eye which leads to electrostatic attraction of particle and provide prolonged drug release. Moreover due to submicron size of particle can reduce eye irritation. Nanoprecipitation technique do not require sophisticated instrument for nanoparticle preparation of variety hydrophilic and hydrophobic drugs .The various factors like drug and polymer ratio, volume of organic and aqueous phase may influence Nanosuspension preparation. In this review article we provide the details of method used for the preparation of nanosuspension. The unique characters of nanoprecipitation technique can be considered as an emerging technique for preparation of nanosuspenion for the treatment of ocular disease.

Conflicts of Interest: We declare that we have no conflict of interest.

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