IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

"Development & Characterization of PLGA-Based Voriconazole Nanosuspension for Opthalmic Application "

Ms. Dipali. R. Khandagale[1] Dr. P. K. Pawar[2]

[1]Assistant Professor at RMP'S Bhalchandra College of Pharmacy Khanapur, Pune.

Abstract

The objective of present investigation to develop and characterize PLGA based voriconazole nanosuspension for the treatment of ocular keratitis. PLGA polymer (75:25) were used to prepare voriconazole loaded nanosuspension by using solvent evaporation technique and characterized physicochemical properties, like particle size, zeta potential and entrapment efficiency, in vitro drug release.. All the formulations of Voriconazole loaded nanosuspension were also evaluated by in vitro drug release profile. The Voriconazole loaded nanosuspension showed uniform particle size (around 167.01±05.00 nm to 491.31±18.94 nm) and shows high entrapment efficiency (78.23 ± 0.57 to 87.34± 0.52%.) and positive zeta potential for all batches. To improve their stability the optimized nanosuspension were lyophilized by using mannitol as a cryoprotectant confirmed that the drug was dispersed inside the particles and there is no only interaction between drug and polymer. The voriconazole was incorporated successfully in nanoparticles of nanosuspension prepared with 1:4 drug polymer ratios by using PLGA (75:25) which provide sustained release profile and follows zero order release kinetic.

Keywords- VCZNS. PLGA, NS,EE.

Introduction

Now days various problems are come in front for researcher to work on othalmic drug delivery. The conventional dosage form are most convienient and patient compliant route but they have some disadvantage like majority of drug eliminated from the eye due to tear turn over, drug elimination like nasolachrymal drainage, conjuctival uptake due to poor bioavailability, less residence time and results into about less than 5% of drug penetrate into cornea. Delivery of drug to the targeted ocular tissue is restricted by various precorneal dynamic and static ocular barriers. Barriers that pose a challenge to anterior segment drug delivery are static (corneal epithelium, corneal stoma, and blood-aqueous barrier) and dynamic barriers (conjunctival blood flow, lymph flow, and tear drainage) and metabolic barriers To overcome this problems various novel drug delivery system for ophthalmic application such as ocular insert, collagen shield, colloidal or particulate system like Nanoparticle, Nano capsules, noisome, liposome have been developed to prolong the residence time & to improve bioavailability Nanosuspension is a submicron colloidal dispersion of drug particles.

A pharmaceutical nanosuspension is defined as very finely colloid, Biphasic, dispersed, solid drug particles in an aqueous vehicle, size below 1*m, without any matrix material, stabilized by surfactants and polymers, prepared

by suitable methods for Drug Delivery applications, through various routes of administration like oral topical, parenteral ,ocular and pulmonary routes. Nanosuspension not only solves the problem of poor solubility and bioavailability but also alter the pharmacokinetic of drug and that improve drug safety and efficacy. The antifungal drug choice of newer triazole i.e. Voriconazole is a novel second generation triazole derivative of fluconazole with excellent broad spectrum activity commercially available for oral and intravenous administration.

The use of bioadhesive polymers play important role that they form coat around the drug and releases drug slowly in sustained manner. Application of PLGA in recent few years due to its mucoadhesive characteristics and presence of positive charge on it significantly bind with negative charge which present on cornea and conjunctiva. The characteristics of PLGA such as biodegradability, non-toxicity, biocompatibility and mucoadhesiveness, PLGA is synthesized by means of ring-opening co-polymerization of two different monomers, the cyclic dimers (1,4-dioxane-2,5-diones) of glycolic acid and lactic acid. 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 molar ratio of the monomers used (e.g PLGA 75:25 identifies a copolymer whose composition is 75% lactic acid and 25% glycolic acid). The crystallinity of PLGAs will vary from fully amorphous to fully crystalline depending on block structure and molar ratio. PLGAs typically show a glass transition temperature in the range of 40-60 °C. PLGA can be dissolved by a wide range of solvents, depending on composition. Higher lactide polymers can be dissolved using chlorinated solvents whereas higher glycolide materials will require the use of fluorinated solvents. The attempts also made to taking above information in view the purpose of present investigation was to formulate voriconazole loaded nanosuspension as sustained ocular drug delivery systems with the aim of improving antifungal activity of voriconazole against Candida Albicans & Aspargillus Flavus compare to marketed formulation

Anatomy and physiology of the eye[13]

There are two segments in human eye: posterior segment and anterior segment.Anterior segment of the eye occupies approximately one-third while the remaining portion is occupied by the posterior segment. Tissues such as cornea, conjunctiva, aqueous humor, iris, ciliary body and lens make up the anterior portion.. The cornea acts as a physical barrier to foreign materials and structural damage even as the sclera maintains the shape of the eye. The iris controls the size of the pupil and determines the amount of light that reaches the retina. The ciliary body produces aqueous humour, even as the choroid contains vessels that supply oxygen and nutrients to the retina. Back of the eye or posterior segment of the eye include sclera, choroid, retinal pigment epithelium, neural retina, optic nerve and vitreous humor. The retina forms the inner section of the eye where neurons capture light and send out processed signals to the brain via the optic nerve. The structure of the eye is shown in figure 1 with the essential barriers for ocular drug delivery further described below.

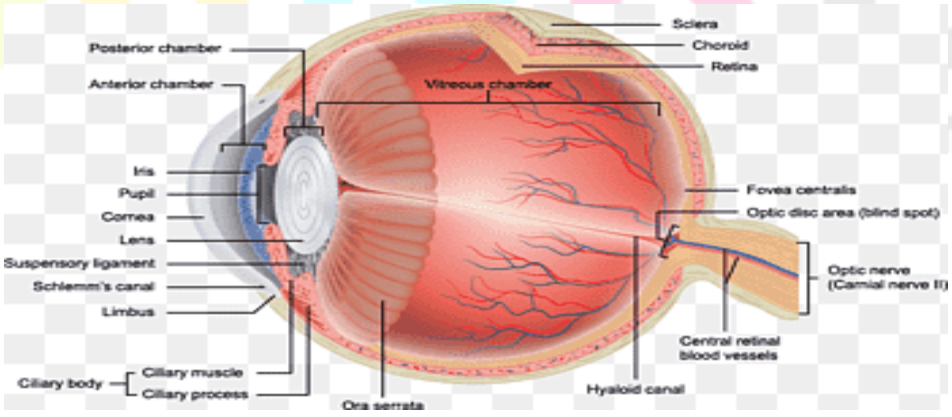

Figure 1: Structure of Eye

Occular Drug Delivery Systems:-

Non-conventional Novel drug delivery systems

The conventional dosage forms having lot of problems thus to overcome this problems, Now days researchers focused on R and D based novel strategies. The colloidal dosage forms, such as microparticles/nanoparticles, liposomes, niosomes, solid lipid nanoparticles (SLN), microemulsions(MEs)/nanoemulsions etc acts as drug reservoirs and can prolongs precorneal time of drug on cornea.

Nanosuspension (NS)

Nano is a Greek word, which means 'dwarf'. Nano means it is the factor of 10^{-9} or one billionth. Some Comparisons of nanoscale are given below,

0.1 nm = Diameter of one Hydrogen atom.

2.5 nm = Width of a DNA molecule

1 micron = 1000 nm.

1 nm = 10^{-9} m= 10^{-7}cm = 10^{-6}mm

Micron = 10^{-6}m= 10^{-4} cm = 10^{-3mm4}

A Nanosuspension is a submicron colloidal dispersion of drug particles. A pharmaceutical nanosuspension is defined as very finely colloid, Biphasic, dispersed, solid drug particles in an aqueous vehicle , size below 1* ,without any matrix material , stabilized by surfactants and polymers , prepared by suitable methods for Drug Delivery applications, through various routes of administration like oral, topical parenteral, ocular and pulmonary routes. A nanosuspension not only solves the problem of poor solubility and bioavailability but also alters the pharmacokinetics of drug and that improves drug safety and efficacy. Nanosuspensions differ from Nanoparticle, which are polymeric colloidal carriers of drug(Nanospheres and nanocapsules), and from solid lipid Nanoparticle (SLN), which are lipidic carriers of drug.In case of drugs that are insoluble in both water and in organic media instead of using lipidic systems nanosuspensions are used as a formulation approach. Nanosuspension formulation approach is most suitable for the compounds with high log P value, high melting point and high dose

Advantages of colloidal based systems

1] Reduced particle size, increased drug dissolution rate, increased rate and extent of absorption, increased bioavailability of drug, area under plasma versus time curve, on set time, peak drug level, reduced variability, reduced fed/fasted effects.

2] Nanosuspensions can be used for compounds that are water insoluble but which are soluble in oil. On the other hand, Nanosuspensions can be used in contrast with lipidic systems, successfully formulate compounds that are insoluble in both water and oils.

3] Nanoparticles can adhere to the mucosa, prolonging the contact time of the drug and there by enhancing its absorption.

4] Nanosuspension of nanoparticles (NPs) offers various advantages over conventional ocular dosage forms, including reduction in the amount of dose, maintenance of drug release over a prolonged period of time, reduction in systemic toxicity of drug, enhanced drug absorption due to longer residence time of nanoparticles on the corneal surface, higher drug concentrations In the infected tissue, suitability for poorly water

5] soluble drugs and smaller particles are better tolerated by patients than larger particles, therefore nanoparticles may represent auspicious drug carriers for ophthalmic applications.

6] Nanosuspension has low incidence of side effects by the excipients.

7] Nanosuspensions overcome delivery issues for the compounds by obviating the need to dissolve them, and by maintaining the drug in a preferred crystalline state of size sufficiently small for pharmaceutical acceptability.

8] Increased resistance to hydrolysis and oxidation, increased physical stability to settling.

9] Reduced administration volumes; essential for intramuscular, subcutaneous, ophthalmic use.

Structure of Nanosuspension

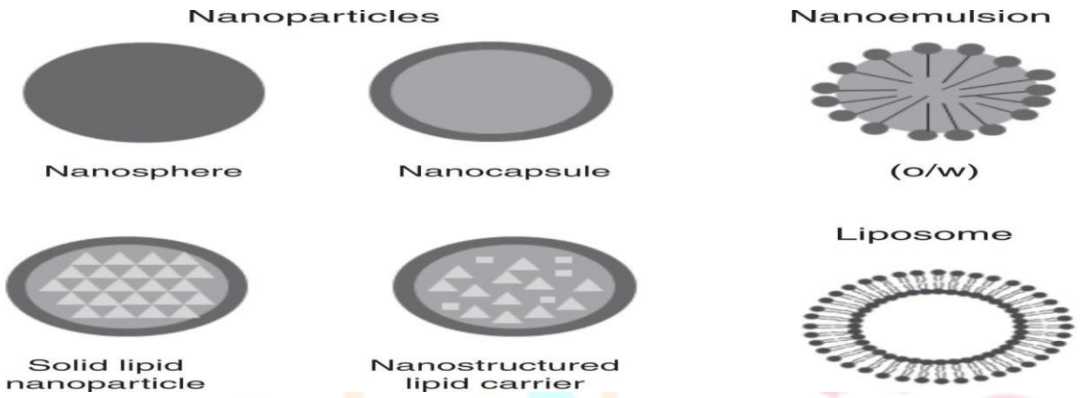

Figure 2 : Structure of Nanosuspension

Materials & Methods

Method of formulation:

Technically preparations of nanosuspensions are simpler alternative than liposome's and other conventional colloidal drug carriers but reported to be more cost effective. it is particularly for poorly soluble drugs and to yield a physically more stable product. For manufacturing nanosuspensions there are two converse methods, "Top-down process technology" and "Bottom-up process technology".

The top-down process follows disintegration approach from large particles, microparticles to Nanosized particles Examples are :-

1. High pressure homogenization
2. Nanoedge
3. Nanopure
4. Media milling (Nanocrystals).

Bottom-up process is an assembly method forms nanoparticles from molecules [15]. Examples includes;

1 .Solvent-Ant solvent method
2 .Super critical fluid process
3. Emulsification Solvent evaporation technique
4. Lipid emulsion/Micro-emulsion template.

DRUG AND EXCIPIENT PROFILE:

1) Voriconazole

Drug category: Triazole Antifungal

Formula: $C_{16}H_{14}F_3N_5O$

Chemical name: (2R, 3S)-2-(2, 4-difluorophenyl)-3-(5-fluoropyrimidin-4-yl)-1-(1H-1, 2, 4-triazol-1-yl) butan-2-ol

Molecular weight: 349.31

Category: Antifungal

Melting point: 127-130°C

Solubility: Freely soluble in acetone and in methylene chloride; soluble in methanol and in chloroform; very slightly soluble in water

.

2) **Poly lactic-co-glycolic acid)- PLGA**

Chemical name poly (D.L-lactide-co-glycolide)

Molecular weight 66,000-107,00

Melting point 40-60ºC

Solubility **solubility** PLGA can be dissolved by a wide range of common solvents, including chlorinated solvents, tetrahydrofuran, acetone or ethyl acetate.

3) **Mannitol**

Chemical name D-Mannitol [69-65-8]

Molecular weight 182.17

Functional category: Diluent; plasticizer; sweetening agent; tablet and capsule diluent; therapeutic agent; tonicity agent.

Melting point 166–1688C

4) Tween 80

Chemical names Polyoxyethylene 20 sorbitan monooleate

Chemical formula $C_{64}H_{124}O_{26}$

Molecular weight: 1310

Functional category Emulsifying agent; non-ionic surfactant; solubilizing agent; wetting, dispersing/suspending agent.

Solubility: Soluble in ethanol, water and insoluble in mineral oil vegetable oil

Method of preparation:

Solvent Evaporation Technique

Voriconazole -loaded PLGA nanosuspension were prepared by a solvent evaporation technique. To prepare the Nanoparticle suspensions, drug and PLGA were dissolved in 10 ml acetone to form a homogenous mixture. Then the prepared solution of the drug and polymers was dropped by using a syringe (26 gauge) with a constant speed (0.5ml/min) into distilled water (20 ml) containing Tween 80 (0.2% w/v). The mixture was homogenized using a magnetic stirring at a constant agitation speed of 1400 rpm for 2 hrs. An excess amount of acetone was evaporated by air-drying and the final volume of the nanosuspension was collected. The resulting in nanosuspension was further sonicated using an ultrasonic bath sonicator.

Formulation for Nanosuspension

FORMULATION CODE	DRUG (mg)	POLYMER (mg)
VCZNS1	30	30
VCZNS2	30	60
VCZNS3	30	90
VCZNS4	30	120

Table 1: Formulation for Nanosuspension

Figure 3 : Bluish Pinch colored Nanosuspension

Evaluation Parameters:

Preformulation studies

I) Characterization of voriconazole

A) Description The sample of voriconazole was analyzed for its nature, color and physical properties.

B) Solubility study Solubility test performed using water, acetone, methanol, methylene chloride and chloroform.

C) Melting point The melting point of voriconazole and polymer was measured by introducing small Amount of substance in the capillary attached to graduated thermometer and continuous heat was supplied with assembly suspended in the theils tube containing paraffin bath. The temperature at which the drug Melted was noted as melting point.

II) Freeze drying of nanosuspension:-

Prepared nanosuspension were frozen and lyophillized using lyophillizer for 24 hr at - 40°c. The mannitol at a 2.5% w/vas a cryoprotectant added in nanosuspension before freeze - drying. The nanosuspension sample divided into small glass vials. These vials were placed inside a Dewar flask containing dry ice (i.e. solid carbon dioxide) in order to supercool and freeze. The frozen sample were placed inside a 600 mi labconaco drying process was carried out. Temperature was kept at -40°c and vaccum was kept at 162mT.After 48 hr lyophilized samples were collected and stored in dessicator for further study.

III) Characterization of Voriconazole Loaded PLGA Nanosuspension:

The developed nanosuspension were further characterized for, Particle Size and Zeta Potential, Polydispersity Index, Drug Entrapment Efficiency

A) Particle size and zeta potential measurement:-

particle size for the formulation was determined by photon correlation spectroscopy (PCS) with a Zetasizer Nano ZS-90 (Malvern Instrument Ltd,). The reading was carried out at 90°angle at 25°c using proper diluted with filtered water (0.5 micrometer filter) with respect to the incident beam. The zeta potential was determined by a laser Doppler anemometer coupled with Zetasizer Nano ZS-90 (Malvern Instrument Ltd; UK).All experiments were done in triplicate.

B) Determination of drug entrapment efficiency:

The voriconazole nanosuspension (10 ml) was centrifuged at 19000 rpm,10ºC using Cooling Centrifuge Instrument (24BL model, Remi, Mumbai, India)for 2 h. The supernant was separated out; the absorbance was measured for the free drug content using UV/Visible spectrophotometer at 257 nm. The entrapment efficiency of Voriconazole nanosuspension was determined by subtracting free drug amount from initial added amount of drug the entrapment efficiency (EE %) could be calculated

Result & Discussion

I) Selection of drug and excipients Rationale behind selection of drug and excipients is depicted in the following table.

Sr. No.	Ingredient	Category
1.	**Voriconazole**	**Anti-fungal agent**
2.	**PLGA (75:25)**	**Polymer**
3.	**Tween 80**	**Non-ionic surfactant**
4.	**Acetone**	**Organic solvent.**
5.	**Water**	**Aqueous solvent**
6.	**Mannitol**	**Cryoprotectant.**

Table 2 Selected excipients and their functional category

II) Preformulation studies

Characterization of Voriconazole

A) Description The sample of Voriconazole was white powder, odourless.

B) Solubility study Voriconazole was freely soluble in acetone and in methylene chloride, soluble in methanol and in chloroform, very slightly soluble in water.

C) Melting Point :- Melting point of Voriconazole was observed at 129°C which complies with that given in the literature i.e. 127-132°C. The reading taken in triplicate and average was taken. The drug sample passed the identification test by capillary method.

III) Particle size, Polydispersity index and Zeta potential measurements:

Formulation code	Drug:: polymer ratio	Particle size (nm ± SD)	Polydispersity index	Entrapment efficiency (% ± SD)	zeta potential
VCZNS1	**1:1**	**491.31±18.94**	**0.521±0.06**	**78.23±0.57**	**11.4±1.59**
VCZNS2	**1:2**	**311.61±20.12**	**0.475±0.05**	**81.54±0.55**	**11.07+2.09**
VCZNS3	**1:3**	**232.21+23.28**	**0.335±0.06**	**83.66±0.51**	**12.25±2.30**
VCZNS4	**1:4**	**167.01±05.00**	**0.271±0.03**	**87.34±0.52**	**16.7±1.22**
VCZNSS	**1:5**	**382.63±08.38**	**0.425±0.04**	**82.14±0.50**	**13.3±1.19**

Table 3: Physicochemical Characterization of Voriconazole Nanosuspension Batches

1. Particle size

The effect of PLGA concentration on the particle size of different formulations is depicted in Table 03. The formulations VCZNS1, VCZNS2, VCZNS3 and VCZNS5 provide higher particle size values except VCZNS4. All the formulations show particle size in the range of 167.01±05.00 nm to 491.31±18.94 nm. Increasing the concentration of PLGA there is decreasing the particle size of voriconazole nanosuspension except the formulation VCZNS4. On the basis of small particle size i.e. 167.01 nm and a lower polydispersity index (0.271 nm), the formulation VCZNS4 was the optimized formulation in comparison with the remaining formulations. The particle size distribution pattern of VCZNS4 batch is given in below Figure

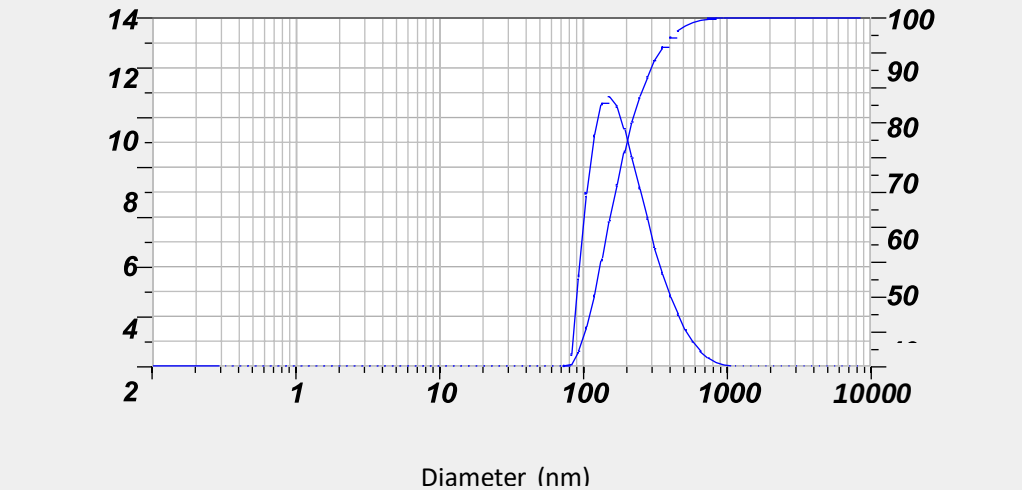

Figure 4 Particle size of voriconazole Nanosuspension(VCZNS4)

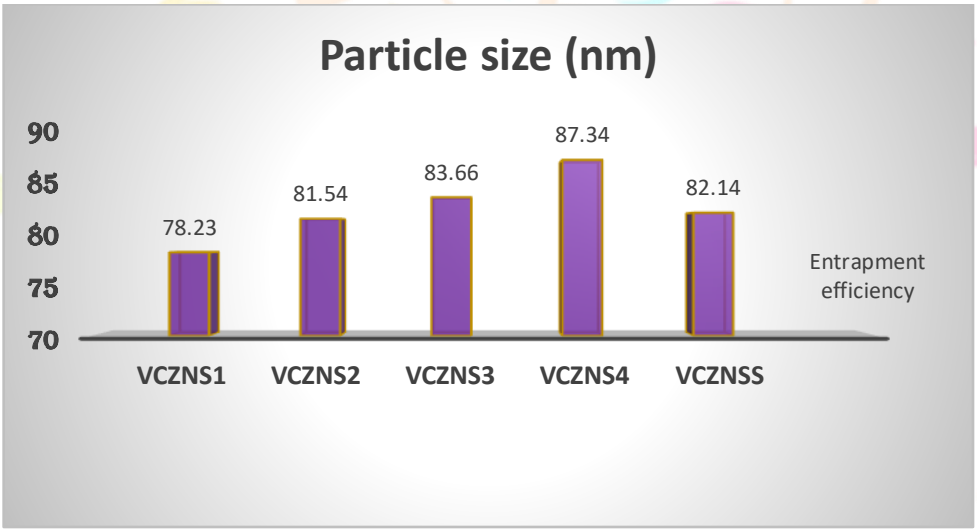

Figure 5 Graphical Representation of particle size of different VCNZNS formulation

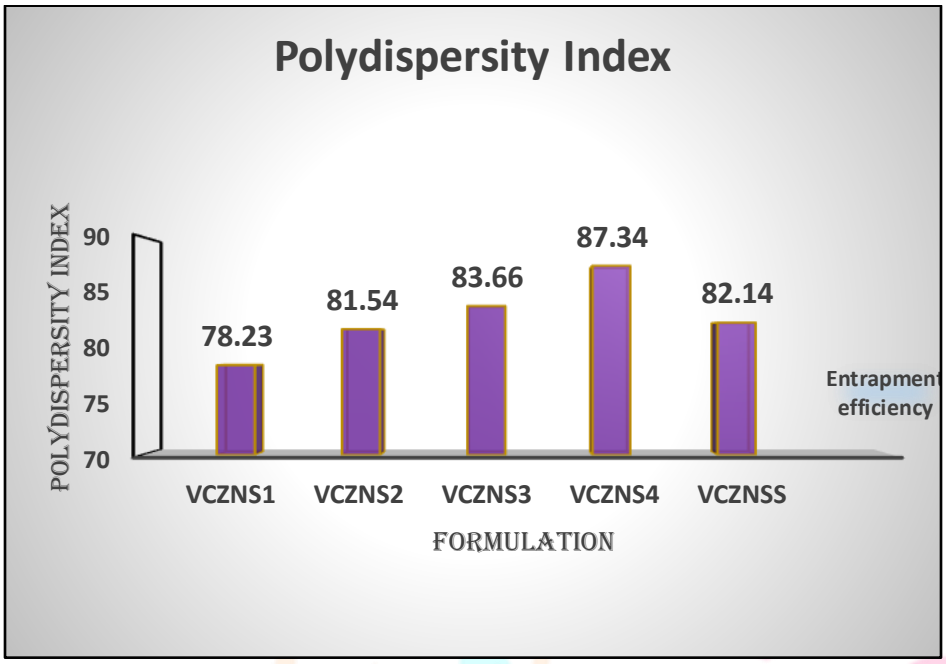

Figure:6 Graphical Representation of Polydispersity Index of different VCNZNS formulation

2. Zeta Potential

Zeta potential is an important parameter to analyses the long term stability of nanoparticles. Generally higher zeta potential values, both (+) or (-), indicate long term stability because of electrostatic repulsion between particles with same charges which avoid aggregation. The zeta potential values for voriconazole nanosuspensions were shown in Table 3. Zeta potential of optimized nanoparticles was found to be around 16.7 ± 1.22 mV which might be indicates the stable formulation (Figure 16). The zeta potential of remaining formulations of voriconazole nanosuspensions was obtained in the range of 11.07+2.09 mV to 16.7±1.22 mV.

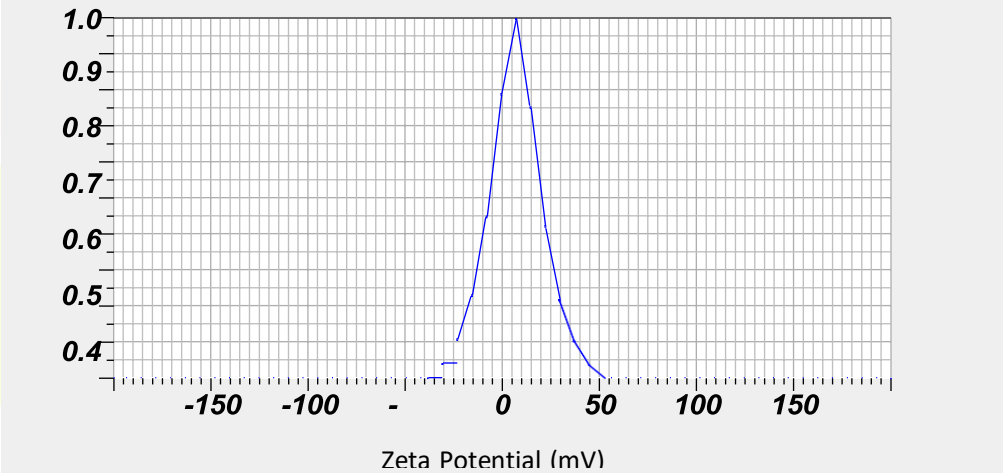

Figure 7 Zeta Potential of voriconazole Nanosuspension(VCZNS4)

7.4.2.Drug Entrapment Efficiency (%)

The entrapment efficiency of all the voriconazole nanosuspension formulations was found to be above 78%. It is evident from table 03 that the percentage entrapment efficiency was affected by the drug to polymer ratio. The results revealed that the entrapment efficiency of all formulation was increased with increasing concentration of polymer in the formulation except, VCZNS4, where the saturation effect of polymer concentration. The optimized formulation i.e. VCZNS4 showed maximum entrapment efficiency of 87.34±0.52%. The % entrapment efficiency of remaining formulations (VCZNS1, VCZNS2, VCZNS3 and VCZNSS) was found to be in the range of 78.23±0.57 to 87.34±0.52%.

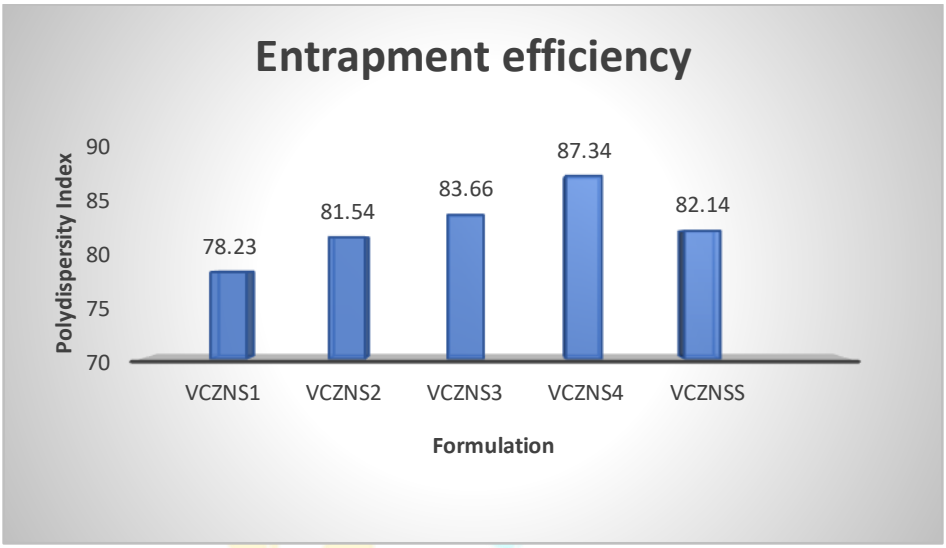

Figure 8 Graphical Representation of Drug Entrapment Efficiency of different VCNZNS formulation

References:

1) N Arunkumar, M Deecaraman, C Rani "Nanosuspension Technology And Its Applications In Drug Delivery" Asian Journal of Pharmaceutics 2009; 168-173.

2) Leonard B. Johnson1 and Carol A. Kauffman "Voriconazole: A New Triazole Antifungal Agent 2002; 630-637.

3) Johnson LB, Kauffman CA: "Voriconazole: A New Triazole Antifungal Agent" Clin Infect Dis 2003; 36(5): 630-637.

4) Ch.Prabhakar "A Review on Nanosuspensions In Drug Delivery" International Journal of Pharma and Bio Sciences 2011;2(1):549-558.

5) Gadad. A.P, Vannuruswamy G, Sharath Chandra P,Dandagi P.M, Mastiholimath V.S. "Review Article Study of Dfferent Properties And Application of Poly Lactic-Co-Glycolic Acid (PLGA)Nanotechnology: An Overview"2012;49(12):5- 17.

6) Cynthia D'Avila Carvalho Erbetta, Ricardo José Alves, Jarbas Magalhães Resende, Roberto Fernando de Souza Freitas, Ricardo Geraldo de Sousa "Synthesis And Characterization Of Poly(D,L-Lactide-Co-Glycolide) Copolymer" Journal of Biomaterials and Nanobiotechnology 2012; 3: 208-225.

7) Brunton LL, Lazo JS, Parker KL: "The Pharmacological Basis Of Therapeutics"New York, McGraw-Hill 2009; 1707-1712, 1712-1715.

8) Rajasekaran A, Kumaran A, Preetha P, Karthika K: "A Comparative Review On Conventional And Advanced Ocular Drug Delivery Formulations" Int J Pharm Tech Res 2010; 2: 668-674.p

9) Tamilvanan S "Oil-In-Water Lipid Emulsions: Implications For Parenteral And Ocular Delivering Systems" Prog Lipid Res 2004, 43: 489-533.

10) Wadhwa S, Paliwal R, Vyas SP " Nanocarriers In Ocular Drug Delivery: An Update Review" Curr Pharm Des 2009; 15: 2724-2750.

11) Rupali L. Shid, Shashikant. N. Dhole, Nilesh Kulkarni, Santosh L.Shid "Nanosuspension: A Review" Int. J. Pharm. Sci. Rev. Res., 2013;22(1):98-106.

12) Roya Yadollahi, Krasimir Vasilev, and Spomenka Simovic "Review Article Nanosuspension Technologies For Delivery Of Poorly Soluble Drug" Journal of Nanomaterials 2015:1-15.

13) Roya Yadollahi, Krasimir Vasilev, and Spomenka Simovic "Review Article Nanosuspension Technologies For Delivery Of Poorly Soluble Drug" Journal of Nanomaterials 2015:1-15.

14) Neely M, Rushing T, Kovacs A, Jelliffe R, Hoffman J "Voriconazole pharmacokinetics and phrmacodynemics in children".Clin Infect Dis 2010;500:27-36. 3007340 (1)

15) Mainardes RM, Cinto PO, Khalil NM, Evangelista RC: "Colloidal Carriers For Opthalmic Drug Delivery"Curr Drug Tar 2005; 6: 363-371.

16) Roya Yadollahi, Krasimir Vasilev, and Spomenka Simovic "Review Article Nanosuspension Technologies For Delivery Of Poorly Soluble Drug" Journal of Nanomaterials 2015:1-15.

References

14)

15) Bhat PA, Dar AA, Rather GM "Solubilization Capabilities Of Some Cationic, Anionic, And Non-Ionic Surfactants Toward The Poorly Water-Soluble Antibiotic Drug Erythromycin" J Chem Engin Data 2008; 53: 1271-1277.

17) Tamilvanan S, Benita S "The Potential Of Lipid Emulsion For Ocular Delivery Of Lipophilic Drugs" Eur J Pharm Biopharm 2004; 58: 357-368.

19)

21) Vishal R. Patel And Y. K. Agrawal "Nanosuspension: An Approach To Enhance Solubility Of Drugs" J Adv Pharm Technol Res. 2011;2(2): 81-87.

24) Karlsson MO, Lutsar I,Milligan PA. "Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies". Antimicrob agent chemother 2009;53:935-944.15221119