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ISSN: 0975-7538
Research Article

Solvent displacement as emerging technique for norfloxacin loaded nanoparticulate suspension for ocular drug delivery

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ABSTRACT

The purpose of the present investigation was to design and evaluate norfloxacin (NRFN) loaded nanoparticulate suspension by solvent displacement technique. Prepared nanoparticulate suspensions were characterized by melting point, FTIR, DSC, TEM, particle size analysis, surface charge measurement, In vitro drug release and stability studies. The optimized nanoparticulate suspension (D1) showed an average particle size (100.7 nm), zeta potential (21.3 mV), high entrapment efficiency (92.13±0.16 %) and drug content (94.46±0.12 %). The invitro drug release of the optimized nanoparticulate suspension showed in the range of 72.16%, after 12 hrs. Stability study revealed that nanoparticulate suspension was more stable at room and refrigerator condition with slight change in particle size distribution after 1month. The release of optimized formulation was found to fit with Higuchi, zero order model and the formulation released by non fickian diffusion mechanism and produce controlled release.These preliminary results indicates that norfloxacin loaded eudragit-RLPO nanoparticulate suspension could be effective in the treatment of ocular disease.

Keywords: Eudragit–RLPO; *In vitro* drug release; Nanoparticulate suspension; Norfloxacin; Ocular delivery; Zeta potential.

INTRODUCTION

Design of safe and effective drug delivery systems that can transport and release the drug components into specific site of action is a tough challenge for pharmaceutical researchers (Sanjeeb K *et al*., 2008). The anterior portion of the eye is the common route of administration of conventional ophthalmic preparations where as 90% of the conventional ophthalmic preparations including eye drops, suspensions or ointments may not be efficient in treating ophthalmic diseases such as conjunctivitis. Easy precorneal elimination of the drugs in these conventional formulations by nasolacrimal drainage, blinking of an eye, tear dilution and/or tear turnover results in their low ocular bioavailability. Further, the conjunctival sac can accommodate approximately up to 20 µL of added fluid (Ripal J *et a*l., 2008).

Different methodologies have been developed to increase the ophthalmic bioavailability of drugs and provide the extended release by reducing precorneal elimination (Swarnali Das et al., 2010). Now a days nanotherapeutics is a quickly advancing range in the field of nanomedicine, which helps to overcome several problems of traditional ophthalmic preparation including poor drug solubility and lack of targeted drug action. Nanocarriers, like lipid solid nanoparticle (SLN), nanosuspension, metal nanoparticles, nanocrystals, magnetic nanoparticles (MNP), nanosponges, and polymeric nanoparticles can be utilized as an alternative to the traditional formulations (Moorthi et al., 2014). Nanoparticle characterized as finely divided solid particles with a size ranges from 10 nm to 1000nm, in which the core drug substance is adsorbed, entrapped, embodied in a carrier. Nanocapsules are a novel carrier in which the drug is filled in a cavity by coating with polymeric substance, while nanospheres are a matrix carrier in which the drug and polymers were uniformly dispersed (Nagavarma B V N et al., 2012).

Nanoparticles can be prepared using a different type of polymers like albumin, chitosan and synthetic polymers. Polylactic- co-glycolic acid is an FDA endorsed biodegradable polymer that is nontoxic and highly biocompatible and have been widely utilized for ophthalmic applications (Soosan *et al*., 2012). Nanoparticulate suspension technology can also be utilized for drugs which are sparingly soluble or insoluble in water (Swamali *et al*., 2011). Nanoparticulate suspensions are defined as colloidal dispersions of nanosize drug particles that are produced by a suitable method and may or may not be stabilized by a suitable stabilizer. Reduction of particle size of the nanoscale range shows to an

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Received on: 18-08-2017
Revised on: 15-09-2017
Accepted on: 20-09-2017

increase dissolution rate due to increase in surface area and saturation solubility (Suzanne A *et al*., 2011). Depending on the characters of the drugs and polymers, nanoparticles can be fabricated through conventional techniques such as solvent evaporation technique, salting out, solvent displacement and other techniques which include emulsion diffusion, quasi emulsion diffusion, double emulsification, nanospray drying, desolvation technique, top down (Ball mill, high pressure homogenization), bottom up (Supercritical fluid technology) and ionic gelation method, and particle replication in non-wetting templates (Hugo *et al*.,2014).

The aim of the present study was to develop norfloxacin (NRFN) loaded nanoparticles with pH independent eudragit-RLPO polymer. Norfloxacin (NRFN) is a third era fluoroquinolone derivative of broad spectrum antibioitics with effective against gram positive and gram negative bacteria by inhibiting the bacterial enzyme DNA gyrase to avoid replicate cell division. As indicated by biopharmaceutical classification (BCS) order, norfloxacin belongs to Class IV with bioavailability range of 35-45%, because of its poor solubility and poor permeability. Eudragit-RLPO is a porous and insoluble ammonio methacrylic corrosive copolymer. It contains quaternary ammonium groups in the range of 8.8-12%. Which capable of prolonging the contact time of drug with corneal membrane. The developed norfloxacin (NRFN) nanoparticulate suspension was characterized to surface characters , size, drug loading and in vitro release etc., (Uivarosi V et al., 2001; Bhosale UV et al ., 2011).

MATERIALS AND METHODS

Norfloxacin and eudragit-RLPO was obtained from microlabs Pvt. Ltd. (Bangalore, India) as a gift sample. Stabilizer Poly vinyl alcohol (PVA) and the dialysis membrane (12,000molecular weight cut off) were purchased from sigma Aldrich Chemicals Pvt. Ltd. (Bangalore, India). All the reagents and solvents used were of analytical grade and obtained from S.D Fine chemicals (Mumbai, India).

Determination of absorption maxima (λ max)

Accurately weigh 100 mg of norfloxacin was dissolved in 100 ml of simulated tear fluid with pH 7.4 and methanol as cosolvent for preparing stock solution. From the stock solutions prepare a solution (6µg/ml) and scanned between 200-400 nm by using a UV spectrophotometer. The resulted norfloxacin UV spectrum as shown in Figure 2.

Melting point determination

Melting point range of pure drug was determined by using capillary method. A little sample of norfloxacin (NRFN) was taken and filled in to the open ended capillary tube. Then a capillary tube tied with thermometer and suspended in a beaker containing liquid paraffin. The temperature at which the sample is started to melt is considered as the melting point (Shruthi M V *et al.,* 2014).

Fourier Transform Infrared Spectroscopy (FTIR) study

The Fourier transform infrared spectroscopic were studied to assess the possibility of chemical interaction between norfloxacin, eudragit-RLPO and lyophilized formulation. The samples was mixed with potassium bromide (1:10 ratio by weight) and pressed at 15,000 psig to make a disc and scanned (FTIR-8300, Shimadzu) in the spectral region between 400 and 4000 cm^{-1}.

Differential scanning colorimetry (DSC) analysis

DSC analysis was conducted for norfloxacin, eudragit–RL100 and lyophilized nanoparticle inorder to characterize the physical state substance by using a DSC analyzer (Shimadzu, Japan). Samples were sealed into aluminum pans and heated in an inert atmosphere of nitrogen with a temperature of 10ºC/min.

Fabrication of nanoparticulate suspension

Norfloxacin (NRFN) nanoparticulate suspension was prepared by the solvent displacement method (Fessi *et al.,* 1989). Briefly a specified quantity of norfloxacin (30 mg) and eudragit-RLPO were dissolved in water miscible organic solvent (mixture of Dichloromethane (DCM) and methanol) to form a homogeneous solution. These organic phase was injected by using a syringe (26 gauge) with a constant speed (0.5ml/min) into the aqueous phase of deionized water containing PVA (Poly vinyl alcohol) as a stabilizer. The mixture was homogenized using a magnetic stirring at 900 rpm for 3 hr until to turn blue colour opalescence are shown in Figure 1(a)**.** Then, it was sonicated (Probe sonicator, Orchid Scientifics) for two minutes to obtain the desired particle size. Then resulting nanoparticulate suspension are subject to evaporate the organic solvent by magnetic stirring for the 12hrs. All formulation of nanoparticulate suspension were prepared according to drug, polymer ratio as shown in Table 1.

Freeze drying and redispersibility of nanoparticulate suspensions

Norfloxacin (NRFN) loaded nanoparticulate suspension were frozen and lyophilized using lyophilizer (Decibel digital, India) for 24h (-40°C) and adding 2.5% w/v mannitol as a cryoprotectant to obtain freeze-dried nanoparticle. The freeze-dried particles as shown in Figure 1(b) were stored at 4-8°C. The freeze-dried samples were diluted with distilled water and redispersibility was observed. The freeze-dried particles were stored at 4-8°C for further characterization (Amruta *et al*., 2014).

Physiochemical evaluation

Particle size and zeta potential analysis

Particle size and zeta potential were determined using Malvern zetasizer (Malvern instruments, UK). To analyse particle size, nanoparticulate suspension was di-

luted with distilled water. Zeta potential of the formulations was analysed to measures the surface charges and the potential physical stability of the nanoparticle. The measurements were performed in triplicates (Bhanu P *et al.*, 2014).

Morphological examination

Outer morphological of nanoparticles was measured utilizing Transmission Electron Microscopy-TEM (Philips EM-CM). In this study a drop of the sample was placed on a copper grid, dried under vaccum pressure before being examined utilizing a TEM. Which helps to characterize the individual particles and its shape (Lakshmana prabu et al., 2014).

Drug Content

Drug content was analyzed by taking a specified volume of nanoparticulate suspension formulation and ultra-centrifuged at 25000xg and the amount of drug in the supernatant was assessed by UV visible spectrophotometer at 277nm. The formula used to calculate the drug content was given below (Masilamani K *et al*., 2012).

$$Drug\ content\,(\%) = \frac{Weight\ of\ drug\ in\ nanoparticles}{Weight\ of\ nanoparticles} \times 100$$

Drug entrapment efficiency

The freshly prepared nanoparticulate suspension was centrifuged for 2 hrs at 10-15°C by using a cooling centrifuge (REMI). The amount of free or unentrapped drug was calculated by taking the absorbance of the appropriately diluted supernatant liquid and analyzed at a 277nm utilizing double beam UV spectrophotometer (ELICO SL-217) against the blank solution. The test was performed in triplicate for each batch and Drug entrapment efficiency (DEE) was calculated by using the following formula (Saieede *et al*., 2017).

$$Drug\ entrapment\ Efficiency\,(\%) = \frac{Actual\ drug\ content\ in\ nanoparticle}{Total\ drug\ used\ in\ formulation} \times 100$$

***In vitro* release study**

The *in vitro* study of the nanoparticulate suspension was carried out in an open ended glass cylinder as shown in Figure 7. A pre-soaked semi permeable membrane (Himedia, Mumbai) was attached to the terminal portion of the glass cylinder. Accurately measure 2 ml nanoparticulate suspension was placed into the open ended glass cylinder (Donar compartment).The magnetic beads were suspended in beaker (Receiver compartment)containing 100 ml of simulated tear fluid (STF pH 7.4) medium maintained at 37ºC ± 5ºC at 100 rpm. Then samples are collected at specified time period with replacement of fresh medium. The withdrawn samples were analysed by a using UV spectrophotometer at 277 nm (Anubha *et al*., 2016)**.**

Drug release kinetics studies

Drug release kinetics used to study the drug release mechanism of nanoparticulate suspension by analyzing 'n' as the diffusion exponent. As per kinetic studies if 'n' value is below 0.45, which indicates fickian mechanism of drug release. If value between 0.45 to 0.89 shows non-fickian mechanism of drug release and if 'n' value is more than 0.89, then release mechanism is governed by case-II transport mechanism respectively (Korsmeyer RW *et al*., 2012).

Short term stability study of nanoparticulate suspension

Optimized nanoparticulate suspension (D1) was selected to conduct a short term stability study for a period of 1month as per ICH guidelines. In the present study sample exposures at 40°C± 2°C and 75%±5% relative humidity (RH). The samples were evaluated for sedimentation and particle size at specific interval. (Khyati *et al.,* 2017).

RESULT AND DISCUSSION

Solvent displacement is a technique used for loading of water insoluble and water soluble drug molecules in nanoparticulate suspension formulation. Solvent displacement is also called by various names such as nanoprecipitation, antisolvent precipitation, interfacial deposition, precipitation, drop by drop addition method, modified solvent evaporation, solvent shifting and solvent extraction method.

The FTIR spectrum of pure drug norfloxacin (NRFN) shows on aromatic C-H stretching at 3000-3500 cm^{-1} and carboxylic acid (C=O stretching) and a distinct absorption band at 1600-1650cm^{-1} for quinolone as shown in Figure 3(a). The same characteristic peak of norfloxacin (NRFN) has observed in the lyophilized nanoparticle formulation as presented in Figure 3(c). Which indicated that there was no chemical interaction between drug and polymers. Differential scanning calorimetry (DSC) imparts information about the physical forms like crystalline or amorphous nature of the samples. The DSC thermogram of pure drug norfloxacin as shown in Figure 4(a) with an exothermic peak at 222.46°C corresponding to its melting temperature, which was not detected in the thermograms for norfloxacin (NRFN) loaded eudragit-RLPO nanoparticles as shown in Figure 4(b).

Moreover it exhibited exothermic peak at 98.47°C, which concluded that in the prepared norfloxacin nanoparticle, the drug was present in the amorphous form and may have been uniformly dispersed in the polymer substance. Which indicate that there was no drug polymer interaction and the drug was compatible with polymer. The melting point of pure norfloxacin (NRFN) drug was measured by capillary method and found in 227-228°C. It confirmed that no impurities in the pure drug substance. The prepared norfloxacin (NRFN) loaded nanoparticulate suspension appearance was appeared as milky or bluish opalescence due to dispersion of colloidal nanoparticle in to the aqueous

Table 1: Composition of Norfloxacin nanoparticulate suspension

S.No	Formulation code	Drug : Polymer	Organic : Aqueous phase	Stirring speed (rpm)	Time (hr)	Stabilizer (%w/v)
1.	A1	1:1	1:2	900	3	0.2
2.	A2	1:1	1:3	900	3	0.2
3.	A3	1:1	1:4	900	3	0.2
4.	B1	1:2	1:2	900	3	0.4
5.	B2	1:2	1:3	900	3	0.4
6.	B3	1:2	1:4	900	3	0.4
7.	C1	1:3	1:2	900	3	0.6
8.	C2	1:3	1:3	900	3	0.6
9.	C3	1:3	1:4	900	3	0.6
10.	D1	1:4	1:2	900	3	0.8
11.	D2	1:4	1:3	900	3	0.8
12.	D3	1:4	1:4	900	3	0.8

(a) **(b)**

Figure 1: a) Norfloxacin nanoparticulate suspension; b) Freeze dried norfloxacin nanoparticle

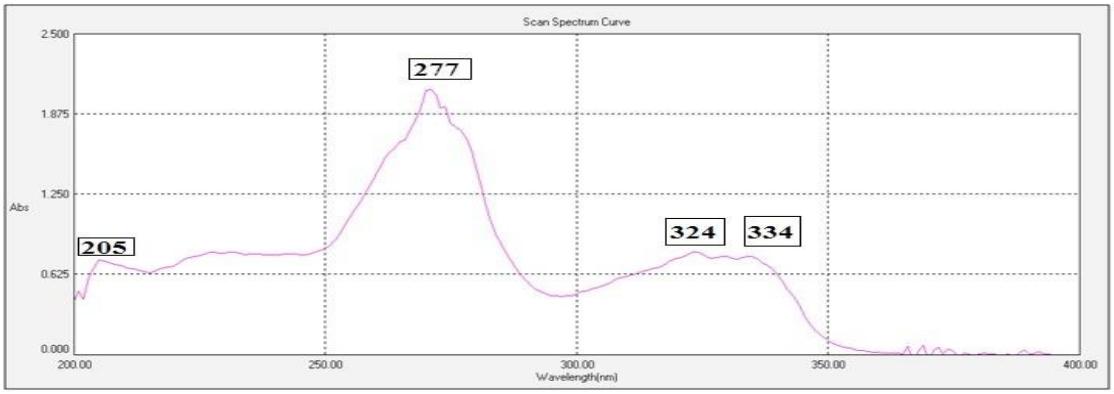

Figure 2: UV absorption spectrum for norfloxacin in simulated tear fluid pH 7.4

phase. This phenomenon was based on the principle of Faraday-Tyndall effect (light scattering particles). The redispersibility of freeze dried nanoparticle was studied by using distilled water. It shows that nanoparticle was redispersed uniformly in water after manual hand shaking without any aggregates due to the presence of cryoprotectant like 2.5% of mannitol.

In solvent displacement technique constant magnetic stirring speed 900 rpm were applied for preparation of nanoparticulate suspension. From the result all batches of the nanoparticulate suspension exhibited average size distribution range below 400 nm, therefore suitable for ocular application. The mean particle sizes of the prepared nanoparticles as measured by zeta sizer were in the size range of 100.7 to 256 nm with less poly dispersity index value of 0.232.Which represented that the particles are in uniform distribution in nanoparticulate suspension as shown in figure 6. Polydispersity index is a measure of scattering homogeneity and more often than not extends from 0 to 1. Values near 0 shows a homogeneous scattering while those more noteworthy than 0.3 demonstrate high heterogeneity. On increase the ratio of polymer gives increase the consistency of organic phase as a result thickness droplet coming out of the needle during nanosuspension preparation. Moreover this causes increase the resistance of drug molecules diffuse from the internal phase to external phase and more drugs gets entrapped with the desirable size of nanoparticles were produced.

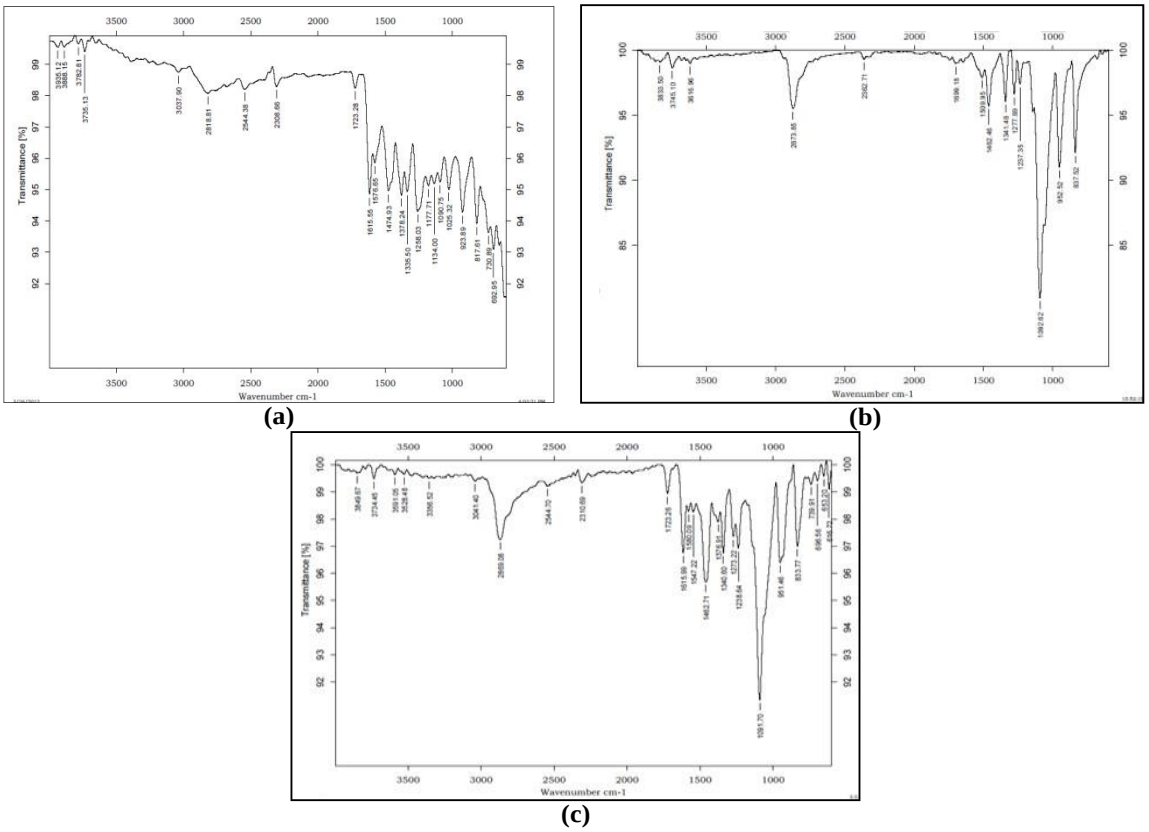

Figure 3: FTIR spectrum of (a) Norfloxacin; (b) Eudragit-RLPO; (c)Norfloxacin lyophilized nanoparticle

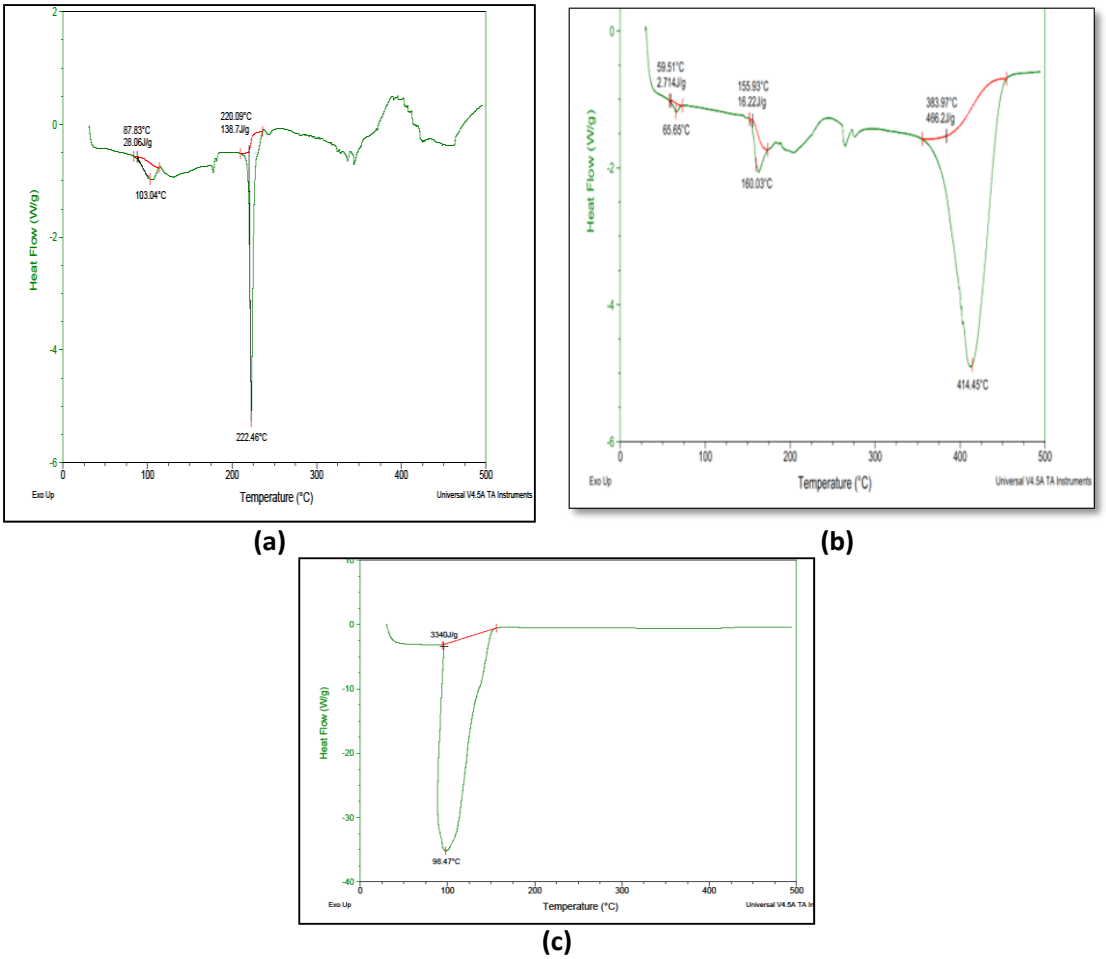

Figure 4: DSC thermogram of (a) Norfloxacin; (b) Eudragit-RLPO; (c) Norfloxacin loaded nanoparticle

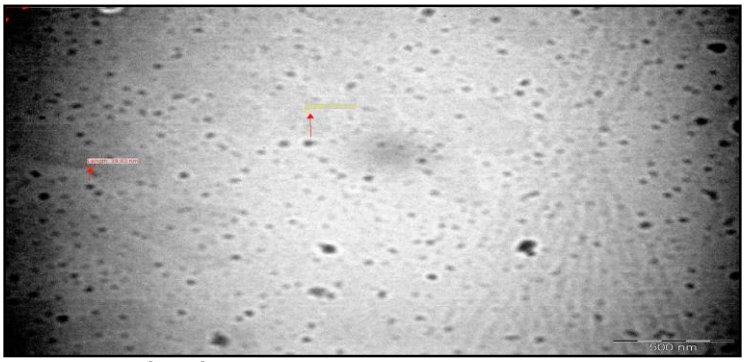

Figure 5: TEM image of norfloxacin loaded optimized nanoparticulate suspension (D1)

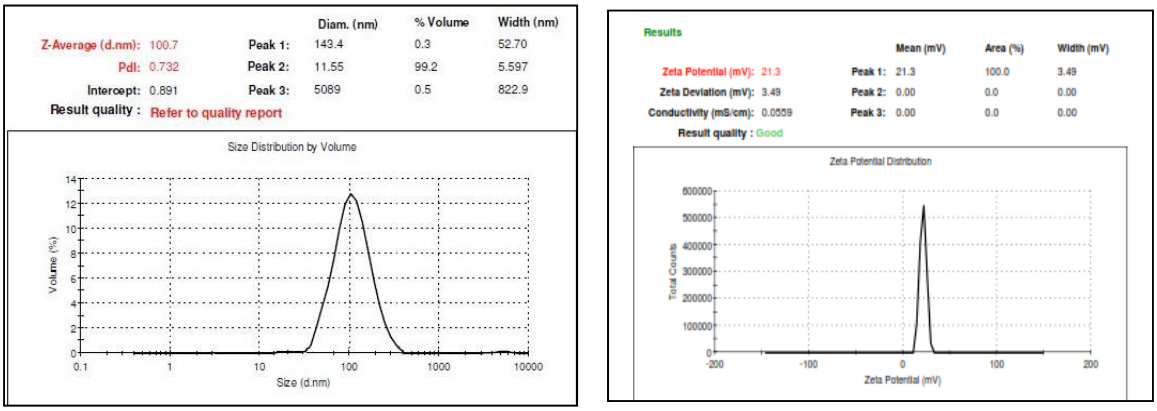

Figure 6: Particle size distribution and zeta potential curve of norfloxacin loaded nanoparticle

Figure 7: Open ended cylinder with dialysis membrane in simulated tear fluid pH 7.4

Table 2: Evaluation parameters of various nanoparticulate suspension

Formulation code	Mean particle size in nm ± SD	Poly dispersity index (PdI ± SD)	Zeta potential (mV ± SD)	Drug Entrapment Efficiency (% ± SD)	Drug con-tent (% ± SD)
A1	192.3± 3.1	0.286± 0.21	19.5±2.6	75.28±0.18	73.26 ± 0.23
A2	175.6±3.8	0.298± 0.30	17.3±1.4	68.43±0.21	88.42 ±0.42
A3	268.4 ±3.5	0.398± 0.18	18.7±2.4	80.14± 0.13	92.34 ±0.13
B1	259.2±1.8	0.495±0.20	16.8±3.6	82.56±0.18	91.64 ±0.52
B2	158.3±2.8	0.332± 0.19	19.2±1.7	76.23±0.16	92.47 ±0.39
B3	142.6±3.2	0.546± 0.25	14.9± 2.8	85.30± 0.23	91.53 ±0.45
C1	112.4 ±4.2	0.212± 0.38	23.3±1.9	90.23±0.22	88.26±0.21
C2	232.5±5.3	0.465±0.26	24.8 ±2.3	88.45±0.21	85.32 ±0.13
C3	188.6±4.5	0.342± 0.31	22.4±2.8	68.29±0.19	79.20 ±0.25
D1	100.7±4.2	0.234± 0.22	21.3±1.9	92.13±0.16	94.46±0.12
D2	225.2±2.7	0.332±0.43	19.5±2.6	89.22±0.23	84.17±0.23
D3	186.4±1.4	0.414±0.14	22.4±2.9	78.16±0.17	79.23±0.19

Data are expressed as mean ± SD (n=3)

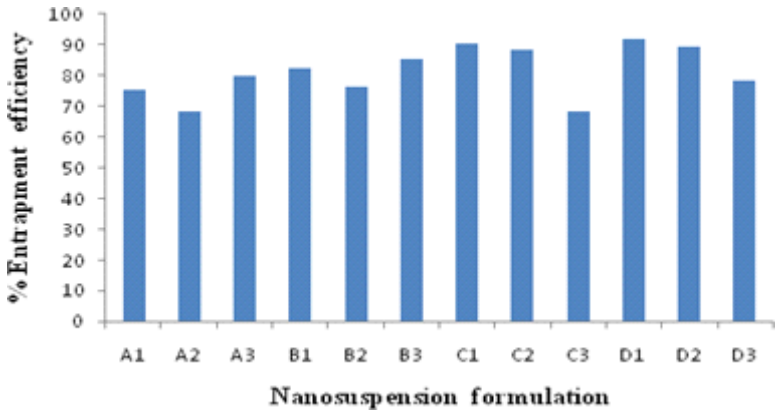

Figure 8: Drug entrapment efficiency of various nanoparticulate suspension

Table 3: In-vitro kinetic release data of optimized nanoparticulate suspension (D1)

Formulation code	Zero order regression	First order regression	Higuchi's model regression	Korsmeyer Peppas model regression & Slope		Best fit model
	(R^2)	(R^2)	(R^2)	(R^2)	Slope(n)	
D1(Optimized formulation)	0.9841	-0.9813	0.9819	0.9969	0.41	Higuchi's model and Zero order

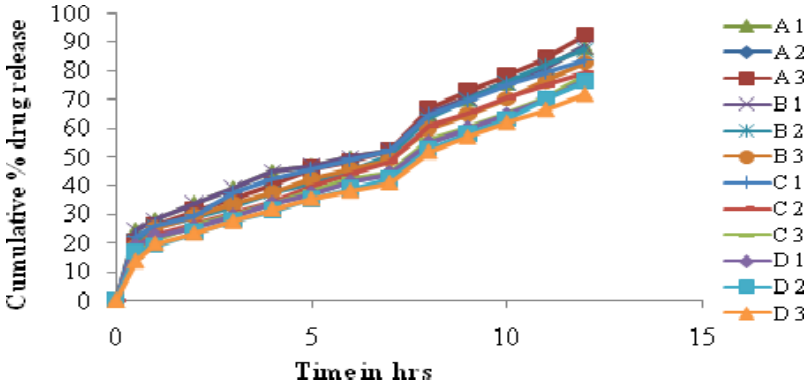

Figure 9: In-vitro drug release

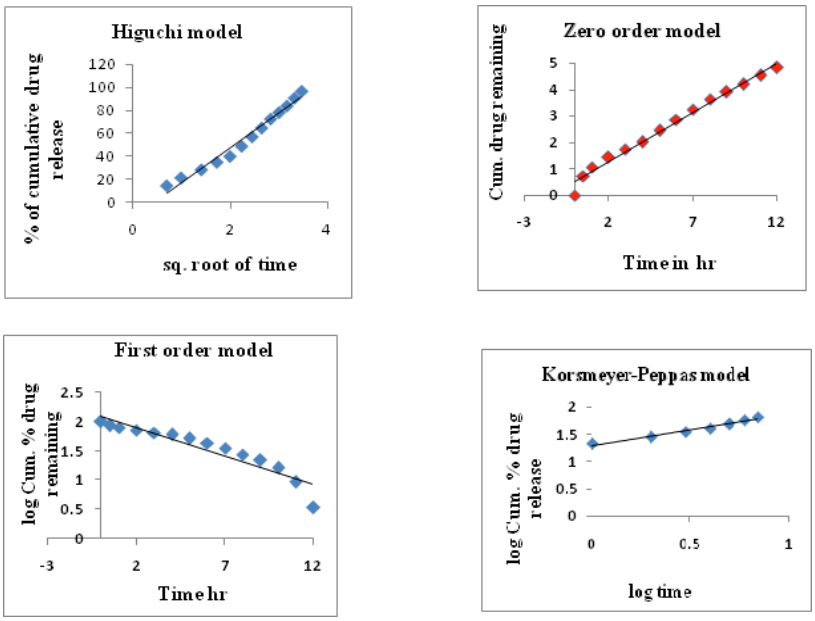

Figure 10: In-vitro drug release kinetic

The morphology characters of nanoparticles formulation (D1) were examined by transmission electron microscope (TEM). It revealed that all nanoparticle particle formulation were smooth spherical shape without aggregation as shown in Figure 5. Generally nanoparticles with zeta potential values greater than +25 mV or less than -25 mV typically have high degrees of stability. Dispersions with a low zeta potential value will eventually aggregate due to vanderwaals inter particle attractions. The zeta potential value of the all formulation has positive value of +16.8 mV to+24.8mV due to the presence of a quaternary ammonium group of eudragit-RLPO polymer in nanoparticle. Which indicate nanoparticulate suspension were stable due to the repulsive forces between nanoparticles, thus preventing agglomeration.

Formulation are prepared with high polymer concentration showed high percentage drug entrapment efficiency as compared to other formulation in the range of 68.29% to 92.13% respectively as shown in figure 8. If the stabilizer polyvinyl alcohol (PVA) concentration is less, aggregation of the polymer occurs, whereas, if more stabilizer is used, drug entrapment could be reduced. Moreover, it was observed that the drug content value of nanoparticle formulation was in the range of 73.26% to 94.46% respectively as resulted in Table 2. *In-vitro* drug release study of all nanoparticulate suspension showed in 12hrs in the range of 72.16% to 92.18% respectively as depicted in Figure 8. The *In-vitro* release data were fitted to various kinetics models in order to calculate the release constant and coefficient of determination (R^2) as represented in table 3. Among the models tested, the drug release profiles of D1 formulation were best fitted with the coefficient of determination (R^2) in the range of 0.9841. The n value 0.41 of D1 formulation indicated that formulation is released by non fickian diffusion mechanism of drug release. Which represents a drug release governed by a combined mechanism of diffusion and erosion. Short term stability study shows that nanoparticulate suspension (D1) was physically and chemically stable, when stored at 40 ±2 ^{o}C and 75 ±5% RH for a period of 1 month. It was observed that there was slight negligible changes in particle size distribution and no sedimentation after stability study.

CONCLUSION

Over the past decade, there has been an increasing interest in using nanoparticulate suspension for ophthalmic drug delivery via solvent displacement technique. Consequently, nanoprecipitation technique is a simple, quick and reproducible technique which is broadly utilized for the preparation of both nanospheres and nanocapsules. From the investigated results, it can be concluded that D1 formulation with concentration (1:3) was optimized as best formulation because of desired particle size, greater stability, maximum entrapment efficiency, low poly dispersity index, produced controlled drug release for prolonged period for 12hrs. Thus, the positive zeta potential and fine particle size would help in prolonging the corneal contact time by minimizing instillation frequency of drug ministration for ocular drug delivery.

ACKNOWLDGEMENT

The authors express gratitude to startech lab, Hyderabad for DSC, TEM analysis and thankful for the QIS College of pharmacy, ongole, Andhra Pradesh for providing facilities for completion of research work.

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