

Sulfacetamide Loaded Eudragit RL100 Nanosuspension with Potential for Ocular Delivery

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ABSTRACT – Purpose. Polymeric nanosuspension was prepared from an inert polymer resin (Eudragit RL100) with the aim of improving the availability of sulfacetamide at the intraocular level to combat bacterial infections. **Methods.** Nanosuspensions were prepared by the solvent displacement method using acetone and Pluronic F108 solution. Drug to polymer ratio was selected as formulation variable. Characterization of the nanosuspension was performed by measuring particle size, zeta potential, Fourier Transform infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), Powder X-Ray Diffraction (PXRD), drug entrapment efficiency and *in vitro* release. In addition, freeze drying, redispersibility and short term stability study at room temperature and at 4°C were performed. **Results.** Spherical, uniform particles (size range below 500 nm) with positive zeta potential were obtained. No significant chemical interaction between drug and polymer were observed in the solid state characterization of the freeze dried nanosuspension (FDN). Drug entrapment efficiency of the selected batch was increased by pH alteration and addition of polymethyl methacrylate in the formulation. The prepared nanosuspension exhibited good stability after storage at room temperature and at 4°C. Sucrose and mannitol were used as cryoprotectants and exhibited good water redispersibility of the FDN. **Conclusion.** The results indicate that the formulation of sulfacetamide in Eudragit RL100 nanosuspension could be utilized as potential delivery system for treating ocular bacterial infections.

INTRODUCTION

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 (1); microparticles (2); nanoparticles (3); liposomes (4); collagen shields (5); ocular inserts/discs (6); dendrimers (7); and transcorneal iontophoresis (8). Nanoparticles have been found to be the most promising of all the formulations developed over the past 25 years of intense research in ocular therapeutics due to their sustained release and prolonged therapeutic benefit. 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 (9). Nanoparticles are solid, submicron, colloidal particles ranging in size from 10 to 1000 nm, in which drug can be dissolved, entrapped, adsorbed or covalently attached (10). These colloidal particles 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.

Sulfacetamide is a sulfonamide antibiotic used to treat conjunctivitis (11), blepharitis (12), trachoma (13), corneal ulcer (14) and other ocular diseased conditions (15). They are bacteriostatic in nature and inhibit bacterial synthesis of dihydrofolic acid by preventing condensation of pteridine with aminobenzoic acid through competitive inhibition of the enzyme dihydropteroate synthetase (16). The drug is marketed as ophthalmic solution of its sodium salt, in a USP concentration of 10% (w/v) under the brand name Bleph-10. The usual adult dose for conjunctivitis is 1 to 2 drops into the conjunctival sac every 2 to 3 hours for 7 to 10 days (17).

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The drug has an ionization constant of 5.4 and an elimination half-life of 7 to 13 hours (17).

Polymeric nanosuspensions, prepared from Eudragit RL100 and RS100, have been investigated extensively for the ocular delivery of ibuprofen (18), flurbiprofen (19), cloridromene (20), piroxicam (21), methyl prednisolone (22), and amphotericin B (23). They are cationic copolymers of methacrylate with 4-12% quaternary ammonium groups. They are inert polymer resins, insoluble at physiologic pH but have swelling properties. Due to their capability to form nanodispersions with smaller particle size, positive surface charge, good stability, absence of any irritant effect on the cornea, iris, and conjunctiva, Eudragit nanoparticles appear to be a suitable inert carrier for ophthalmic drug delivery.

The simplest method to prepare drug loaded nanoparticles is the solvent displacement method also known as nanoprecipitation method, developed by Fessi *et al.* (24). The method is based on the interfacial deposition of a polymer following displacement of a semi-polar solvent miscible with water. The technique is easy, less complex, less energy consuming as well as widely applicable without any additives for the manufacturing of defined nanospheres (25). However, entrapment of hydrophilic drug substances is very difficult in this method. Ideally, nanoparticles with high drug entrapment efficiency would reduce the quantity of carrier required for the administration of a sufficient amount of drug at the target site, as well as drug wastage during manufacture. There are several methods already reported in the literature to improve drug entrapment efficiency of the nanoprecipitation method. These include: changing the pH of the inner/external phase; addition of excipients (fatty acids, oligomers); replacing the salt form of the drug with the base form; and the addition of salt to the aqueous phase (26).

Currently, no attempt has been made to encapsulate sulfacetamide inside a polymeric nanoparticulate carrier which could facilitate the drug delivery to the ocular surface. Sensoy *et al.* (27) have recently reported the treatment of ocular keratitis in rabbit eye using bioadhesive sulfacetamide sodium microspheres consisting of pectin, polycarbophil and hydroxypropylmethyl cellulose. The rabbit eyes treated with microspheres demonstrated significant decrease in the number of viable bacteria in infection models when compared

to sulfacetamide alone. In another study, it was reported that uptake of polymeric particles into primary cultured rabbit conjunctival epithelial cells (RCEC) was dependent on particle size (28). Interestingly, the uptake of polymeric nanoparticles into RECE was found to be significantly higher compared to microparticles after topical application to the albino rabbit eye.

Therefore, an attempt was made to prepare and characterize sulfacetamide loaded Eudragit RL 100 nanosuspensions intended for the treatment of ocular infections. Nanosuspensions were prepared by the solvent displacement method using acetone and 1 % (w/v) Pluronic F108 solution. Physicochemical characterization of the nanosuspension was performed by measuring particle size, zeta potential, drug entrapment efficiency and in vitro drug release. Solid state characterization of the freeze dried nanosuspension was performed by Fourier Transform Infrared spectroscopy (FTIR), Differential Scanning Calorimetry (DSC) and Powder X-Ray Diffraction analysis (PXRD). These techniques allow understanding the thermal behavior, drug crystallinity and possible occurrence of drug polymer interaction for the freeze dried nanosuspension. The effect of changing polymer content, pH of the external media and addition of polymethyl methacrylate (PMMA) on drug entrapment efficiency was studied for the selected batch. Freeze drying and redispersibility of the lyophilized samples were performed for the selected batch. Short term stability for 1 month for the selected batch was also carried at room temperature (20°C) and at 4°C.

MATERIALS AND METHODS

Materials

Eudragit RL 100 was purchased from Röhm GmbH & Co. KG, Germany. Sulfacetamide (purity of 99 to 100.5%) was supplied by Spectrum Chemical Mfg. Corp, CA. Pluronic F 108 was purchased from BASF Wyandotte Corp, NJ. All other chemicals were of reagent grade. Eudragit RL 100 and Pluronic F 108 were used as supplied by the manufacturers.

Preparation of Nanosuspension

The Eudragit RL 100 nanosuspensions were prepared by the solvent displacement method

similar to that employed by Fessi *et al.* (24). Four different weight ratios of drug and polymer, namely 10:100 (batch B1), 20:100 (batch B2), 30:100 (batch B3) and 40:100 (batch B4) were used as shown in Table I. Briefly, a 100 mg portion of Eudragit RL100 and various proportions of drug (10-40% by weight of the total polymer) were dissolved in 10 mL of acetone. This organic phase was poured drop wise into 20 mL of a 1% w/v of Pluronic F-108 solution with moderate magnetic stirring at room temperature. Nanoparticles were spontaneously formed and turned the solution slightly turbid. Then, acetone was removed by continuous stirring for 20 hrs. The resulting particle suspension was filtered through a 1.2 μm cellulose nitrate membrane filter in order to remove larger particle aggregates. The prepared suspension was centrifuged at 19,000 rpm at 15°C for 2 hours (Sorvel RC-5B refrigerated super speed centrifuge, rotor SS-34, 33300g, K 446). For selected batch, the supernatant was removed and the sediment was freeze dried for 48 hrs for further analysis.

Particle size analysis and zeta potential measurement

The mean particle size for the formulations was determined by Photon Correlation Spectroscopy (PCS) with a Zetasizer Nano ZS-90 (Malvern Instruments Ltd., UK) equipped with the DTS software. The reading was carried out at a 90° angle with respect to the incident beam. The zeta potential was measured by a laser doppler anemometer coupled with the same instrument. A potential of ± 150 mV was set in the instrument. Disposable cuvettes of 0.75 mL capacity were used for all measurements. All measurements were run in triplicate.

Scanning Electron Microscopy (SEM)

In order to examine the particle surface morphology and shape, SEM was used. A concentrated aqueous suspension was spread over a slab and dried under vacuum. The sample was shadowed in a cathodic evaporator (also known as "Sputter coater") with a gold layer of 20 nm thick in an argon gas environment at 45 mA current for 5 seconds. Photographs were taken using a JSM-5200 Scanning Electron Microscope (Tokyo, Japan) operated at 10 kV.

Transmission Electron Microscopy (TEM)

TEM helps to visualize the inherent matrix of individual particles and its shape. A drop of the suitably diluted sample was placed onto a holey carbon coated 400 mesh copper grid and dried in an oven at 40°C for 20 minutes. The images were taken using a Hitachi Ultra-thin film evaluation system (HD-2300A) in Phase contrast, Z contrast, Scanning Electron (SE) modes.

Differential Scanning Calorimetry (DSC)

DSC (model 822e, Mettler Toledo, OH, USA) with a Mettler MT50 analytical balance was used in order to analyze the thermal behavior of different samples. Indium (3-5 mg, 99.999% pure, onset 156.6°C, heat of fusion of 107.5 J/g) was used to calibrate the instrument. Samples (3-5 mg) were accurately weighed into 100 μl aluminum pans and then crimped. The thermograms were recorded over a temperature range of 10-200°C at a rate of 10°C/min under nitrogen purge gas at 50 mL/min. Mettler Toledo STARe software (version 8.10) was used to analyze data.

Powder X-Ray Diffractometry (PXRD)

The crystalline state of the drug in the polymer sample was evaluated by PXRD analysis. The X-ray spectra were recorded with an X'Pert-PRO multipurpose X-Ray diffractometer (PANalytical, Tokyo, Japan) using Ni-filtered, CuK α radiation with a voltage of 45 kV, and a current of 40 mA with a scintillation counter. The instrument was operated in the continuous scanning speed of 4°/min over a 2 θ range of 5° to 40°. The samples were ground using a mortar and pestle, placed into the cavity of an aluminum sample holder and packed smoothly using a glass slide. The results were evaluated using the X-Pert Data collector version 2.1 software.

Fourier Transform Infrared spectroscopy (FTIR)

The Fourier transform infrared analysis was conducted to verify the possibility of interaction of chemical bonds between drug and polymer. The FTIR spectrum was performed using a PerkinElmer 1600 spectrophotometer with a resolution of 2 cm^{-1} . The samples were scanned in the spectral region between 4000 and 400 cm^{-1} by taking an average of 8 scans per sample. Solid powder samples were

oven dried at around 30⁰C, finely crushed, mixed with potassium bromide (1:10 ratio by weight) and pressed at 15000 psig (using a Carver Laboratory Press, Model C, Fred S. carver Inc., WIS 53051) to form disc. The detector was purged carefully using clean dry nitrogen gas to increase the signal level and reduce moisture. For the analysis of the data, the spectrum GX series model software was used.

Drug entrapment efficiency (DEE)

A 20 mL portion of the freshly prepared nanosuspension was centrifuged at 19,000g for 2 hrs at 10-15⁰C temperature using a Sorvel RC-5B refrigerated super speed centrifuge with rotor SS-34 at 33300 g and K 446. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted supernatant solution at a 260 nm using single beam UV spectrophotometer (Genesis 10 UV, Thermoelectron Corporation, USA) against blank/control nanosuspension. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated.

***In vitro* drug release study**

The Static Franz diffusion cell was used for studying the *in vitro* release of the nanosuspension. A cellulose acetate membrane (dialysis membrane with a molecular weight cut off value of 12,000-14,000, Spectra/por molecular porous membrane tubing, 25 mm diameter, Spectrum Medical Industries Inc., CA 90060) was adapted to the terminal portion of the cylindrical donor compartment. A 10 mL portion of the nanosuspension containing drug, sufficient for establishing sink conditions for the assay was placed into the donor compartment. The receptor compartment contained 90 mL of 0.2M phosphate buffer solution of pH 7.4 maintained at 37⁰C under mild agitation using a magnetic stirrer. At specific time intervals, aliquots of 1mL were withdrawn and immediately restored with the same volume of fresh phosphate buffer. The amount of drug released was assessed by measuring the absorbance at 256 nm using a single beam UV spectrophotometer (Genesis 10 UV, Thermoelectron Corporation, USA).

Kinetics of drug release

In order to analyze the drug release mechanism, *in vitro* release data were fitted into a zero-order (29), first order (30), Higuchi (31), Hixon-Crowell cube root law (32), and Korsmeyer-Peppas model (33).

Freeze drying and redispersibility of nanosuspension

All of the four batches (B1, B2, B3, B4) were freeze dried to obtain a dry powder. Additionally, selected batch (B3) was taken to study the effect of cryoprotectant on freeze drying as well as the redispersibility of the drug loaded nanosuspension. Two cryoprotectants were used; sucrose and mannitol both at a 2.5% and 5% w/v concentration level. The nanosuspension sample was divided into four 2 mL parts and placed individually into small glass vials. The vials were placed inside a Dewar flask containing dry ice (i.e. solid carbon dioxide) in order to supercool and freeze. The frozen samples were placed inside a 600 mL Labconco fast-freeze flask with attached adapter. The freeze-drying process was carried out in the Virtis Freezemobile model 12EL. Temperature was kept about - 70⁰C and the vacuum was kept at 162 mT. After 48 hours, the lyophilized samples were collected and stored in a desiccator for further analysis.

Redispersibility of lyophilized products was carried out by manual hand shaking in small glass vial with distilled water. Visual observation was done to investigate formation of any aggregates or precipitates after shaking. Particle size and size distribution after redispersion of the sample was performed using Zeta potential/Particle sizer (model NicompTM 380 ZLS, CA, USA).

Short term stability study of nanosuspension

Prepared nanosuspension (batch B3) was chosen to perform short term stability study of the nanosuspension. Samples were stored in glass vials for 1 month at room temperature (20⁰C) and at 4⁰C in freeze. After 1 month, samples were visually observed for any sedimentation. The particle size and size distribution was performed using Zeta potential/Particle sizer (model NicompTM 380 ZLS, CA, USA).

RESULTS

Particle size and size distribution

The presence of bluish opalescence indicated the formation of colloidal nanosuspension (figure not shown). The effect of the drug-to-polymer ratio on the size of the nanoparticles was studied using four different weight ratios of drug and polymer, namely 10:100 (B1), 20:100 (B2), 30:100 (B3) and 40:100 (B4), as shown in Table I. Batch B0 in which no drug was added showed a mean particle size of 398.1 nm and mean polydispersity index (PI) of 0.414. The mean particle size for drug loaded batches (B1 to B4) varied in the narrow range from 112.4 nm to 140.6 nm although standard deviation was higher for batch B1 and B4. The mean PI values for the drug loaded formulation varied in the range of 0.456 to 0.67. It could be inferred from the results that there was no significant impact of the drug-to-polymer ratio on the mean particle size of the drug loaded nanosuspensions ($p < 0.05$). One way ANOVA followed by the Tucky test showed that batch B0 showed significant difference in particle size as compared to drug loaded batches ($p < 0.05$). A trend of increasing drug content in the formulation with decreasing mean size of nanoparticles was observed. This observation is in conformity with the findings of Das *et al.* for Amphotericin B loaded Eudragit RL 100 nanoparticles (23). The probable spatial interaction (due to electrostatic charges) between drug and polymer forming more compacted structure at higher drug concentrations have resulted in decreasing particle size. The phenomenon may be related to viscosity.

Zeta potential

The zeta potential remained in the range of positive values for all batches and varied between + 9.16

mV to + 24.1 mV (Table 1). This result is consistent with the findings of Pignatello *et al.* (34). The positive surface charge for the nanoparticles was observed due to the presence of the quaternary ammonium groups of Eudragit RL 100.

SEM and TEM

Nanoparticle surface morphology and shape were visualized using SEM and TEM. SEM revealed that the drug loaded nanoparticles were found to be distinct, spherical with a smooth surface (Figure 1A). TEM images were also in conformity with the SEM and dynamic light scattering data for particle size. All particles were found to be spherical with a smooth surface for the various batches (Figure 1B and 1C). Magnification of a single particle showed the internal cage like structure in which the drug molecules are dispersed uniformly throughout the polymer matrix (Figure 1D).

DSC

From the overlay of the DSC thermograms, it has been observed that sulfacetamide is crystalline in nature (Figure 2). It exhibited a sharp melting endotherm at an onset temperature of 180.1⁰C, a peak temperature of 182.31⁰C and a heat of fusion of 119.7 J/gm. The drug recrystallized at an onset temperature of 241.76⁰C, a peak temperature of 245.09⁰C and had energy of activation of about 80.16 J/gm. Eudragit RL 100 polymer exists as a completely amorphous form with a glass transition temperature (T_g) of about 60⁰C (35). The amorphous polymer did not show any fusion peak or phase transition, apart from a broad signal around 55–60⁰C due to a partial loss of residual humidity. The thermal behavior of the freeze dried nanoparticles suggested that the polymer inhibited the melting of the drug crystals.

Table 1. Mean size, Polydispersity index and zeta potential of blank and Sulfacetamide-loaded Eudragit RL100 Nanosuspensions (σ is Standard deviation, $n=3$)

Batch	Drug to Polymer ratio (by wt)	Mean size (Z average) $\pm \sigma$ (nm)	Polydispersity Index $\pm \sigma$	Zeta potential $\pm \sigma$ (mV)
B0	0:100	398.1 \pm 21.84	0.414 \pm 0.095	13.03 \pm 0.32
B1	10:100	140.6 \pm 49.94	0.456 \pm 0.075	18.77 \pm 0.45
B2	20:100	127.9 \pm 28.82	0.501 \pm 0.145	24.1 \pm 1.58
B3	30:100	118.9 \pm 8.17	0.67 \pm 0.162	9.16 \pm 0.43
B4	40:100	112.4 \pm 40.25	0.467 \pm 0.137	16.47 \pm 0.29

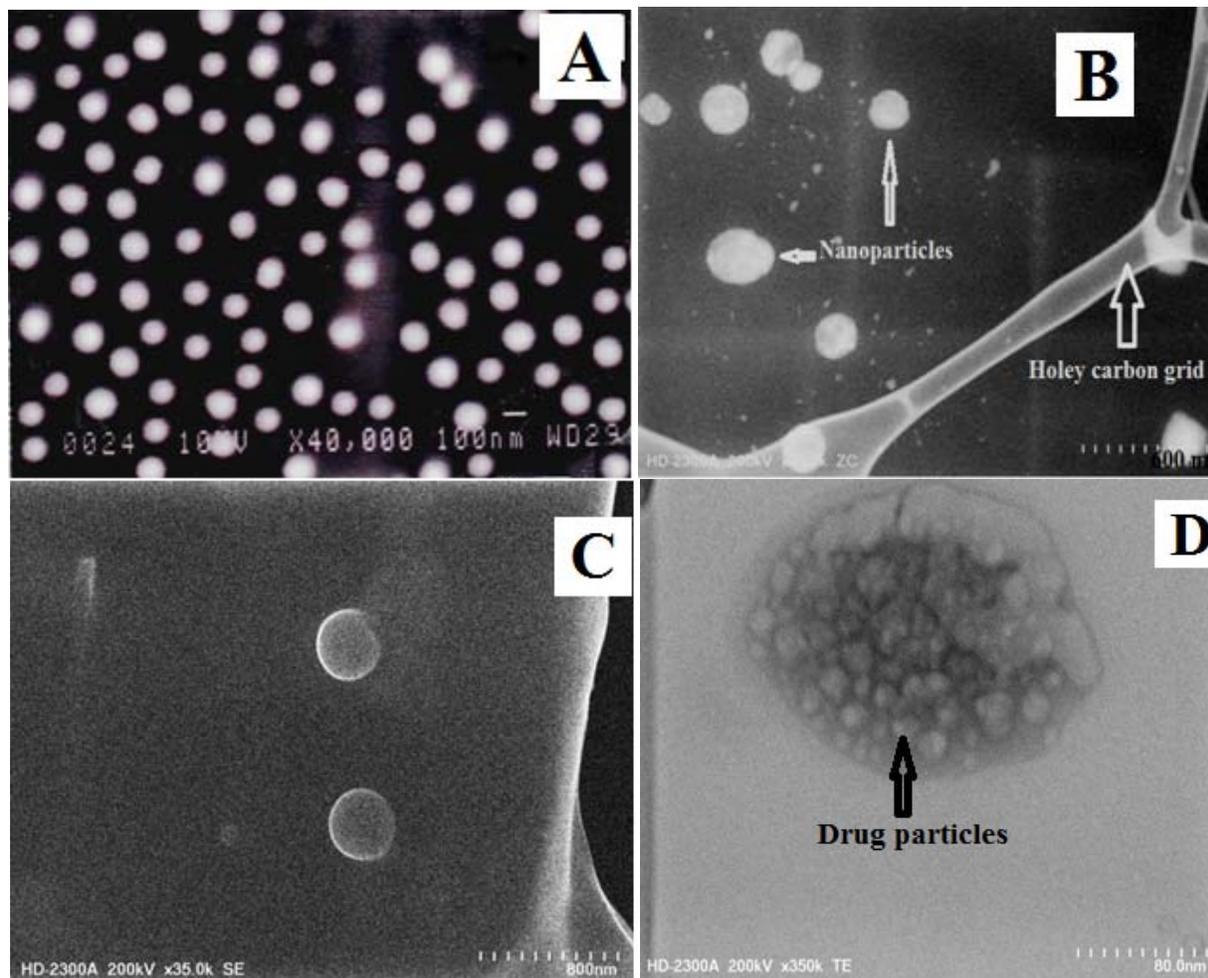


Figure 1: (A) SEM image of Sulfacetamide loaded Eudragit RL 100 nanosuspension (batch B3) taken at 40,000 magnification and acceleration voltage of 10 kv, (B) TEM image of Blank Eudragit RL 100 nanosuspension taken at Z contrast mode, (C) Sulfacetamide loaded Eudragit RL 100 nanosuspension (batch B3) taken at Scanning Electron mode, (D) TEM image of a single nanoparticle (batch B3) showing internal structure and dispersed drug molecules in the polymer matrix.

However, the physical mixture of drug/polymer (1:1) did not show any drug melting peak or crystallization peak. Freeze dried drug loaded nanosuspension (batch B3) showed a broad endothermic transition at an onset of 21.57^oC, a peak at 50.89^oC. Similar observation was noted for other three batches (not shown in thermograms). This observation can be explained from the effect of adsorbed poloxamer as surfactant onto the drug loaded nanoparticles. Pluronic F108 exhibited a melting onset of 55.52^oC, a peak of 58.51^oC. The exothermic crystallization peak of Pluronic F108 was observed at an onset of 169.86^oC and a peak of

175.05^oC. The most probable reason for the appearance of slightly shifted broad endothermic and exothermic peaks is due to melting and crystallization of the adsorbed poloxamer present on the nanoparticle surface.

PXRD

In order to investigate the physical nature of the encapsulated drug, the Powder X-ray Diffraction technique was used. Solid state analysis of the nanosuspension system after freeze drying showed the probability of the drug to disperse in the polymeric matrices as microcrystalline (36) or

could be in semicrystalline form (37). According to Boyer, the semicrystalline system is characterized by amorphous and crystalline phases that are closely associated, leading to the establishment of a three-dimensional interphase associated with the paracrystalline phase (crystalline phase with low degree of organization) and a constrained amorphous phase (38). While the polymer is completely amorphous in nature, entrapment of crystalline sulfacetamide (sharp intense peaks as seen in Figure 3) into the polymeric nanoparticles reduced its crystallinity to a greater extent. A similar observation was noted for the other three batches. This is evident from the disappearance of most peaks in the nanoparticles compared to the drug or the physical mixture of drug/polymer (1;1). Thus, it can be inferred that the drug is present inside the nanoparticles in a semicrystalline or microcrystalline form. This finding was also in agreement with the flurbiprofen loaded acrylate polymer nanosuspension prepared by Pignatello *et al.* (34).

FTIR

Pure sulfacetamide has characteristic IR peaks at 3471.93 cm^{-1} (NH stretch), 1686.3 cm^{-1} (CO), 1642 cm^{-1} , 1596.18 cm^{-1} , 1505.61 cm^{-1} , 1440.51 cm^{-1} , 1375.01 cm^{-1} , 1322.8 cm^{-1} (sym SO₂), 1233 cm^{-1} , 1155 cm^{-1} (asym SO₂). The peaks were in conformity with the findings of Nagendrappa (39). Figure 4 showed that the characteristic bands of the ester groups at $1,150 - 1,190\text{ cm}^{-1}$ and $1,240 - 1,270\text{ cm}^{-1}$, as well as the C = O ester vibration at $1,730\text{ cm}^{-1}$. In addition, CHX vibrations can be discerned at 1385 cm^{-1} , 1450 cm^{-1} , 1475 cm^{-1} and $2,950 - 3,000\text{ cm}^{-1}$. Eudragit has characteristics IR absorption frequency at 3437.91 cm^{-1} (OH stretch), 2952.37 cm^{-1} (sp³ CH stretch), 1733.89 cm^{-1} (CO stretch). The observed peaks were comparable with the findings of Basu *et al.* (40). Freeze dried solid sample of sulfacetamide loaded nanosuspension (batch B3) exhibited mainly the Eudragit absorption peaks with few overlapping peaks from the sulfacetamide. Slight broadening of few peaks near 3500 cm^{-1} for the physical mixture of drug/polymer (1:1) was observed. It can be concluded that no strong chemical interaction occurred between drug and polymer inside the nanoparticles. Similar observation was noted for other three batches of drug loaded nanosuspensions.

DEE

DEE for the sulfacetamide loaded nanosuspension was found to be in the range of 28.26 % to 35.74% for the four batches. The low DEE values indicate relatively low affinity of the drug with the polymer matrix. Another explanation for poor entrapment is probably related to the solubility and ionization of the drug (26).

Three strategies were used to enhance DEE for the batch B3 and included the effect of changing polymer content, changing external phase pH and the addition of Polymethyl methacrylate (PMMA) in the formulation (29). Changing the content of polymer in the formulation B3 did not improve the DEE of nanosuspension (data not shown). When the pH of the aqueous phase was adjusted to 3.4, significant improvement in DEE (~ 50%) was observed. This finding may be due to the suppression of ionization and decrease in solubility of drug during the formation of nanodroplets in solvent displacement method (26). Thus, drug molecules did not escape from the particles when the external aqueous surfactant solution phase was adjusted to acidic pH (3.4) which is two units below the pKa (5.4) of the drug. When, 30 parts of PMMA was incorporated in B3, DEE increased to about 50%.

In vitro drug release

In vitro drug release from the nanosuspension in phosphate buffer pH 7.4 was performed by the dialysis experiment using the static Franz diffusion cell. The *in vitro* drug release profiles obtained from the dialysis experiment was shown in Figure 5. The amount of drug incorporation in the formulation and drug entrapment efficiency has a direct effect on the drug release profile from the four batches. As the content of the drug in the formulation increased, the release rate also increased. Batch B4 had the lowest drug entrapment efficiency (DEE) of 28.26% with a smaller average particle size (112.4 nm) and gave 100% drug release within 2 hours. Batch B1 had a DEE of 31.35 % with a larger average particle size (140.6 nm) and exhibited a prolonged drug release profile with only about 54.22% drug release after 3 hours. A similar tendency was observed for Batch B2 (DEE 32.24% and particle size 127.9 nm) which released about 60.46% of the drug after 3 hours. Batch B3 with a particle size of 118.9 nm and DEE of 35.74% showed 91.17% drug release after 3 hrs.

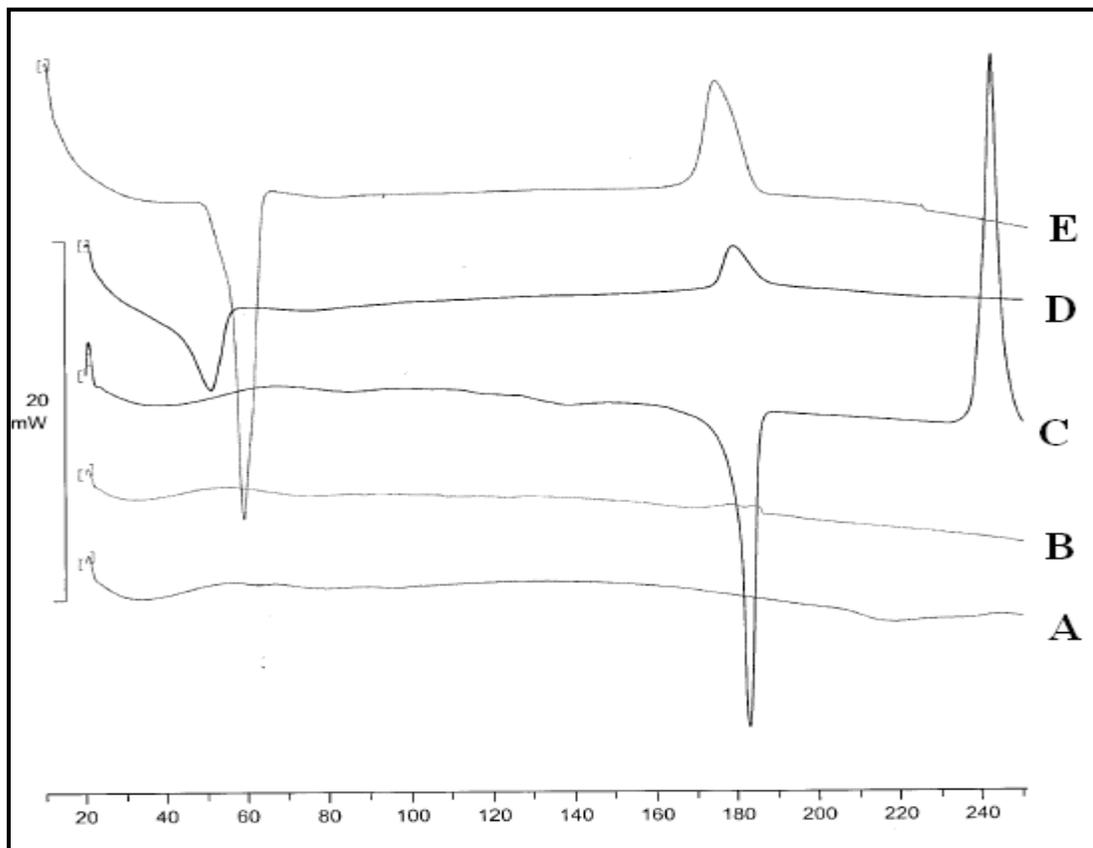


Figure 2: DSC thermograms of EudragitRL100 (A), physical mixture of Sulfacetamide/Eudragit RL100 at 1:1 ratio (B), sulfacetamide (C), Freeze dried nanosuspension batch B3 (D), Pluronic F108 (E).

Kinetics of drug release

The release data were fitted to various kinetic models in order to calculate the release constant and regression coefficients (R^2) as seen in Table II. Among the models tested, the drug release profiles for batch B1 and B2 were best fitted with the Hixon-Crowell cube root model based on the regression coefficients of R^2 of 0.97 and 0.95, respectively. Batches B3 and B4 followed a zero order model with a R^2 of 0.98 and 0.99, respectively. Using the Korsmeyer-Peppas equation, which plots the logarithm of cumulative percentage of drug release up to 60% versus the logarithm of time, showed an excellent fit for the model ($R^2 \sim 0.97$). The diffusion exponent (n) values for all batches were within 0.4.

Freeze drying and redispersibility of nanosuspension

Batch B3 (drug to polymer ratio of 30/100) was selected for freeze drying since it had the highest drug entrapment efficiency with a small particle size and sustained release behavior. The effect of using cryoprotectants on redispersibility in distilled water was investigated visually to observe the formation of any aggregates upon manual hand shaking. 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 a white spongy, cotton-like material upon lyophilization. The freeze dried sample without cryoprotectants did not redisperse in water after manual hand shaking. Large aggregates were observed.

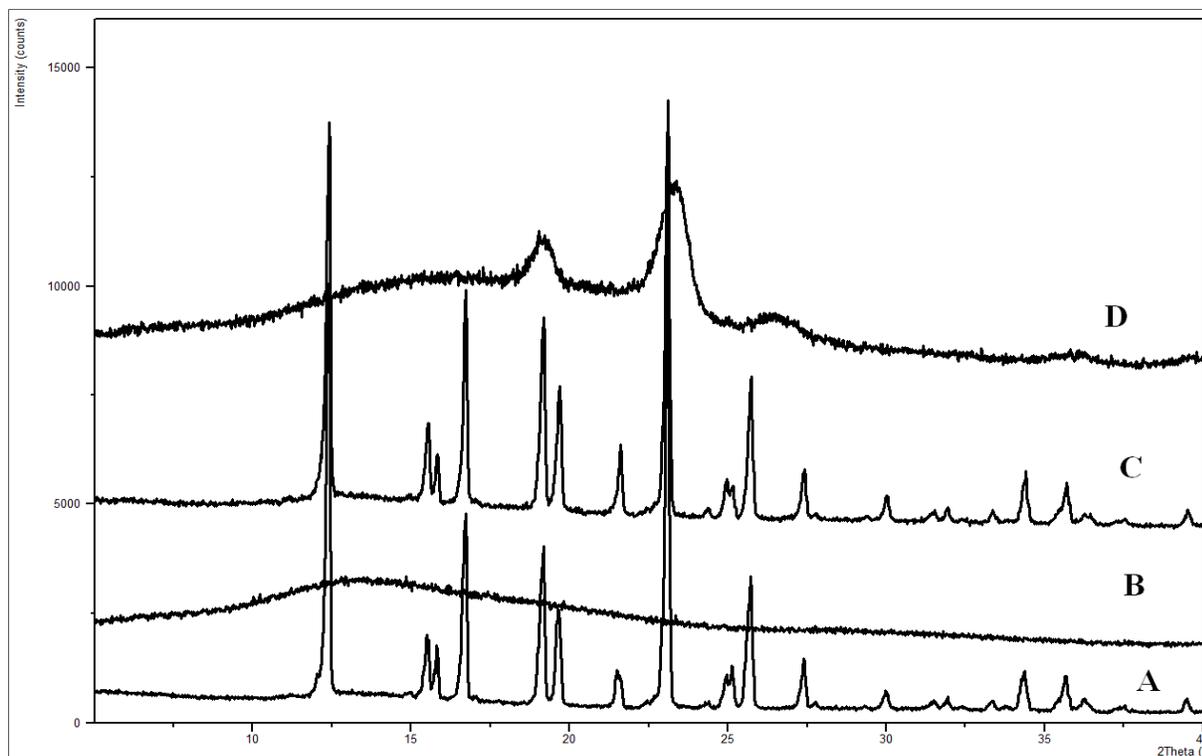


Figure 3: PXRD of Sulfacetamide (A), Eudragit RL100 (B), physical mixture of Sulfacetamide/ Eudragit RL100 at 1:1 ratio (C), freeze dried nanosuspension batch B3 (D)

Mannitol containing samples showed good redispersibility upon manual shaking. No difference was observed for 2.5% and 5% mannitol containing samples. Sucrose containing samples showed excellent redispersibility within a few minutes of shaking for samples with 5% sucrose. Samples containing 2.5% sucrose formed slight turbidity and foaming upon shaking.

Particle size of the 5% sucrose containing batch was 304.7 ± 30.4 nm whereas the 5% mannitol containing batch contained 156.2 ± 18.1 nm average particle size. Therefore, the 5% mannitol containing batch appeared to be the most suitable cryoprotectant for batch B3.

Short term stability study of nanosuspension

The physical appearance of the B3 nanosuspension did not change when samples were stored at 4°C for 1 month. A loose, thin layer of sediment was observed when the nanosuspension was stored at room temperature for 1 month. However, the sediment disappeared with slight hand shaking. The average particle diameters were 125.2 ± 25.1 nm and 98.2 ± 21.3 nm when samples were stored at

room temperature and 4°C , respectively. The particle size for the batch B3 was 118.9 ± 8.17 nm before performing the stability study. It can be inferred from the observed data that the prepared nanosuspension B3 was stable after 1 month of storage at room temperature and 4°C .

DISCUSSION

Eudragit RL100 Nanosuspensions were successfully prepared by the solvent displacement technique. In this process, nanoparticles were spontaneously formed when the organic phase (acetone) containing Eudragit RL 100 with/without sulfacetamide was added dropwise into stirred aqueous surfactant solution (1% Pluronic F 109), resulting in a transparent solution with a bluish opalescence. Instantaneous formation of a colloidal suspension occurred as a result of the polymer deposition on the interface between the organic phase and water when partially water miscible organic solvent (acetone) diffused out quickly into the aqueous phase from each transient particle intermediate.

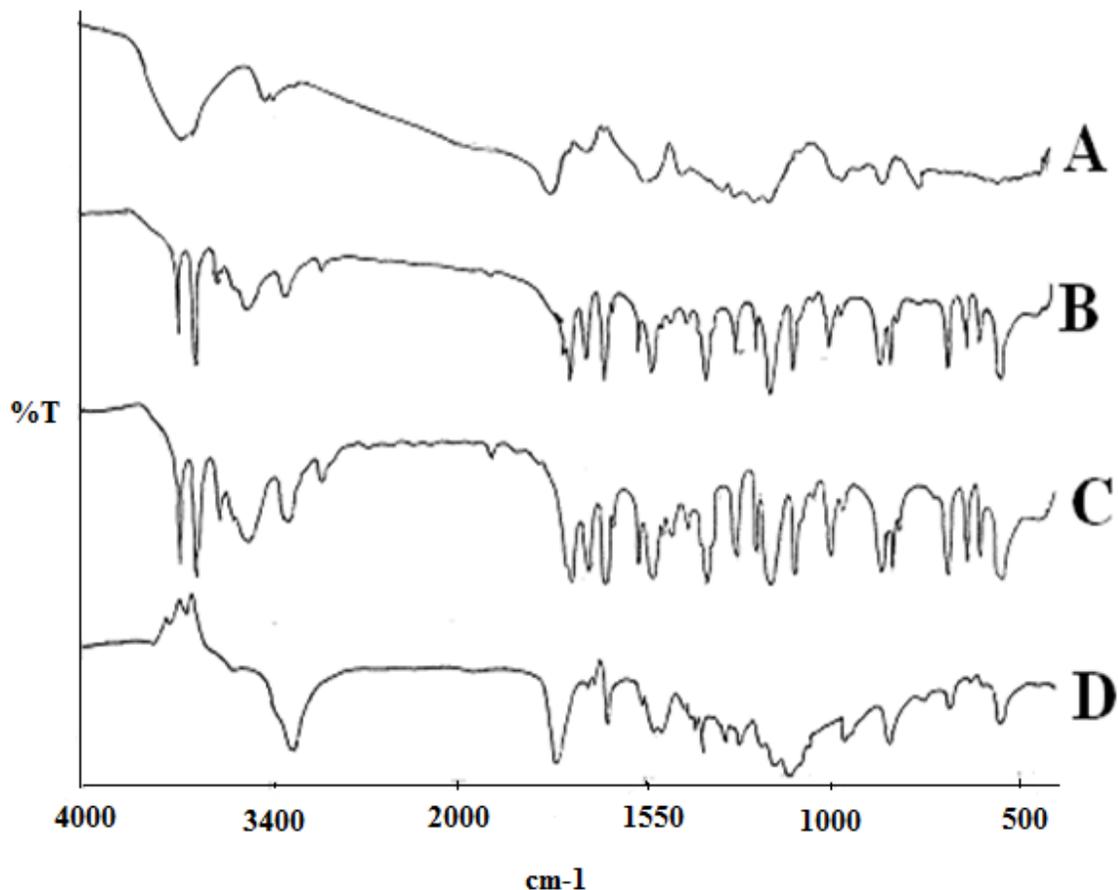


Figure 4: FTIR spectra of EudragitRL100 (A), Sulfacetamide (B), physical mixture of Sulfacetamide/ Eudragit RL100 at 1:1 ratio (C), freeze dried nanosuspension batch B3 (D).

According to the “Marangoni effect”, the transient particle intermediate causes a size reduction to the nano range (41). Formation of a colloidal nanodispersion can be visualized by the bluish opalescence.

This phenomenon is known as the Tyndall effect which results from scattering of light caused by the dispersed colloidal particles (42).

The particle size and size distributions are critical parameters for ocular delivery purposes in order to avoid irritation to the ocular surfaces. Particle size for ophthalmic application should not exceed 10 μm (43). 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 (44). In this study, all batches of the nanosuspension exhibited size distribution range which was below 500 nm, therefore suitable for ocular application. From the formulation point of view, incorporation of the drug above 40% in the formulation resulted in aggregation and separation of the particles to form immediate white sediment. Therefore, the study was carried out in the range of 10-40% drug incorporation in the formulation. Electron microscopy (SEM, TEM) revealed that particles are smooth, regular and spherical in nature. Additionally drug particles were found to be dispersed inside the particles when TEM was performed.

The positive surface charge for the nanoparticles could allow for a longer residence time for the particles by ionic interaction with the negatively charged sialic acid residues present in the mucous of the cornea and conjunctiva (45).

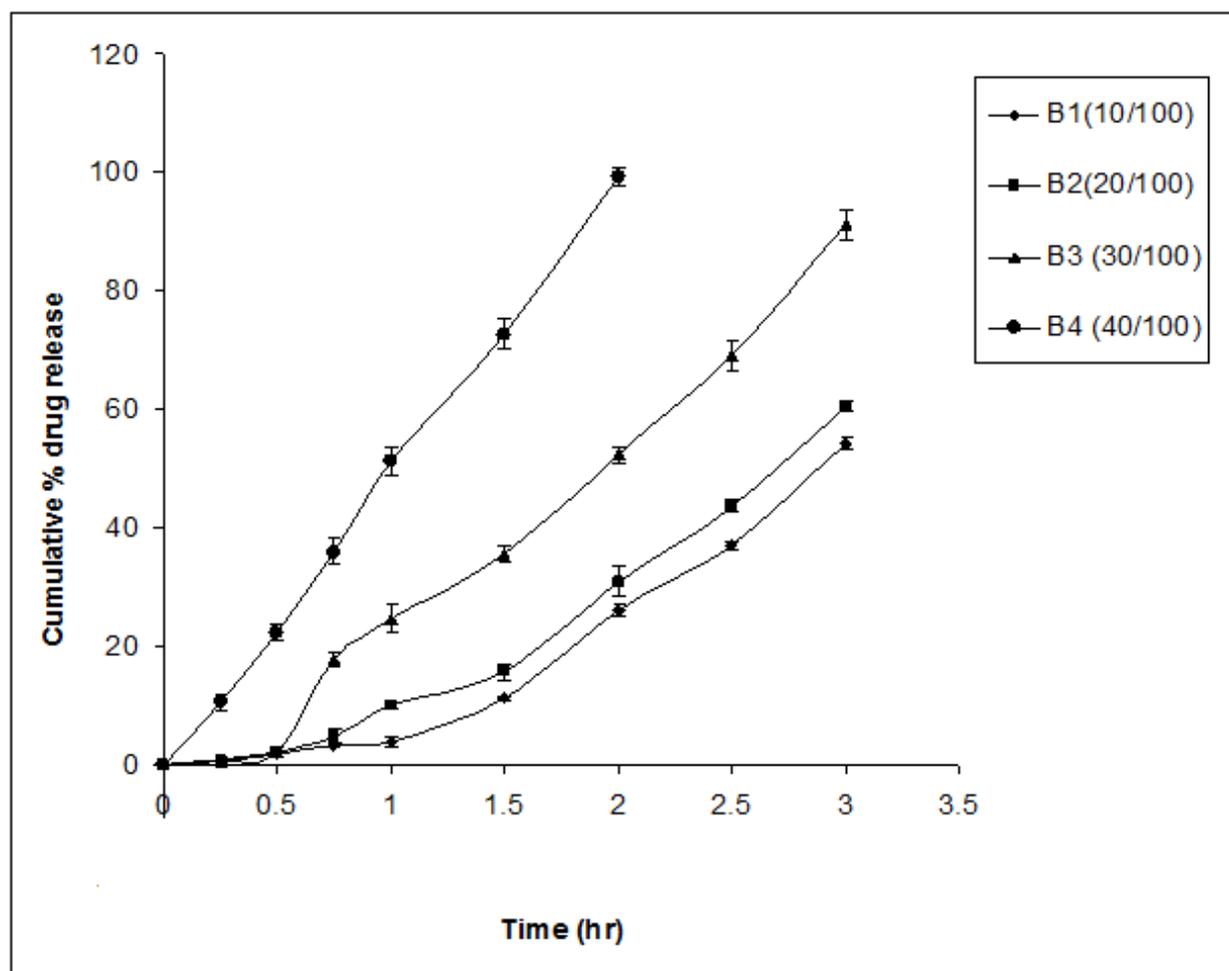


Figure 5: In vitro release of Sulfacetamide loaded nanosuspensions in phosphate buffer pH 7.4 at 37°C (n=3).

Table 2. Kinetic release rate constants, correlation coefficient and diffusion exponent of various models (n=3)

Batch	Zero order		First order		Higuchi model		Hixon-crowell		Korsmeyer peppas		
	K_0	R^2	K_1	R^2	K_h	R^2	K_H	R^2	K	n	R^2
B1	17.876	0.915	0.784	0.868	70.587	0.892	0.630	0.975	0.057	1.953	0.986
B2	20.228	0.948	0.747	0.894	54.035	0.845	0.645	0.953	0.080	1.856	0.995
B3	30.942	0.982	0.498	0.836	34.213	0.765	0.745	0.879	0.159	2.28	0.921
B4	50.036	0.998	0.122	0.750	29.745	0.715	1.036	0.792	0.502	1.14	0.999

Sulfacetamide belongs to a class of secondary sulfonamides in which the hydrogen on the nitrogen atom is acidic. Thus, in basic medium, the nitrogen acquires a negative charge on the conjugate base stabilized by resonance. The adsorbed surfactant (Pluronic F108) present on the nanoparticles surface may shield the particle surface, thus covering it with the electrically neutral layers and causing a slight shift in the surface charge (46). The relative

constancy of zeta potential with slight variation indicates that sulfacetamide was encapsulated within the nanoparticles and a major part of the drug is not present on the nanoparticle surface.

Solid state characterization of FDN was performed by DSC, PXRD and FTIR techniques. These techniques allow us to confirm the possibility of any significant or insignificant chemical or physical interaction among drug, polymer,

surfactants or other additives of the formulation. No significant chemical interaction was observed among sulfacetamide, Eudragit and Pluronic. Therefore, the inactive formulation ingredients were compatible with the drug.

The indirect method was used to determine DEE as described by Hou *et al.* (47). After preparing the fresh nanosuspension, it was centrifuged and the free drug present in the supernatant was analyzed by UV-Visible spectrophotometer using a calibration curve. Subtracting this value from the initial amount of drug, DEE was calculated. The method is suitable for determining entrapment efficiency of nanosuspension when fairly high concentrations of free drug are present in the supernatant after centrifugation (48). Sulfacetamide is slightly soluble in water and has an ionization constant of 5.4. The aqueous 1% Pluronic (surfactant) solution has a pH of about 6. Therefore, when the organic phase is added dropwise into the aqueous surfactant solution, part of the drug is ionized and escapes from the nanoparticles during diffusion of the acetone into the aqueous phase. Increasing the drug content in the formulation increased the DEE inside the nanoparticles. However, when the drug content is 40% in the formulation (batch B4), saturation of the polymer particles occurs with such a high drug loads. The excess drug escapes from the acetone phase into the water. Therefore, DEE dropped in batch B4. Another possibility for the decreased DEE at high drug content in the formulation can be explained by possible saturation of the cationic sites on the Eudragit by anionic drug molecules (49). Therefore, excess drug is being lost from the particles during its formation process.

Drug content and average particle size has impact on drug release profile of nanosuspensions. As the content of the drug in the formulation increased, the release rate also increased. Batch with the lowest drug entrapment efficiency (DEE) and smaller average particle size gave faster drug release. The progressive saturation of the quaternary groups in the polymer by drug molecules (occurred at high drug content) increased drug release from the formulation (23). On the other hand, batch with a larger average particle size exhibited a prolonged drug release profile. A correlation between drug release from the nanosuspensions with mean particle size was observed. Thus, it can be inferred that larger

particles have a small initial burst release and a longer sustained release than smaller particles (50).

Among the models tested, the drug release profiles were best fitted with the Hixon-Crowell cube root model (for B1 and B2) and zero order model (for B3 and B4) based on R^2 values. The diffusion exponent (n) values calculated from Korsmeyer-Peppas equation for all batches were within 0.4 which indicated that the drug release mechanism followed pure Fickian diffusion. Pignatello *et al.* (34) showed that the drug release from Eudragit RL100 particles was complex in nature which involves the occurrence of dissolutive and diffusive phenomena. Overall the drug release rate was faster which were probably due to the high water permeability and swellability characteristics of Eudragit RL 100 (51). The presence of a high content of quaternary ammonium groups makes the polymer more permeable to water.

CONCLUSION

In this study, the potential of Eudragit RL 100 nanosuspension intended for ocular delivery of Sulfacetamide was investigated. Nanosuspension was prepared by the solvent displacement technique which is the easiest and most reproducible method to prepare nanoparticles without need of any sophisticated instruments. Due to formation of nanosuspension with suitable particle size, positive surface charge, good short term stability, redispersibility and sustained release characteristics, the delivery system appears to be promising for treating ocular bacterial inflammation and infection. Although we improved the drug entrapment efficiency of the formulation to some extent by pH alteration and additional of another excipient in the formulation, the issue warrants further attention. Additionally, long term stability study and in vivo study should be performed in order to evaluate the clinical potential of the delivery system.

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