ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Development and In-Vitro Evaluation of Nanosuspension Formulation Containing Acyclovir for the Treatment of Ocular Infections

Uma maheswari R[1], and Mullaicharam AR[2]

[1]Research Scholar, Sastra University, Thanjavur, Tamilnadu, India
[2]Research Supervisor, Sastra University, Thanjavur, Tamilnadu, India

ABSTRACT

Nanosuspensions were prepared by the solvent displacement method using acetone and 1% (w/v) Pluronic® F108 solution. Physicochemical characterization of the nano suspension was performed by measuring particle size, zeta potential, drug entrapment efficiency and in vitro drug release. The delivery system was intended to enhance ocular availability without blurring vision and reducing the frequency of dosing in conjunctivitis leading to patience compliance. Positive surface charge of nanoparticles can allow longer residence time for the drug on the eye surface by increasing the interaction of nanoparticles with the glycoprotein of the cornea and conjunctiva.

Keywords: Nanosuspension- Acyclovir-Ocular infection.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Conventionally, most ocular diseases or disorders are treated with water-soluble drugs in aqueous solution while water- insoluble drugs in ointments or aqueous suspension [1]. However, there are several disadvantages such as: frequent installation of highly concentrated solutions due to rapid tear turnover and precorneal loss [2]; large volume of the instilled dose [20-50 µl vs 7-8 µl of the tear film [3]; irritation caused by drug penetration; drug solubility and stability in the eye fluids, difficulty in passing the blood-corneal barrier [4]. The precorneal half- life is considered to be 2-3 minutes after installation of an excess volume of fluid [5]. Typically less than 5% of the topically applied drug penetrates the cornea and reaches the posterior segment of the eye [6]. A major fraction of the instilled dose is absorbed systematically via the nasolachrymal duct. This may cause systemic adverse effects such as trachycardia, hypertension, bronchial asthama e.g. Timolol ophthalmic solution [7].There are several new ophthalmic drug delivery systems under investigation such as: hydrogels [8]; microparticles [9]; nanoparticles [10]; liposomes [11]; collagen shields [12]; ocular inserts/discs [13]; dendrimers [14]; and transcorneal iontophoresis [15]. 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. 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 [16]. Polymeric nanosuspensions, prepared from Eudragit® RL 100 and RS 100, have been investigated extensively for the ocular delivery of ibuprofen[17,18], flurbiprofen [19,20], cloriocromene [21], piroxicam[22], methyl prednisolone [23], and amphotericin B [24]. They are approved by USFDA as a excipient for controlled drug delivery. 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 nanoprecipitation or solvent displacement method, developed by Fessi et al [25]. Currently, no attempt has been made to encapsulate acyclovir inside a polymeric nanoparticulate carrier which could facilitate the drug delivery to the ocular surface. Therefore, an attempt was made to prepare and characterize Acyclovir loaded Eudragit® RL 100 nanosuspensions intended for the treatment of ocular infections.

MATERIALS AND METHODS

Materials

Acyclovir [Chemical Name: N-[[4-aminophenyl]sulonyl]acetamide], Eudragit® RL100 [Chemical Name: Ammonio Methacrylate Copolymer, Type A USP/NF Molecular Weight: 150,000 [approx]], Pluronic® F108 [Chemical Name: 2-methyloxirane], Acetone [Chemical Name: Dimethyl ketone, 2-propanone]

ISSN: 0975-8585

Methods

Preparation of Nanosuspension

The Eudragit® RL 100 nanoparticles were prepared by nanoprecipitaion method similar to that employed by Fessi et al [26] and other authors [27-31]. Briefly, a 100 mg portion of Eudragit® RL100 and various proportions of drug were dissolved in 10 mL of acetone. This organic phase was poured dropwise 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 continuing stirring for 20 hrs. The resulting particle suspension was filtered through 1.2 µm cellulose nitrate membrane filter in order to remove larger particle aggregates. The prepared suspension was centrifuged at 19,000 rpm at 150C f or 2 hours [Sorvel RC-5B refrigerated superspeed centrifuge, rotor SS-34, 33300g, K 446]. 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.

Scanning Electron Microscopy

In order to examine the particle surface morphology and shape, Scanning Electron Microscopy [SEM] was used. Photographs were taken using a JSM-5200 Scanning Electron Microscope [Tokyo, Japan] operated at 10 kV.

Transmission Electron Microscopy

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 40^0C for 20 minutes. The images were taken using a Hitachi Ultra-thin film evaluation system [HD-2300A] in Phase contrast, Z contrast, Secondary Electron [SE] modes.

Drug Entrapment Efficiency

A 20 mL portion of the freshly prepared nanosuspension was centrifuged at 19,000g for 2 hrs at 10-150˚C temperature using 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 appropriately diluted supernatant solution at 260 nm using single beam UV spectrophotometer [Genesis 10 UV, Thermoelectron Corporation, USA]

against blank/control nanosuspension. By substraction from the initial amount of drug taken, entrapment efficiency was calculated. The experiment was performed in triplicate for each batch and the average was calculated.

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 behaviour of different samples. Samples [3-5 mg] were accurately weighed into 100 µl aluminium pans and then crimped. Mettler Toledo STARe software [version 8.10] was used to analyze data.

Powder X-Ray Diffractometry [PXRD]

The drug crystalline state in the polymer sample was evaluated by Powder X-Ray Diffraction [PXRD] analysis. X-ray spectrawere recorded with X'Pert-PRO multipurpose X-Ray diffractometer [PANalytical, Tokyo, Japan] using Ni-filtered, CuKα radiation, a voltage of 45 kV, and a current of 40 mA with a scintillation counter. 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 by using a PerkinElmer 1600 spectrophotometer with a resolution of 2 cm-1. For the analysis of the data, the spectrum GX series model software was used.

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 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 37°C 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].

Freeze drying and redispersibility of nanosuspension

All the four batches [B1, B2, B3, B4] were freeze dried to obtain dry

powder. Additionally, selected batch [B3] was taken to study effect of cryoprotectant on freeze drying and redispersibility of drug loaded nanosuspension. Two cryoprotectants were used: sucrose and mannitol both at 2.5% and 5% w/v concentration level. The nanosuspension sample was divided into four 2 mL parts and taken individually in small glass vial. Required amounts of cryoprotectants were added in each vial and shaken to dissolve. A 2 mL portion of the nanosuspension without the cryoprotectant was taken in vial as a control. The opening of the vial was covered with tissue paper wrapped by a cotton thread. 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 600 mL Labconco® fast-freeze flask with attached adapter. Freeze-drying process was carried out in the Virtis Freezemobile model 12EL. Temperature was kept about - 700C and vacuum was kept at 162 mT. After 48 hours, lyophilized samples were collected and stored in dessicator 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 Nicomp^{TM} 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 [20^{o}C] and at 4 ^{o}C 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 Nicomp^{TM}380 ZLS, CA, USA].

RESULTS AND DISCUSSIONS

Preparation of Nanosuspension

Eudragit® RL100 Nanosuspensions were successfully prepared by the solvent displacement or nanoprecipitation technique [32]. Nanoparticles were spontaneously formed when the organic phase [acetone] containing Eudragit® RL 100 with/without Acyclovir 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. According to the "Marangoni effect", the transient particle intermediate causes a size reduction to the nano range. Formation of a colloidal nanodispersion can be visualized by the bluish opalescence [Figure 1]. This phenomenon is known as the Tyndall effect. It is a phenomenon in which the scattering of light is caused by the dispersed colloidal particles [33].

ISSN: 0975-8585

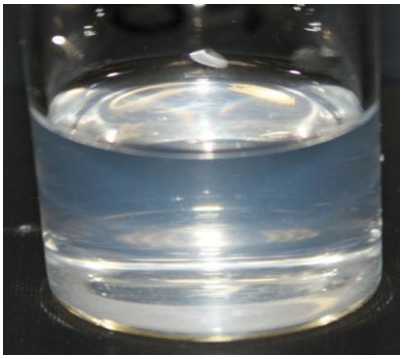


Figure 1: Photograph of the nanosuspension B4 showing bluish opalescence

Particle size and size distribution

The particle size and size distributions are critical parameters for ocular delivery purposes in order to avoid irritation to the ocular surface. Particle size for ophthalmic application should not exceed 10 µm [34]. The United States Pharmacopoeia [USP] specifies that ophthalmic solutions should contain not more 50 particles with a diameter more than 10 µm, 5 particles with adiameter 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 [35]. The experimental output of DLS experiments are seen in Figures 3 to 7.

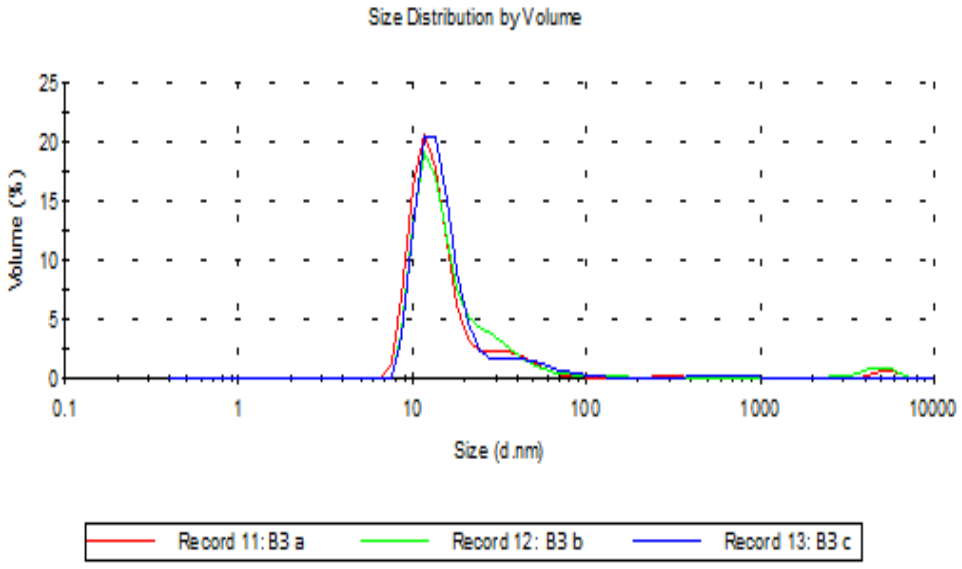


Figure 2: DLS plot for the size distribution vs number for batch B3 [n=3]

The effect of the drug to polymer ratio on size of the nanparticles were studied using four different weight ratios of drug and polymer, namely 10:100, 20:100, 30:100 and 40:100.

Incorporation of the drug above 40% in the formulation resulted in aggregation and separation of particles to form white sediment immediately. Therefore, the study was carried out in the range of 10-40% drug incorporation in the formulation. Particle size data for nanosuspension matches was shown in Table 1. The 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 [Z-average diameter] for drug loaded batches [B1 to B4] varied in the narrow range from 112.4 nm to 140.6 nm.

Table 1: Particle size, Polydispersity index [PI], zeta potential of blank and Acyclovir-loaded Eudragit® RL100 Nanosuspensions [σ is Standard deviation, n=3]

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

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 nanosuspension [$p < 0.05$]. One way ANOVA followed by Tucky test showed that batch B0 showed significant difference in particle size compared to drug loaded batches [$p < 0.05$]. Surprisingly, 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 [36]. All batches of the nanoparticles showed mean sizes which were below 500 nm, therefore suitable for ocular application.

Zeta potential

The zeta potential values for nanosuspensions were shown in Table.1. The zeta potential remained in the range of positive values for all batches [+ 9.16 mV to + 24.1 mV] which is consistent with the findings of Pignatello et al [37]. The positive surface charge of the nanoparticles was observed due to the presence of the quarternary ammonium groups of Eudragit® RL100. 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 [38]. Acyclovir belongs to a class of secondary sulfonamides in which the hydrogen on the nitrogen atom is acidic. Thus in basic medium, the nitrogen acquires negative charge on the conjugate base stabilized by resonance [39]. The adsorbed surfactant [Pluronic® F108] present onto the nanoparticles surface may shield the particle surface, thus covering with the electrically neutral layers and causes a slight shift in surface charge [40]. The

ISSN: 0975-8585

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

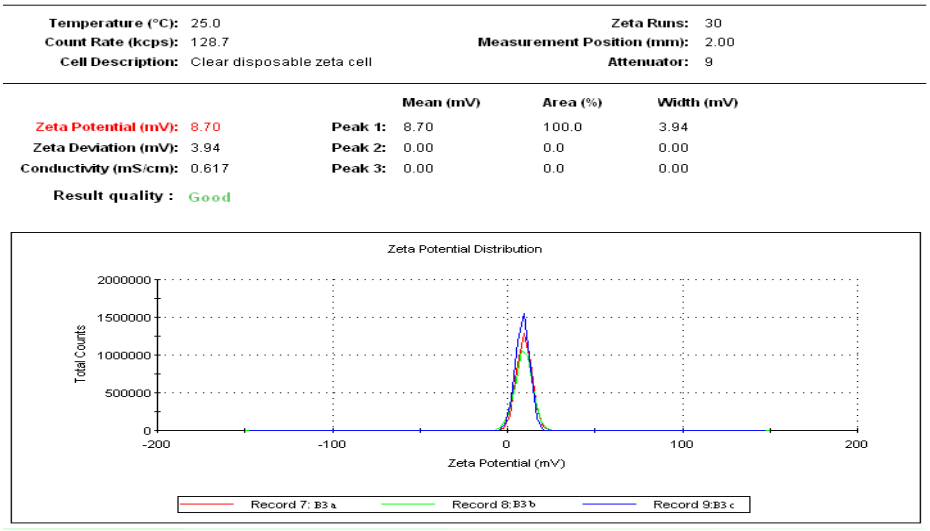


Figure 3: Plot of Zeta Potential distribution for the batch B3 [n=3]

SEM and TEM

Nanoparticle surface morphology and shape were visualized using SEM and TEM. SEM revealed that the blank nanoparticles were spherical to oval in shape with a larger size whereas, the drug loaded nanoparticles were found to be distinct, spherical with a smooth surface [Fig.4]. 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. Magnification of a single particle showed the internal cage like structure where the drug molecules are dispersed uniformly throughout the polymer matrix. The drug appears as white spots on the surface. It was observed that when a high energy electron beam were passed to scan the particles in TEM, the polymer burns out leaving the drug particles viewed as a cage like structure.

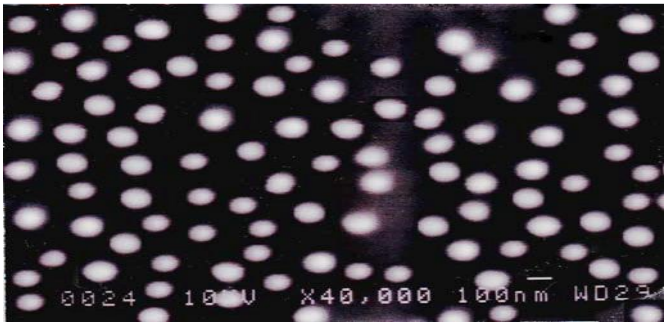


Figure.4: SEM image of drug loaded Eudragit® RL100 nanosuspension [batch B3] taken at 40,000 magnification and acceleration voltage of 10 kv

ISSN: 0975-8585

Drug Entrapment Efficiency

The indirect method was used to determine drug entrapment efficiency [DEE]. After preparing the fresh nanosuspension, it was centrifuged and the free drug present in the supernatant was analyzed by UV-Visible spectrophotomer using a calibration curve. The calibration curve was constructed by measuring the absorbance at 260 nm of solutions of five different concentrations of drug in water. By subtracting form initial amount of drug, DEE was calculated. The method is suitable for determining entrapment efficiency of nanosuspension when fairly high concentration of free drug is present in the supernatant after centrifugation [41]. DEE of the Acyclovir 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 solubility and ionization of the drug. Acyclovir is 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 DEE inside the nanoparticles [Figure.19]. 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 saturation of the cationic sites on the Eudragit® by anionic drug molecules. Therefore, excess drug is being lost from the particles during its formation process.

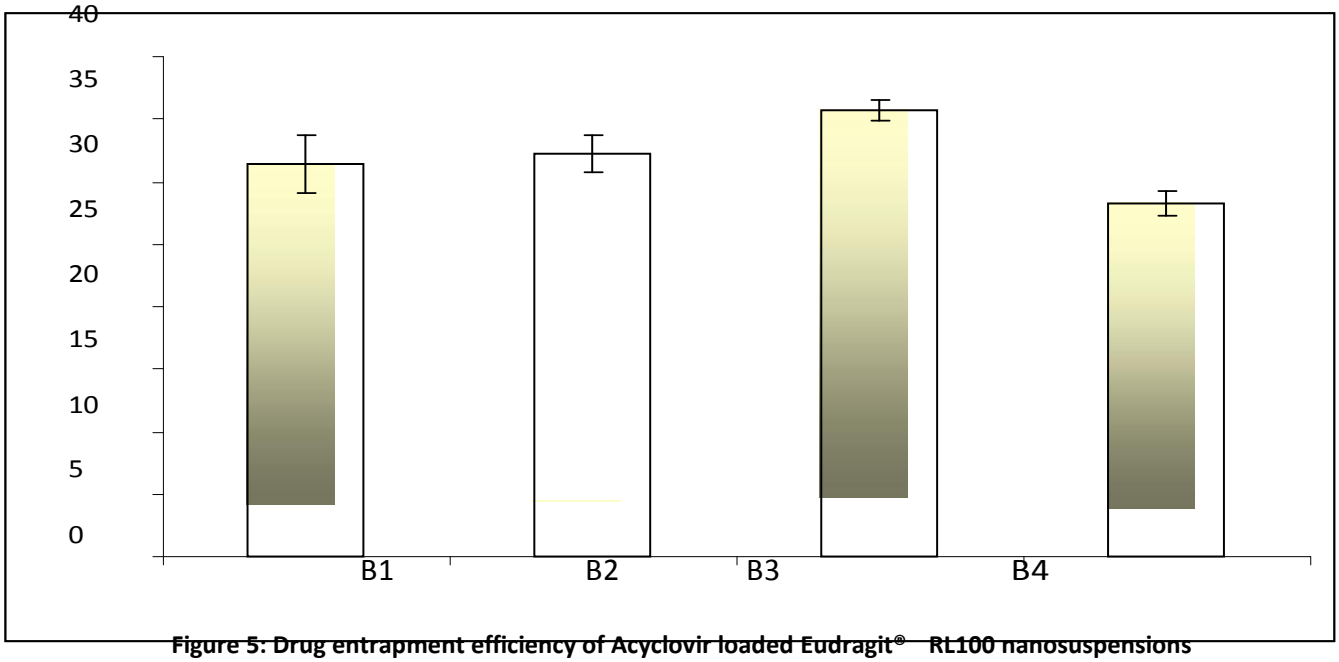


Figure 5: Drug entrapment efficiency of Acyclovir loaded Eudragit® RL100 nanosuspensions

Three strategies were used to enhance DEE of the batch B3 such as effect of changing polymer content, changing external phase pH [42] and addition of Polymethyl methacrylate [PMMA] in the formulation [14]. 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 insolubility of acyclovir during the formation of nanodroplets in solvent displacement method. Thus, drug molecules did not escape from the particles when the external aqueous surfactant solution phase was adjusted to acidic pH of 3.4, which is below the pKa [5.4] of the drug. When, 30 parts of PMMA was incorporated in B3, DEE increased to about 50%

DSC

From the overaly of DSC thermograms, it has been observed that Acyclovir is crystalline in nature [Figure.20]. It exhibited a sharp melting endotherm at an onset temperature of 180.1 ^{o}C, a peak temperature of 182.31 ^{o}C and a heat of fusion of 119.7 J/gm. The drug recrystallized at an onset temperature of 241.76, a peak temperature of 245.09 and had an energy of activation of about 80.16 J/gm. Eudragit® RL 100 polymer exists as a completely amorphous form with a glass transition temperature [Tg] of about 60 ^{o}C [43]. The amorphous polymer did not show any fusion peak or phase transition, apart from a broad signal around 55–60 ^{o}C due to a partial loss of residual humidity [44].

The thermal behavior of the freeze dried nanoparticles suggested that the polymer inhibited the melting of the drug crystals. The possible occurrence of ionic interaction may have existed in the physical mixture as observed for the furosemide and Eudragit® RL 100 system [45]. However, the physical mixture of drug and polymer did not show any drug melting peak or crystallization peak. Freeze dried drug loaded nanosupension [batch B3] showed an broad endothermic transition at an onset of 21.57, a peak at 50.89^{o}C. Similar observation was noted for other three batches. 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 ^{o}C, a peak of 58.51 ^{o}C consistent with the finding of Passerini et al [46]. The exothermic crystallization peak of Pluronic® F108 was observed at an onset of 169.86 ^{o}C and a peak of 175.05 ^{o}C. The most probable reason for the appearance of slightly shifted broad endothermic peak and exothermic peak 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 that the drug is dispersed in the polymeric matrices in a

semicrystalline to microcrystalline form. While the polymer is completely amorphous in nature, entrapment of crystalline acyclovir [sharp intense peaks as seen in Figure.21] into the polymeric nanoparticles reduced its crystallinity to a greater extent. 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. There may also be the possibility of overlapping of drug peaks by the background diffraction pattern of the amorphous structure [47]. Thus, it can be inferred that the drug is present inside the nanoparticles in a semicrystalline to microcrystalline form. This finding was also in agreement with the flurbiprofen loaded acrylate polymer nanosuspension prepared by Pignatello et al [37].

FTIR

Pure acyclovir 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]. This finding is in agreement with the findings of Nagendrappa G [48]. Figure 12.23 showed that the characteristic bands of the ester groups at 1,150 - 1,190 cm^{-1} and 1,240- 1,270 cm^{-1}, as well as the C = O ester vibration at 1,730 cm^{-1}. In addition, CHX vibrations can be discerned at 1385 cm^{-1}, 1450 cm^{-1}, 1475 cm^{-1} and 2,950 - 3,000 cm^{-1}. Eudragit has characteristics IR absorption frequency at 3437.91 [OH stretch], 2952.37 [sp3 CH stretch], 1733.89 [CO stretch].

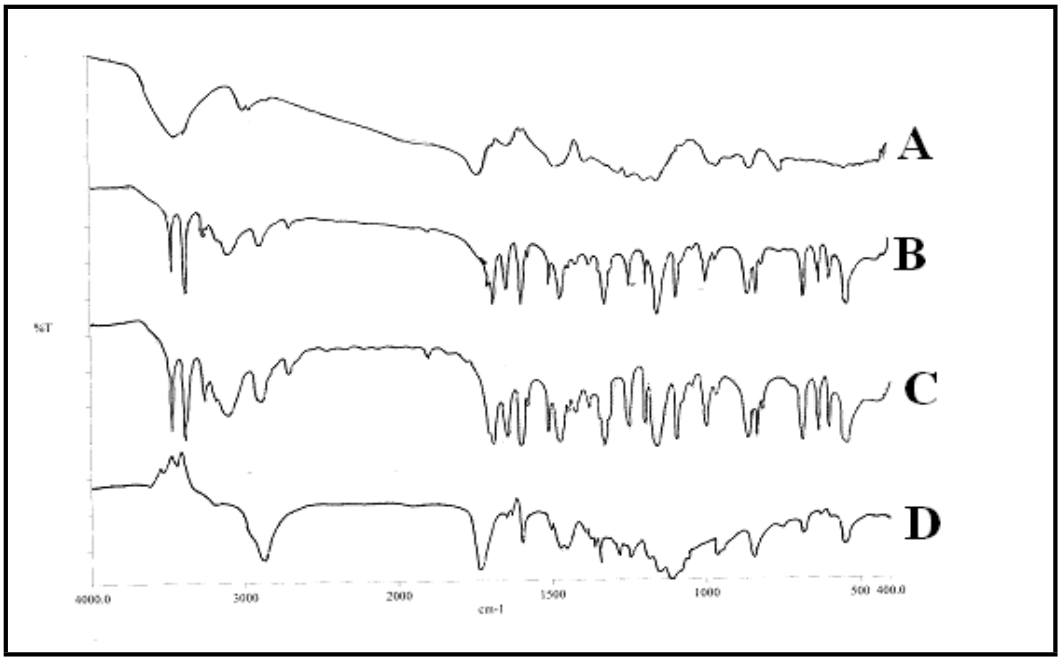


Figure 6: FTIR spectra of Eudragit®RL100 [A], Acyclovir [B], Physical mixture of Acyclovir and Eudragit® RL100 [C], Freeze dried nanosuspension batch B3 [D]

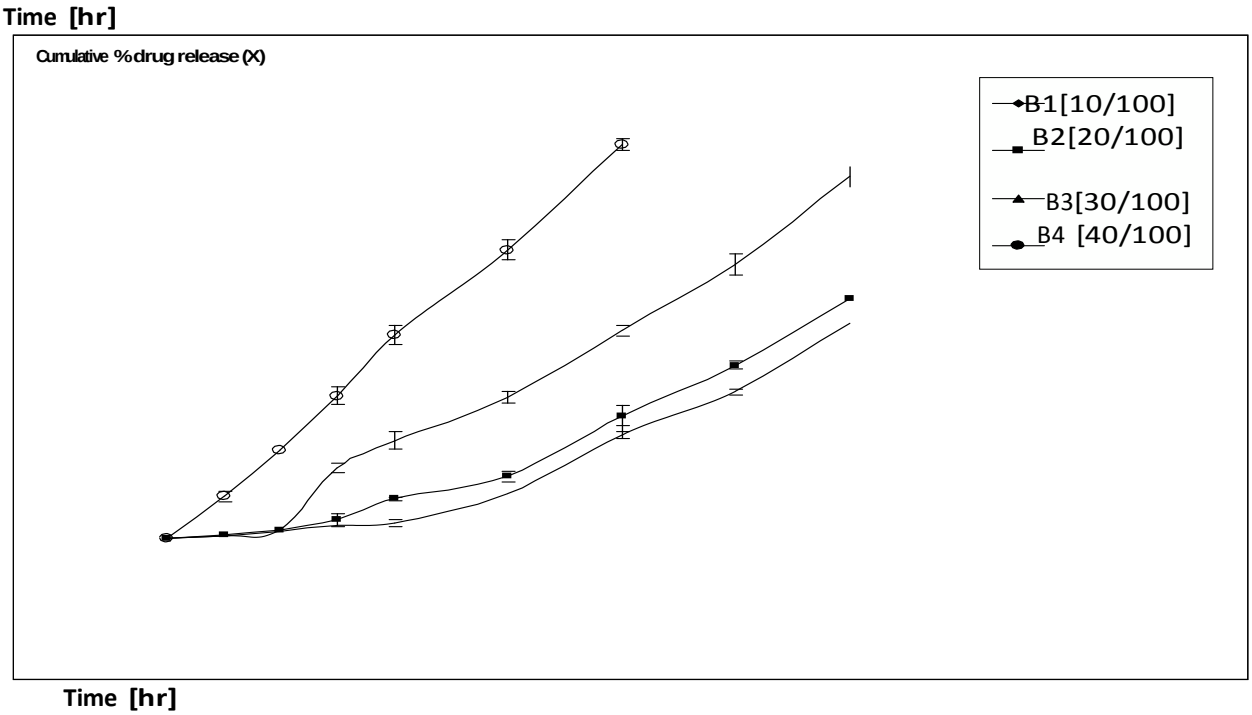
Freeze dried solid sample of acyclovir loaded nanosuspension [batch B3] exhibited mainly the Eudragit® absorption peaks with few overlapping peaks from the acyclovir. It can be concluded that no strong drug polymer interaction occurred inside the nanoparticles. Similar observation was noted for other three batches of drug loaded nanosuspension.

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 .23. The amount of drug incorporation in the formulation and drug entrapment efficiency have a direct effect on the drug release profile from the four formulations. 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] gave 100% drug release within 2 hours. The progressive saturation of the quaternary group in the polymer by drug molecules, occurred a at high drug content which increased drug release from the formulation [7]. Batch B1 had a DEE of 31.35 % with a larger average particle size [140.6 nm], gave 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. Thus, 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 [49].

In vitro release of acyclovir loaded nanosuspensions in phosphate buffer pH 7.4 at 37^{o}C

ISSN: 0975-8585



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 12.2. Among the models tested, the drug release profiles for the batch B1 and B2 were best fitted with Hixon crowell cube root model based on the regression coefficients [R^2 of 0.97 and 0.95 respectively]. Batches B3 and B4 followed zero order model [R^2 of 0.98 and 0.99 respectively]. With Korsemeyer-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~ 0.97]. The diffusion exponent [n] values for all batches were within 0.4 which indicated that drug release mechanism followed pure Fickian diffusion. Pignatello et al, showed that the drug release from Eudragit RL 100 particles was complex in nature which involves the occurrence of dissolutive and diffusive phenomena [50]. Overall the drug release rate was faster which is probably due to the high water permeability and swellability of Eudragit. The presence of a high content of quaternary ammonium groups makes the polymer permeable to water.

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		Korsemeyer 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

Freeze drying and redispersibility of nanosuspension

The 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.

Short term stability study of nanosuspension

Physical appearance of the B3 nanosuspension did not change when samples were stored at 4^{o}C for 1 month. A loose, thin layer of sediment was observed when 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 ^{o}C respectively. The particle size for the batch B3 was 118.9 ±8.17 nm before performing 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 ^{o}C.

CONCLUSION

In this study, the potential of Eudragit® RL 100 nanosuspension with potential for ocular delivery of Acyclovir was investigated. Nanosuspension was prepared by solvent displacement technique which is the easiest and reproducible method to prepare nanoparticles without need of any sophisticated instruments. Size range of all the batches was within 500 nm with polydispersity index of 0.4 to 0.6 suitable for ocular administration. Additionally, SEM and TEM images showed almost spherical particles with smooth surface. The positive surface charge on the particle would provide ionic interaction with the mucous membrane of cornea, resulting in sustained drug release and improved ocular penetration. No major drug polymer interaction was detected using FTIR, DSC, PXRD studies done for solid state characterization. Batch B1 and B2 showed sustained drug release profile whereas release from batch B3 and B4 were comparatively faster. Drug entrapment efficiency was found to be in the range of 28.28% to 35.74% which is low due to the ionization and solubility of acyclovir. In terms of entrapment efficiency, batch B3 containing 30 parts acyclovir to 100 parts Eudragit® RL 100 showed relatively higher drug entrapment efficiency. This batch was selected to study the effect of three approaches to increase drug entrapment efficiency. There are three strategies employed to increase drug entrapment such as: changing polymer content in formulation, changing external phase pH and addition of another polymer in the formulation. Changing pH to about 3.4 suppressed ionization and increased drug entrapment efficiency. Similarly, addition of 30 parts of PMMA in the formulation B3 increased drug entrapment efficiency to about 50%. Overall the study objectives are fulfilled based on the experimental results. Freeze dried nanosuspension using sucrose and mannitol as cryoprotectant exhibited good redispersibility upon manual hand shaking. Short term stability study revealed stable nanosuspension with no significant change in particle size distribution. Several strategies are currently under investigation in order to increase entrapment efficiency of the nanoparticles. Sterilization, long term stability

and in vivo studies could further be performed in order to characterterize the delivery system for clinical use.

REFERENCES

[1] Ding S. "Recent developments in ophthalmic drug delivery". Pharmaceutical Science and Technology Today 1998; 1(8): 328-335.

[2] Desai SD and Blanchard J. Encyclopedia of P harmaceutical Technology, Swarbrick, J and Boylan, JC, Marcel Dekker, New York 1995; 43–75.

[3] Mishima S, Gasset A, Klyce SDJ and Baum JL. "Determination of tear volume and tear flow". Investigative Ophthalmology and Visual Science. 1966; 5(3):264-276.

[4] Peyman GA, Ganiban GJ. "Delivery systems for intraocular routes". Advanced Drug Delivery Reviews. 1995; 16(1): 107-123.

[5] Hull DS, Green K, Boyd M and Wynn HR. "Corneal endothelium bicarbonate transport and the effect of carbonic anhydrase inhibitors on endothelial permeability and fluxes and corneal thickness". Investigative Ophthalmology and Visual Science. 1977; 16(10): 883-892.

[6] Mezei M and Meisner D. Biopharmaceutics of Ocular Drug Delivery. Edman P[ed] CRC Press, Boca Raton, FL, USA 2003; 91–104.

[7] Vander ZJA, Valuck RJ, Bunch CL, Perlman JI, Anderson C and Wortman GI. "Systemic adverse effects of ophthalmic beta-blockers". The Annals of Pharmacotherapy. 2001; 35(12): 1633-1637.

[8] Jennifer JKD and William FM. "Thermoresponsive hydrogels as a new ocular drug delivery platform to the poterior segment of eye". Transactions of the American Ophthalmological Society 2008; 106: 206-214.

[9] Joshi A. Microparticulates for Ophthalmic Drug Delivery. J of Ocular Pharmacology and Therapeutics. Spring 1994; 10(1): 29-45.

[10] Nagarwal RC, Kant S, Singh PN, Maiti P and Pandit JK. Polymeric nanoparticulate system: A potential approach for ocular drug delivery. 2009; 136(1): 2-13.

[11] Nagarsenker MS, Londhe VY and Nadkarni GD. Preparation and evaluation of liposomal formulations of tropicamide for ocular delivery". Int J Pharmaceutics. 1999; 190(1): 63-71.

[12] Yoel G, Guy K. "Use of collagen shields for ocular-surface drug delivery". Expert Review of Ophthalmology. 2008; 3(6): 627-633.

[13] Bloomfield SE, Miyata T, Dunn MW, Bueser N, Stenzel KH, Rubin AL. "Soluble Gentamicin Ophthalmic Inserts as a Drug Delivery System". Archives of Ophthalmology. 1978; 96(5): 885-887.

[14] Vandamme TF, Brobeck L. "Poly [amidoamine] dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide". Journal of Controlled Release 2005; 102(1): 23-38.

[15] Monti D, Saccomani L, Chetoni P, Burgalassi S, Saettone MF. "Effect of iontophoresis on transcorneal permeation 'in vitro' of two β-blocking agents and on corneal hydration". Int J Pharmaceutics 2003; 250(2): 423-429.

[16] Kreuter J. "Nanoparticles. In Colloidal drug delivery systems", J. K. Ed. Marcel Dekker:

New York, 1994; 219-342.
[17] Bucolo C, Maltese A, Puglisi G, Pignatello R. "Enhanced ocular anti-inflammatory activity of ibuprofen carried by an Eudragit RS100 nanoparticle suspension". Ophthalmic Research. 2002; 34(5): 319-323.
[18] Pignatello R, Bucolo C, Ferrara P, Maltese A, Puleo A, Puglisi G. "Eudragit RS100 nanosuspensions for the ophthalmic controlled delivery of ibuprofen". European Journal of Pharmaceutics. 2002; 16(1-2): 53-61.
[19] Castelli F, Messina C, Sarpietro MG, Pignatello R, Puglisi G. "Flurbiprofen Release from Eudragit RS and RL Aqueous Nanosuspensions: a Kinetic Study by DSC and Dialysis Experiments". AAPS PharmSciTech 2002; 3(2): 9.
[20] Pignatello R, Bucolo C, Spedalieri G, Maltese A, Puglisi G. "flurbiprofen-loaded acrylate polymer nanosuspensions for ophthalmic application". Biomaterials 2002; 23(15): 3247-3255.
[21] Pignatello R, Ricupero N, Bucolo C, Maugeri F, Maltese A, Puglisi G. "Preparation and Characterization of Eudragit Retard Nanosuspensions for the Ocular Delivery of Cloricromene". AAPS PharmSciTech 2006; 7(1): 27.
[22] Adibkia K, Siahi Shadbad MR, Nokhodchi A, Javadzedeh A, Barzegar-Jalali M, Barar J, Mohammadi G, Omidi Y. "Piroxicam nanoparticles for ocular delivery: physicochemical characterization and implementation in endotoxin-induced uveitis". J Drug Targeting 2007; 15(6): 407-416.
[23] Adibkia K, Omidi Y, Siahi MR, Javadzadeh AR, Jalali MB, Jaleh Barar, Maleki N, Mohammadi G, Nokhodchi A, Kreuter J. "Nanoparticles. In Colloidal drug delivery systems", JK Ed. Marcel Dekker: New York 1994; 219-342.
[24] Das S, Sureshb PK, Desmukhc R. "Design of Eudragit® RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery". Nanomedicine: Nanotechnology, Biology and Medicine, October 2009. [in press]
[25] Bucolo C, Maltese A, Puglisi G, Pignatello R. "Enhanced ocular anti-inflammatory activity of ibuprofen carried by an Eudragit RS100 nanoparticle suspension". Ophthalmic Research 2002; 34(5): 319-323.
[26] Fessi H, Puisieux F, Devissaguet JP, Ammoury N and Benita S. "Nanocapsule formation by interfacial polymer deposition following solvent displacement". International Journal of Pharmaceutics 1989; 55:R1-R4.
[27] Bilati U, Allémann E and Doelker E. "Development of a nanoprecipitation method intended for the entrapment of hydrophilic drugs into nanoparticles". Europian Journal of Pharmaceutical Science. 2005; 24(1): 67-75.
[28] Galindo-Rodriguez S, Allémann E, Fessi H and Doelker E. "Physicochemical Parameters Associated with Nanoparticle Formation in the Salting-Out, Emulsification-Diffusion, and Nanoprecipitation Methods". Pharmceutical Research 2004; 21(8):1428-1439.
[29] Peltonen L, Aitta J, Hyvonen S, Karjalainen M and Hirvonen J. "Improved Entrapment Efficiency of Hydrophilic Drug Substance During Nanoprecipitation of Poly[l]lactide Nanoparticles". AAPS PharmSciTech 2004; 5(1): 16.
[30] Hornig S, Heinze T, Becer CR and Schubert US. "Synthetic polymeric nanoparticles by nanoprecipitation". Journal of Materials Chemistry 2009; 19: 3838 – 3840.
[31] Govender T, Stolnik S, Garnett MC, Illum L and Davis SS. "PLGA nanoparticles prepared by

nanoprecipitation: drug loading and release studies of a water soluble drug". Journal of Controlled Release 1999; 57(2): 171-185.

[32] Ter VR, Fromell K and Caldwell KD, "Shifts in polystyrene particle surface charge upon adsorption of the Pluronic F108 surfactant". Journal of Colloid and Interface Science 2005; 288(1):124-128.

[33] Mecklenburg W. The relation between the Tyndall effect and the size of the particles of colloidal solutions. Kolloid-Zeitschrift 1915; 16: 97-103.

[34] Zimmer A, Kreuter J. Microspheres and nanoparticles used in ocular delivery systems. Advanced Drug Delivery Reviews. 1995; 16(1): 61-73.

[35] Kelly JW. [PPI05] Parenteral Products- Industrial 05 USP 29-NF 24; 2729.

[36] Das S, Sureshb PK, Desmukhc R. Design of Eudragit® RL 100 nanoparticles by nanoprecipitation method for ocular drug delivery. Nanomedicine: Nanotechnology, Biology and Medicine. 2009 [in press]

[37] Pignatello R, Bucolo C, Spedalieri G, Maltese A and Puglisi G. "Flurbiprofen-loaded acrylate polymer nanosuspensions for ophthalmic application". Biomaterials 2002; 23(15): 3247-3255.

[38] Marchal-Heussler L, Maincent P, Hoffman M, Spittler J and Couvreur P. Antiglaucomatous activity of betaxolol chlorhydrate sorbed onto different isobutylcyanoacrylate nanoparticle preparations. International journal of pharmaceutics 1990; 58(2):115-122.

[39] Cairns D. Essentials of pharmaceutical chemistry. Ed 2; 2003: 75.

[40] Ter VR, Fromell K and Caldwell KD. "Shifts in polystyrene particle surface charge upon adsorption of the PluronicF108 surfactant". Journal of Colloid and Interface Science 2005; 288(1): 124-128.

[41] Xu X, Fu Y, Hu H, Duan Y and Zhang Z. "Quantitative determination of insulin entrapment efficiency in triblock copolymeric nanoparticles by high- performance liquid chromatography". Journal of Pharmaceutical and Biomedical Analysis 2006; 41(1): 266-273.

[42] Peltonen L, Aitta J, Hyvönen S, Karjalainen M and Hirvonen J. "Improved Entrapment Efficiency of Hydrophilic Drug Substance During Nanoprecipitation of Poly[l]lactide Nanoparticles". AAPS PharmSciTech. 2004; 5(1): 16.

[43] Gupta VK, Beckert TE, Deusch NJ and Hariharan M. "Price JC. Investigation of Potential Ionic Interactions between Anionic and Cationic Polymethacrylates of Multiple Coatings of Novel Colonic Delivery System". Drug Development and Industrial Pharmacy 2002; 28(2):207-215.

[44] Passerini N, Albertini B, González-Rodríguez ML, Cavallari C and Rodriguez L. "Preparation and characterisation of ibuprofen–poloxamer 188 granules obtained by melt granulation". European Journal of Pharmaceutical Science 2002; 15(1): 71-78.

[45] Pignatello R, Spadaro D, Vandelli MA, Forni F and Puglisi G. "Characterization of the Mechanism of Interaction in Ibuprofen-Eudragit RL100® Coevaporates". Drug Development & Industrial Pharmacy 2004; 30(3): 277-288.

[46] Heun GN, Lambov N and Gröning R. "Experimental and molecular modeling studies on interactions between drugs and Eudragit® RL/RS resins in aqueous environment". Pharmaceutica Acta Helvetiae 1998; 73(1): 57-62.

[47] Eerikainen H, Kauppinen EI and Kansikas J. “Polymeric Drug Nanoparticles Prepared by an Aerosol Flow Reactor Method”. Pharmaceutical Research 2004; 21(1): 136-143.
[48] Nagendrappa G. “An elegant example of chemoselective reaction”. Resonance 2008; 13(10): 929-940.
[49] Hans ML and Lowman AM. “Biodegradable nanoparticles for drug delivery and targeting”. Current Opinion in Solid State and Materials Science 2002; 6(4): 319-327.
[50] Pignatello R, Consoli P and Puglisi G. “In vitro release kinetics of Tolmetin from tableted Eudragit microparticles”. Journal of Microencapsulation 2000; 17(3): 373-83.