Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2016, 6(3), 345-352
doi: 10.15171/apb.2016.046
http://apb.tbzmed.ac.ir

TUOMS Publishing Group

Research Article

Comparison of Different Nanosuspensions as Potential Ophthalmic Delivery Systems for Ketotifen Fumarate

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Article info

Article History:
Received: 9 November 2015
Revised: 8 July 2016
Accepted: 11 July 2016
ePublished: 25 September 2016

Keywords:
· Ketotifen fumarate
· Nanosuspension
· Transcorneal
· Permeability

Abstract

***Purpose*:** The objective of this study was to develop, characterize, and comparatively investigate the ketotifen fumarate (KF) nanosuspensions (NS_S) to enhance the permeability of KF.

***Methods*:** In the present work, the NS_P and NS_E were prepared by double-emulsion solvent evaporation/nanoprecipitation methods with poly (D,Llactide-co-glycolide) and Eudragit RL100 polymers, respectively. The loading efficiency, particle size, and polydispersity index of prepared different NSs were evaluated with scanning electron microscopy (SEM), X-ray diffraction, differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), and in vitro release and transcorneal permeation . NSs were also compared on the basis of particle size and polydispersity index.

***Results*:** Particle size, polydispersity index, and loading efficiency of NS_{P1} and NS_{E3} showed the best value (158 nm, 117 nm, 0.21, 0.43 and 43%, 95.23%, respectively). SEM showed spherical globules and DSC results showed the reduction in crystallinity. The NS_{E3} formulations demonstrated significantly ($p<0.05$) higher drug release rates than the NS_{P1} due to increases in the surface area. Comparative studies showed that NS_E release and permeability are higher than NS_P.

***Conclusion*:** It is concluded that both NS_P and NS_E provide a useful dosage form for the ocular drug delivery which can enhance the permeability of KF.

Introduction

Eye is the most exclusive organ of the body and a wide range of drug delivery systems are employed to deliver the drug into the eye. Presently, conventional eye drops encompass more than 90% of the marketed ophthalmic formulations. However, after using an eye drop, normally up to 5% of the instilled drug passes the cornea and reaches the intraocular tissues. This happens because of quick and vast precorneal drop loss afforded by blinking and high tear fluid output. To this end, controlled drug delivery to the eye has been suggested as one of the remarkable fields of pharmaceutical research. The major problems associated with conventional systems consist of low drug contact time and poor ocular bioavailability as a result of drainage of drug solution, tear turnover and dilution or lacrimation. Moreover, the anatomical barriers and physiological conditions of the eye are also considerable criteria which dominate designing of drug delivery systems. Numerous novel ocular drug delivery systems such as nanoparticles (NPs), nanoemulsions (NEs), nanosuspensions (NSs) have been developed to achieve higher bioavailability, controlled ocular delivery, patient compliance, and less side effects.[1,2]

NSs and polymeric NPs are more valuable approaches over the current methods in delivering the highly hydrophilic or highly lipophilic molecules across the ocular mucosa. For instance, nanocrystal drug suspensions (NS) entitle an increased dissolution velocity along with saturation solubility of poorly water soluble drugs which is accompanied by an increase in ocular bioavailability.[3]

As a colloidal dispersion of nanosized particles, NSs are stabilized by other excipients like surfactants (as polyvinyl alcohol), viscosity enhancers, or charge modifiers.[4] NSs can also be described as a biphasic system consisting poorly water soluble drug particles dispersed in an aqueous media in which the diameter of the dispersed particles is below 1µm. Size reduction of drug particles conducts to increasing the dissolution rate (enhanced surface area and saturation solubility). The increment in the saturation solubility rate of nanoparticles is related to increase of vapour pressure of the particles.[5] A nanosuspension formulation like this can be prepared by pearl milling, high-pressure homogenization, and precipitation techniques.[6,7]

The precipitation method is the most currently used technique in which the drug is solved in an organic solvent

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and this solution is admixed with a miscible anti-solvent. In this method, mixing leads to precipitation of drug in the solution, and producing a very fine amorphous or crystalline drug. Precipitation has also been accompanied with the high shear proceeding.[5,8] Several NS formulations have been developed and successfully used for topical ocular drug delivery.[9,10]

Kassem *et al*. formulated NSs for prednisolone, hydrocortisone, and dexamethasone for topical ocular delivery and evaluated them.[11] Studies on the *in vivo* tissue distribution of the glucocorticoid NSs certified remarkably higher levels in anterior chamber tissues in comparison with solution and microcrystalline suspension of similar compounds.[12]

Aksungur *et al*. demonstrated NPs of cyclosporine (CsA) loaded PLGA and /or Eudragit RL-100 and PLGA coated with Carbopol for intensive dry eye syndrome therapy. The ultrafine NPs were supplied with Eudragit RL polymer. It was obtained that the NPs size reduction with Eudragit RL concentration increasing resulted from physicochemical characteristics of the polymer.[13] Mandal *et al.* showed that cloricromene loaded Eudragit RL100 polymeric NPs enhance the ocular bioavailability. They suggested cloricromene-loaded NPs system for clinical trial.[14]

Gupta *et al.* supplied PLGA nanoparticles containing sparfloxacin for ophthalmic delivery using nanoprecipitation technique and showed modified precorneal residence time and ocular penetration for NPs. The improved lyophilized NPs were stable for longer period of time than traditional commercial formulation.[15]

The use of ketotifen fumarate (KF) for the treatment of allergic conjunctivitis behaves as a histamine H1-receptor antagonist, mast cell stabilizer, and eosinophil inhibitor in that it decreases the chemotaxis and activation of eosinophils. Eudragit RL 100 polymers are referred to as ammonium methacrylate copolymers, which are synthesized from acrylic acid and methacrylic acid esters with 10% of functional quaternary ammonium groups.[16] Biodegradable poly (DL-lactic-co-glycolic acid) (PLGA) copolymers have been broadly used as carriers of bioactive molecules.[17] The biocompatibility and biodegradability of PLGA have been proved, and also approved by the FDA for specific human clinical applications.[18] Polymeric carrier systems using Eudragit and PLGA have been investigated for the ophthalmic release of gentamicin,[19] cloricromene,[20] acetazolamide[21] and non-steroidal anti-inflammatory drugs such as ibuprofen.[22] These carrier systems showed good stabilizing properties and narrow size distribution.

NSs of KF may overcome the problems observed in conventional drops. These nanocarriers may prolong the corneal contact time (higher bioavailability), controlled ocular delivery, rapid penetration of active ingredients, patient compliance, and ocular effect of KF.

The preparation of KF-loaded NS systems and evaluating the effect of polymer type and composition of formulations on the nanocarriers formation have targeted in this study. The feasibility of using the KF-loaded nanoparticulate system as an ocular formulation was demonstrated through extensive characterization of the size, charge, loading efficiency, drug release, and transcorneal permeability.

Materials and Methods

Materials

For this study, the KF was supplied by Behansar Co. (Iran). Eudragit RL 100 was kindly a gift from Akbarie Co. (from RÖhm Pharma GMBh, Weiterstadt, Germany). PLGA polymer Resomer® 502 H (MW 7000-17000) was purchased from Sigma-Aldrich (Sigma-Aldrich Co. US). Polyvinyl alcohol (MW 72000), D-mannitol, dichloromethane (DCM), ethanol, sodium chloride, calcium chloride, and potassium chloride were obtained from Merck (Germany). All solvents and reagents were of analytical grade. Commercial eye drop (Zaditen®, 0.025%) was purchased from Thea pharma (France).

Methods

Preparation of KF-NS_P and KF-NS_E

Two nanocarriers, NS_P (Nanosuspension of PLGA polymer) and NS_E (Nanosuspension of Eudragit RL100 polymer) were produced using PLGA and Eudragit RL 100, described polymers.[23] Briefly, NS_E and NS_P were prepared by nanoprecipitation method and double emulsion solvent evaporation technique ($W_1/O/W_2$) at different drug to polymer ratios, respectively (Table 1).

Table 1. Selected preparation parameters and their investigated range

Preparation parameter	Selected formulation		Investigation range	
	NS_{P1}	NS_{E3}	NS_P	NS_E
Polymer type	PLGA	Eudragit RL100	PLGA	Eudragit RL100
Drug to polymer ratio	1:5	1:15	1:5 - 1:10	1:7.5 - 1:15
Amount of drug (mg)	10	10	10	10
Concentration of polymer (mg/ml)	5	12.5	5-10	6.25-12.5
PVA (1%w/v)/ NaCl (0.8 %w/v) (ml)	25	-	25	-
PVA (1%w/v) (ml)	-	25	-	25
Theoretical drug content (%)	16.67	6.25	9.10-16.67	6.25-11.76
Mean drug entrapped (%±SD)	10.58±0.85	9.52±6.35	4.07-10.58	9.39-9.52
Drug loading efficiency (%±SD)	43.00±8.00	95.23±8.45	43-55	93.95-95.23
Mean particle size (nm)	158±2.24	117±16.00	158-754.6	117-182
Zeta Potential (mV±SD)	-3.30±3.21	+13.40±0.28	-3.30 - -2.99	+6.58-13.40
Polydispersity Index (±SD)	0.21±0.29	0.43±0.18	0.21-0.83	0.34-0.73

Formulation of KF-NS_P: An aqueous 0.5 % w/v KF solution was added to 10 ml PLGA in organic solvent (dichloromethane) by using an ultrasound probe (Hielscher, UP200H, amplitude 80%) in an ice bath for 3 min. This solution was added drop by drop using syringe needle to 25 ml aqueous phase of PVA (1%w/v), NaCl (0.8% w/v) and sonicated for 3 min. Then this emulsion was diluted in 50 ml distilled water. The organic solvent was allowed to evaporate at room temperature under magnetic stirring and NS_P were collected by centrifugation (Eppendorf, Centrifuge 5810 R, Germany) at 12000 rpm, 4°C for 60 min and washed and freeze-dried .

Formulation of KF-NS_E: KF and Edragit RL 100 were dissolved in 12 ml ethanol. The solution was mixed with 25 ml of 1% w/v PVA aqueous solution using ultrasound probe (Hielscher, UP200H) for 3 min. Then the mixture of drug and polymer was diluted in 50 ml distilled water. Finally, the resulted nanosuspension was stirred at room temperature to extract the organic solvent. NS_E was separated under the same conditions of NS_P. Prepared NPs were mixed with 10ml 5%w/v mannitol solution as a cryoprotectant and then lyophilized.

Characterization of NS_P and NS_E

Particle size and zeta potential

Particle size and zeta potential of freshly prepared NS_P and NS_E were determined by Dynamic Light Scattering (Malvern, UK) using a Zetasizer. The zeta potential is used to measure the electric charge at the surface of the particles, showing the physical stability of colloidal systems. For this study, the formulated NS_P and NS_E were diluted with distilled water. Visual observations were made immediately after dilution for evaluation of NS_P and NS_E efficiency, appearance (transparency), phase separation, and precipitation of drug. The polydispersity index of the resulting NS_P and NS_E were determined by dynamic light scattering with Zeta sizer.

Morphology

The outer macroscopic structure of NS_P and NS_E were investigated by scanning electron microscopy (SEM). SEM (MIRA3 TESCAN, Czech Republic) was used to examine the surface morphology of Eudragit and PLGA nanoparticles. The samples were stationed on a metal stub with a double adhesive tape and coated with the platinum/palladium alloy under the vacuum.

Drug loading and production yield of NS_P and NS_E

To determine the amount of drug loaded in prepared nanocarriers, NS_P and NS_E, the supernatant was UV analyzed for the unloaded drug at wavelength 298 nm. Calibration curve was performed by means of KF in 1% PVA aqueous solution. The drug loading efficiency was determined using the following equations:[24]

$$\text{Drug loading efficiency} = \frac{\text{Total amount of drug} - \text{unincorporated drug amount}}{\text{Total amount of drug}} \times 100$$

Evaluation of physical state and polymer-drug interaction of NS_P and NS_E

Physical state and polymer-drug interaction of NS_P and NS_E were examined by XRD, DSC and FTIR analyses.

X-ray diffraction (XRD): XRD analysis was performed using Bruker Axs, D8 Advance diffractometer with nickel-filtered CuKα radiation (operating at 40KV, 20mA). The scanning rate was 4 °C/min over a 2θ range of 10°-90°.

Differential scanning calorimetry: Differential scanning calorimetry (DSC) (Shimadzu, Japan) measurements were carried out on drug, polymers and different formulations. The weighed samples were put in aluminum pans and scanned for 30°C-300°C with heating rate of 10°C/min.

Fourier transform infrared spectroscopy: The Fourier transform infrared spectroscopy (FT-IR) spectra for KF loaded nanocarriers, blank NPs, polymer and drug were obtained by a computerized FT-IR (Bruker, Tensor 27, and USA) operating in the scanning wavenumber range of 400-4000 cm^{-1} at 1 cm^{-1} resolution.

In vitro release study

In vitro release

In vitro release experiments were performed on the NS_P and NS_E using dialysis bag diffusion method.[20] Fifty milligrams of particles were suspended in 4 ml SLF (simulated lacrimal fluid) buffer (pH 6.8) in the dialysis bag (cutoff 12,000 Da), which was immersed in 300 ml of the same buffer as dissolution medium. The medium was preheated to 32±1°C and stirred at 100 rpm. At preset intervals, 3.5 ml of medium were withdrawn and replaced with 3.5 ml of fresh SLF to keep the sink condition. The amount of KF in the samples was determined by UV spectrophotometer (Shimadzu, Japan) analysis at wavelength 298 nm. The experiments were repeated for each formulation in triplicate.

Ex-vivo transcorneal permeation studies

The *in vitro* permeation study of the KF-loaded NS_P and NS_E through the bovine cornea was performed using Franz diffusion cell at 32 °C. Freshly obtained scleral layer was mounted between the donor and the recipient compartments. The nanocarriers suspended in 5 ml distilled water were placed on the epithelial faced surface and the compartments were clamped together. The NS_P and NS_E was stationed on the cornea, and the opening of the donor compartment was sealed with a glass coverslip and soaked with simulated lacrimal fluid (SLF, composition: 8.3 g of NaCl, 0.084g of CaCl2·2H 2O, 1.4g of KCl, and distilled deionized water to 1000 mL).
The recipient compartment was filled with 22-25 ml SLF at pH 6.8 and stirred with a magnetic bead at 200 rpm.[25] Three milliliters of the sample were withdrawn at predetermined time intervals and analyzed for drugs at 298 nm.

Permeability coefficient was calculated using the following equation:

$$Kp = \frac{Jss}{C_0}$$

Where Jss is the steady state flux per unit area, Kp is the permeability coefficient for a given solute in a given vehicle (cm h^{-1}), and C_0 is the concentration of the solute in the donor compartment.

Statistical analysis

Where appropriate, all results were evaluated using a one-way ANOVA or t-test at the 0.05 level of error.

Results & Discussion

The composition of NS_P and NS_E formulations are listed in Table1, where the amount of the different compounds is expressed as % (w/w). As shown in the table, NS_P was prepared by using PLGA and DCM as the organic phase, PVA as stabilizer and sodium chloride as osmosis pressure agent.[26] NS_E was produced by using Eudragit RL100 and ethanol as organic phase and PVA as emulsifier. Both formulations, NS_P and NS_E, were loaded with the same amount of KF (10 mg) and stabilized with the same surfactant in the same concentration (1% w/v).

In the NS, water was used as an anti-solvent whereas polyvinyl alcohol was used as the surfactant. Nanoprecipitation occurs at the interface of the organic phase (ethanol) and anti-solvent phase (water) due to diffusion of the solvent by forming local disturbances followed by precipitation of nanoparticles which were governed by surfactant system.

Under the sonication, the mixture of ethanolic was injected into the aqueous phase so the nanosuspension (polymeric solutes become aggregated to produce nanosized particles) was formed by precipitation with diluted organic solution in the aqueous phase leading to the production of nanoparticles. The sonication was operated for several minutes to let the system reach equilibrium. The solvent displacement method for fabrication of NS was adopted from the nanoprecipitation method applied for polymeric nanoparticles. The organic phase (O) was poured into an aqueous phase (W) containing a surfactant to yield nanosuspension.[4]

The highest loading efficiency for NS_P and NS_E formulations were 43% and 95.23%, respectively. The results showed that the drug was uniformly distributed throughout the NS_E formulations (Table 2).

Table 2. Comparison of various release characteristics, flux and permeability coefficient of KF from different NPs formulations and commercial® drop

Formulation code	[a]$Rel_{0.25}$ (%)	[b]Rel_8 (%)	[c]DE	[d]$T_{50\%}$ (min)	[e]f_1	[f]Flux (mg/cm^2.min)*10^{-3}	[g]Kp (cm/min) *10^{-4}
NS_{P1}	15.81±3.04	82.57±4.87	74.49	248.84	44.68	0.2	5.82
NS_{P2}	12.30±0.52	65.15±7.75	62.72	352.61	54.72	0.06	3.44
NS_{P3}	10.67±0.26	58.77±8.53	54.04	383.74	63.38	0.06	4
NS_{E1}	30.67±1.54	65.51±4.10	61.28	93	49.24	0.6	10.7
NS_{E2}	55.74±5.28	80.31±3.48	77.77	45.64	28.19	0.6	16.78
NS_{E3}	65.14±1.44	88.82±3.33	85.88	46.61	20.22	0.5	17.30
KF drop®	97.77±0.00	101.62±1.81	101.03	8.31	0	0.1	28.99

[a] $Rel_{0.25}$ = amount of drug release after 0.25 h; [b] Rel_8 = amount of drug release after 8 h; [c]DE = dissolution efficiency; [d]t 50% = dissolution time for 50% fractions; [e] f_1 = Differential factor ($0<f_1<15$), [f]flux and [g]permeability coefficient.

Particle size diameter (Z-Ave), polydispersity index (PI), and zeta potential of NS_P and NS_E were determined just after preparation (reported in Table 1). Freshly prepared NS_{P1} showed a Z-Ave of 158 nm (0.21 PI) and a zeta potential of −3.30 mV while freshly prepared NS_{E3} presented a Z-Ave of 117 nm (0.43 PI) and a zeta potential of +13.40 mV. As can be observed, NS_P and NS_E, showed negative and positive zeta potential values, respectively. The positive zeta potential of NS_E may a longer residence time of NPs on the corneal surface. As shown in Table 2, freshly prepared NS_{P1} was well homogeneously dispersed with reduced particle size and PI, compared to the NP_{E3} that was briefly homogeneously dispersed. However, Table 2 clearly shows that NS_E was less stable than the NS_P. The low stability of this formulation (NS_E) was also confirmed by the increasing of polydispersity index value from 0.34 to 0.73. On the basis of PDI, we found that KF-loaded NS_P was better than KF-loaded NS_E. A higher value of polydispersity index indicates a broad particle size distribution.[27] NS_P and NS_E data were confirmed by SEM images (Figure 1).

SEM is used to assess the microscopic surface morphology of the formulations. Prepared formulations were present in the form of a rough surface which might have led to the enhanced dissolution rate.[27] Moreover, the particle showed a satisfactory regular spherical shape in the case of NS_P, which is probably the reason for its best results even in the dissolution.

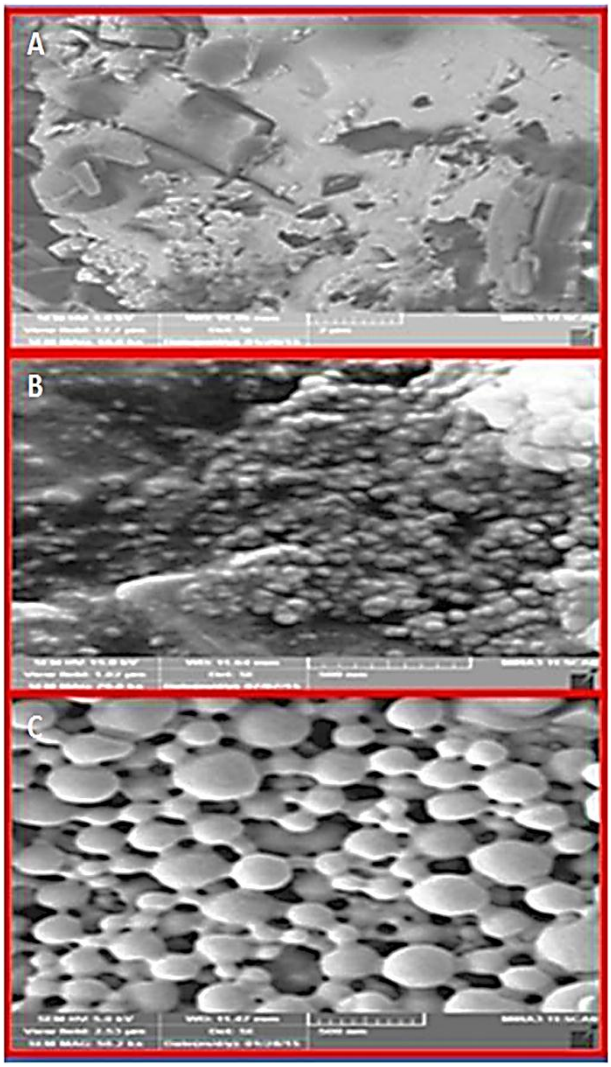

Figure 1. SEM images of KF (A); NS_{E3} (KF:EU) 1:15 ratio (B); NS_{P1} (KF:PLGA) 1:5 ratio (C) at 1000× magnification.

The influence of preparation method on the KF degree of crystallinity and melting point was evaluated by DSC characterization of NS_P and NS_E. The freeze dried drug loaded NS_E exhibited a sharp melting endotherm at an onset temperature of 197.69 °C, a peak temperature of 201.09 °C, and a heat of fusion of 176.14 J/g (Figure 2). The freeze dried drug loaded NS_E showed a broad endothermic transition at an onset of 216.68 °C, a peak at an onset of 197.93°C, and a peak at 213.96˚C (from F_1 to F_3). Eudragit RL 100 and PLGA polymers are found as an utterly amorphous form with a glass transition temperature (Tg) of about 60°C.[28] No fusion peak or phase transition was observed in the amorphous polymer, apart from a broad signal around 55–60°C owing to a partial loss of residual humidity.[29] The thermal behavior of the freeze dried NPs proposed that the polymer prevented the melting of drug crystals. The ionic interaction may have occurred in the NPs as observed for the KF and Eudragit RL 100 system.[30] However, the NPs of KF shows drug melting peak. The thermal profile comparison between NS_P and NS_E KF confirmed that the solid state transition that occurred during NS preparation did not influence the drug behavior.

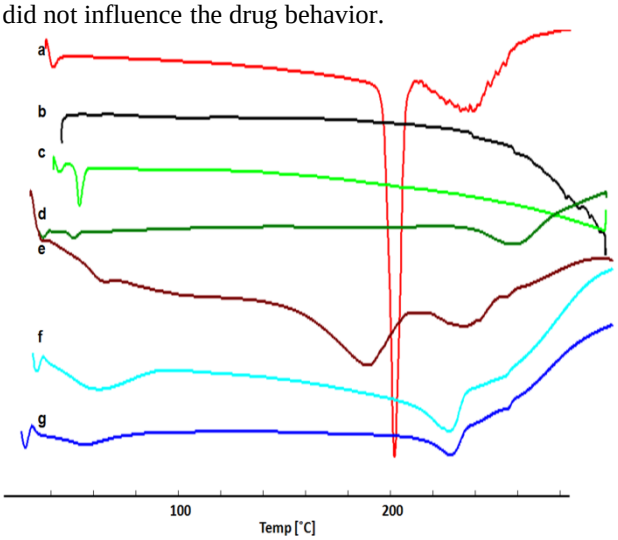

Figure 2. DSC thermogram of KF (a); PLGA (b); NS_{P1} (KF:PLGA) 1:5 ratio (c); blank NS_{P1} (d); Eudragit RL100 (e); NS_{E3} (KF:EU) 1:15 ratio (f); blank NS_{E3} (g), respectively.

In the XRD, spectra are obvious and the NS_P with lower polymer concentration would show similar peaks as the blank NS_P. For NS_P, some of the identifying peaks for KF are detectable at a high concentration of polymer; though these peaks hold very low intensity due to the presence of lower concentration of drug in the sample compared to pure KF sample (Figure 3). Eudragit RL polymer is completely amorphous in nature, and entrapment of crystalline KF (sharp intense peaks as seen in Figure 3) into the polymeric NS_E reduced its crystallinity to a greater extent. This is evident from the disappearance of most peaks in the NS_E compared to the drug. There may also be the possibility of overlapping of drug peaks by the background diffraction pattern of the amorphous structure.

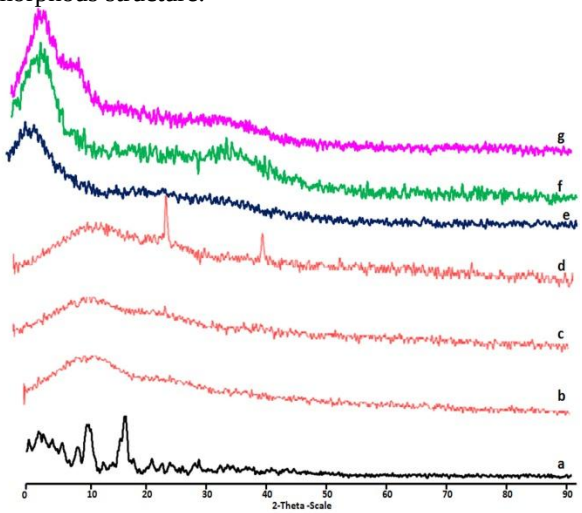

Figure 3. XRD thermogram of KF (a); PLGA (b); NS_{P1} (KF:PLGA) 1:5 ratio (c); blank NS_{P1} (d); Eudragit RL100 (e); NS_{E3} (KF:EU) 1:15 ratio (f); blank NS_{E3} (g), respectively.

The FT-IR spectrum of KF alone showed that the principal peaks were observed at wave numbers

stretching vibration N-H at 3424.64 cm^{-1}, aromatic stretching vibration C=C at 1649.70 cm^{-1}, bending vibration CH3 at 1476.99 cm^{-1}, bending vibration phenolic OH at 1397.14, and CH out of plane bending vibrations in substituted ethylenic system (-C=CH- (cis) at 754.15 cm^{-1} (Figure 4). The spectra obtained by FT-IR for the PLGA are presented in Figure 4. The strong bands in the region between 1760 and 1750 cm^{-1} could be observed, in the spectra, due to the stretch of the carbonyl groups within the PLGA. Moreover, stretching bands are observed because of asymmetric and symmetric C-C(=O)-O vibrations between 1300 and 1150 cm^{-1}. The presence of bands in these regions is of benefit in the characterization of esters. The 3525 and 3459 cm^{-1} bands in the FT-IR spectra for lactide and glycolide are ascribed to moisture in the sample (OH group). The absorption bands between 3600 and 3400 cm^{-1} in the spectra presented in Figure 4, showing the hydroxyl group, indicate that the PLGA copolymers are hydrous. FT-IR studies showed characteristic peaks of KF, confirming the purity of the drug. For NS_P, stretching vibration N-H is seen at 3400-3423, a stretch of the carbonyl groups at 1760, asymmetric and symmetric C-C(=O)-O vibrations at 1390 and bending vibrations in substituted ethylenic system (-C=CH- (cis) at 725-752 cm^{-1}.

For Eudragit RL 100, in the spectra, the strong bands are observed in the region between 1150-1190 cm^{-1} and 1240-1270 cm^{-1}, due to the stretch of carbonyl (ester) groups present in the Eudragit (Figure 4). There are also stretching bands in view of the C (=O) ester vibration at 1734.01 cm^{-1}. The 1388.22, 1449.97, 2953, and 2992.11 cm^{-1} bands in the FT-IR spectra can be discerned to CHx vibration. IR absorption frequency at 3437.91cm-1 (OH stretch) presented in Figure 3 and showing the hydroxyl group, indicates that the Eudragit RL100 is hydrous.[29,31]

FT-IR spectral studies showed that there was an interaction between KF and polymers used. For NS_E, stretching strong band C-H (alkyne group) are seen at 3293.88-3298cm^{-1}, stretch band strong C-H (alkane group) at 2936.61, 2937.50 and 2939.24 cm^{-1}, N-H stretch band of the amine group at 3000 cm^{-1}, stretch band of carbonyl group at 1728.54, 1728.79, and 1729.01 cm^{-1}, bending vibrations in -C-H at 1436.12, 1436.62, and 1438.91 cm^{-1}, stretch band of ester group C-O at 1089.41, 1089.61, and 1090.74 cm^{-1}, and stretch band in -C-Cl at 844.76, 845.12, and 845.57 cm^{-1} (Figure 4). The available differences in the positions of the absorption bands of KF were seen in spectra of the prepared formulations, proving the presence of chemical interactions in the solid state between the drug and the polymers (PLGA and Eudragit RL100).

In vitro dissolution studies

In vitro dissolution data of all best formulations (NS_P and NS_E) were compared together. The NS_P formulation showed that 82.57% of drug was released in 480 min (NS_{P1}). NaCl increased the solubility of drug by entrapping the KF in the network interstitial spaces of NaCl molecule and also reducing the particle size. In NSs, increasing the osmotic pressure of W_2 (external phase of second emulsion) directs water migration from W_1 to W_2 as well as a rapid shrinkage of the droplets. This phenomenon results in smaller nanoparticles and increases drug release.

In the NS_E formulation 88.82% drug was released in 480 min (NS_{E3}). Increasing the dissolution kinetics of KF from NS_E may be due to the conversion of the drug from crystalline to amorphous state. Also presence of surfactant (PVA) and co-surfactant (ethanol) in NS_E reduces the interfacial tension and helps to solubilize the drug in the formulation of NS_E. According to the literature, the drug release amount and behavior as well as the drug absorption are influenced by the particle size. The particle size of NS_{E3} (117 nm) and NS_{P1} (158 nm) are the smallest which may be the reason for their highest releases (Figure 5).

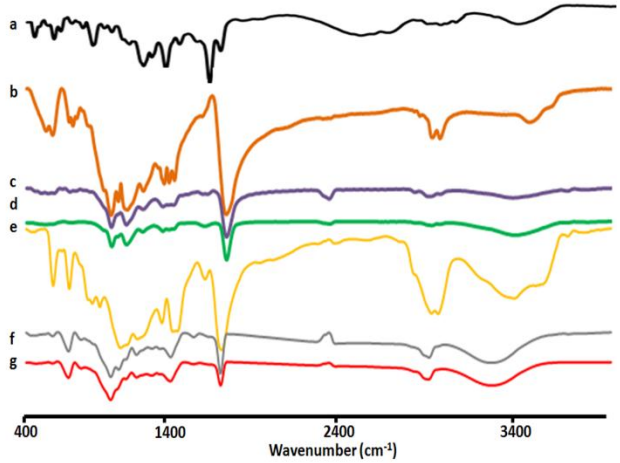

Figure 4. FT-IR thermogram of KF (a); PLGA (b); NS_{P1} (KF:PLGA) 1:5 ratio (c); blank NS_{P1} (d); Eudragit RL100 (e); NS_{E3} (KF:EU) 1:15 ratio (f); blank NS_{E3} (g), respectively.

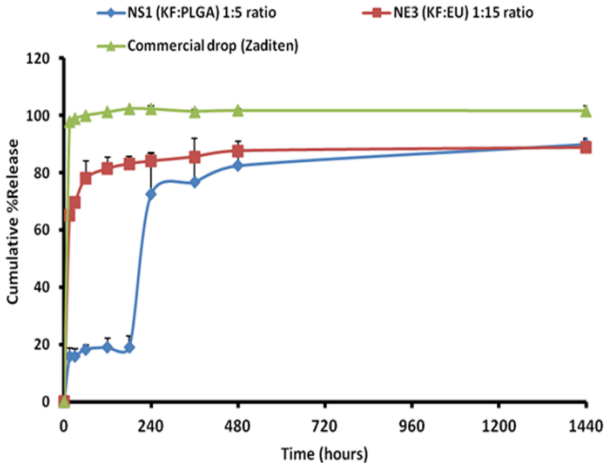

Figure 5. Cumulative percent release of KF from naoparticles with different polymer ratios and KF commercial drop.

KF delivery into and through the bovine cornea was evaluated in *ex vivo* conditions, by the use of Franz vertical diffusion cells. During this study, transcorneal

delivery of prepared KF nanosuspensions was compared with commercial (Zaditen®) eye drop as control. Comparison of data obtained from NS_P and NS_E (Table 2) highlights a different KF delivery into and through the bovine cornea. As expected, the NS_E showed a higher drug permeation and transcorneal delivery than the NS_P. However, differences of drug permeability in two types of formulations (as NS_P and NS_E) were statistically significant (p <0.05). Comparison of data obtained from NS_P and NS_E underlines the influence of different formulations on the *ex vivo* drug availability; NS_E is useful for improving the transcorneal delivery. NS_E is able to favor KF permeation into the eye and at the same time to prolong the contact time with the cornea and increase the efficacy of drug delivery.

In NSs, solid drug is dissolved in the vehicle (lacrimal fluid) and diffuses through the vehicle to the cornea. On the other side, when a nanocarrier is applied onto the eye, two consecutive physical events may limit corneal absorption, namely, the drug release (from nanocarrier) into lacrimal fluid and its penetration through the corneal barrier. These two processes are intimately intertwined, and both are due to the physicochemical properties of drug (type of nanocarrier) and barrier.

The degree of partitioning of the drug into the cornea relies on the relative affinity for the vehicle and for the intercellular environment. In the present investigation, the higher drug permeability may be due to the polymer type (Table 2), surfactant, and the method of preparation, which taken together act as penetration enhancers.[32,33] In addition, as shown for NS_E, the small particle size of the NS_E (in comparison with NS_p) makes it an excellent carrier for promoting *ex vivo* corneal KF permeation. Overall results show that NS_E is suitable nanoparticles for corneal delivery of KF. NSs are almost exclusively formed from drug nanoparticles with small amounts of biocompatible and safe surfactants, such as PVA used in this work. This leads to a highly fast dissolution process that favors drug penetration into the cornea. Moreover, compared to other colloidal carriers, NSs show extra advantages such as simplicity, biodegradability of polymer (PLGA), and scalable preparation methods.[34,35]

Conclusion

On the whole, this work showed the high potential of NS_P and NS_E in ocular drug delivery of KF. Indeed, NS_s has been established to be able to localize the drug into the cornea *ex vivo*. Besides, the NS_P was shown to give comparable ocular KF delivery as the NS_E, which strongly enhanced *in vitro* ocular drug delivery. Furthermore, the application of NS_E in ocular KF delivery showed the advantage of increasing permeability and retention time of the drug in comparison with the NS_P. To conclude, results of this work evinces that NS_E formulation approach could be a potentially valuable tool of use in the design of new KF nanomedicines for the treatment of eye diseases.

Acknowledgments

The authors report that they have no conflicts of interests. The financial support from the Drug Applied Research Center of Tabriz University of Medical Sciences under the grant No. 42 is greatly acknowledged. Mitra Jelvehgari received the research grant from Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Ethical Issues

Not applicable.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this article.

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