RESEARCH ARTICLE

Formulation and evaluation of dorzolamide hydrochloride-loaded nanoparticles as controlled release drug delivery system

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This study aimed to prepare anti-glaucomatous dorzolamide hydrochloride-(Dorzo) loaded nanoparticles as a controlled release system. Eudragit RS 100 (RS) and/or RL 100 (RL) were used in formulations by an opportunely adapted Quasi-emulsion solvent diffusion technique. The formulations were evaluated in terms of particle size, zeta potential, drug entrapment, and release profile. All formulations showed tiny particle size varying from 114 to 395 nm for RS and 65 to 277 nm for RL. Positive zeta potential was +19 to +32 mV for RS and +23 to +42 mV for RL formulations. It was demonstrated that increasing polymer concentration lead to increase the percentage of drug entrapped in all batches, to a certain extent (drug: polymer 1:4). Nanoparticles prepared using RL showed lower entrapment efficiency than RS. In contrast, increasing the stirring rate resulted in an increase in the percentage of Dorzo entrapped. A prolonged drug release was shown by all the formulations. Increasing the polymer concentration caused a decrease in the release rate. Moreover, it was evident that increasing RL content increased the amount of Dorzo released. Dorzo-loaded nanoparticles could represent promising drug ophthalmic carriers, due to small particle size, positive zeta potential, and sustained release profile; hence, expecting prolonged corneal contact time, more therapeutically efficient, decreased frequency of administration per day, and better patient compliance.

***Key words*:** *Dorzolamide hydrochloride, eudragit, nanoparticles, ocular drug delivery*

INTRODUCTION

Glaucoma, the second most common cause of blindness in the world, has been observed to spread increasingly with increasing age. Elevated intraocular pressure (IOP) appears to be the main mechanism that leads to optic neuropathy. At the current time, there is no curative treatment that could reverse the pathogenesis of optic nerve atrophy secondary to chronic elevation of IOP. Medical or surgical treatment to lower IOP by even a few mm Hg can slow progression of the disease.[1,2]

Carbonic anhydrase (CA) is responsible for generation of bicarbonate anions secreted by the ciliary process into the posterior chamber of the eye. Inhibition of CA results in reduction of IOP.[3] Dorzolamide hydrochloride [(4S-*trans*)-4-(ethylamino)-5,6-dihydro-6-methyl-4H-thieno[2,3-b]thiopyran-2-sulfonamide 7,7-dioxide monohydrochloride; Dorzo] is a CA inhibitor used in the treatment of glaucoma. Dorzo has been shown to be topically active and 20 times more potent than acetazolamide in lowering IOP.[4]

Efficient ocular drug delivery remains a challenge for pharmaceutical scientists. The majority of ocular disorders are treated by topical drug preparations in the form of solutions, suspensions, and ointments. Unfortunately, these conventional forms have a disadvantage of poor ocular bioavailability, because of various anatomical and patho-physiological barriers present in the eye.[5,6] The anti-glaucoma therapy requires continued administration of a drug; so,

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controlled release system is needed in order to permit a lowering of the dose over time and to enhance the effect.

The development of various nanoparticulate-based drug delivery systems, like nanoparticles, nanoemulsions, nanosuspensions, liposomes, dendrimers, niosomes, cyclodextrins, and others can enhance the rate of ophthalmic drug delivery.[5,7,8] These submicron particles are better than conventional ophthalmic dosage forms to enhance bioavailability without blurring the vision. In addition, drug-loaded nanoparticles have the ability of targeting the site of action, leading to a decrease in the dose required and side effects.[7,9-12]

Eudragit RS 100 (RS) and Eudragit RL 100 (RL) are biocompatible co-polymerizates based on esters of acrylic and methacrylic acids with a low content of quaternary ammonium groups. The ammonium groups are present as salts, and they are responsible for permeability, which is independent of pH in the physiological region. The molar ratio of these hydrophilic components to the other neutral methacrylic acid esters is 1/20 for Eudragt RL and 1/40 for Eudragit RS.[13] Polymeric nanosuspensions prepared from RL and RS have been investigated extensively for the ocular delivery of ibuprofen,[14,15] flurbiprofen,[16,17] cloriocromene,[18] piroxicam,[19] methyl prednisolone,[20] and amphotericin B.[8,21] These literatures showed that nanoparticles prepared from RS and RL were simple to manufacture and exhibited an excellent tolerance when topically administered, devoid of any irritant effect on cornea, iris, and conjunctiva, and thus appeared to be a suitable inert carrier for ophthalmic drug delivery. Also, the positive zeta potential and fine particle size helped to prolong the corneal contact time.

Our study aimed to formulate topical nanoparticle preparations of Dorzo with sustained release effect in the target area and to evaluate the *in vitro* performance of these preparations compared to free drug solution.

MATERIALS AND METHODS

Materials

Eudragit® RS 100 (RS) and RL 100 (RL) were kindly supplied by Evonic RÖhm GmbH, Darmstadt, Germany. Dorzolamide hydrochloride was a gift from Jamjoom Pharma, K.S.A. All other chemicals and solvents were of analytical grade and obtained from Sigma-Aldrich Chemicals.

Preparation of nanoparticles

All Dorzo-loaded nanoparticles were prepared from Dorzo (2.22% w/w), Eudragit RS or Eudragit RL in different drug / polymer ratios w/w [Tables 1 and 2]. Benzalkonium chloride was added as a preservative in all prepared formulations in a concentration of 0.01% w/w. Sodium hydroxide was added to the final preparations to adjust pH (approximately 5.6).

Nanoparticles were prepared by an adaptation of the Quasi–emulsion solvent diffusion method (QESD).[22-24] Drug and polymer were co-dissolved at room temperature in 4 ml of ethanol. Ethanol solution produced by co-dissolving the drug and the polymer was perfectly clear. Since Dorzo is slightly soluble in ethanol, the presence of polymers aided the dissolution of the drug.

The solution was slowly injected (0.5 ml / min) with a syringe connected to a thin teflon tube, in 10 ml of water containing tween 80 (0.02%, w/v) as a hydrophilic emulsifier in a cylindrical vessel maintained at low temperature by means of an ice-cooled water bath to avoid a rapid ethanol evaporation. During injection, the mixture was stirred at 10,000, 20,000, and 24,000 rpm by a mechanical stirrer (an Ultra-Turrax T 25, Ika Labortechnik, Staufen, Germany). Stirring was kept at the same rate for 15 min. The solution immediately turned into a pseudo-emulsion of the polymer ethanol solution in the external aqueous phase. The counter diffusion of ethanol and water out of and into the emulsion micro droplets, respectively, and the gradual evaporation of the organic solvent determined the

Table 1: Properties of Dorzo- loaded RS nanoparticles. Stirring rate was 10,000 rpm, 20,000 rpm and 24,000 rpm, for batches A, B and C, respectively

Batch code	Dorzo versus RS (w/w)	Mean size, nm (Z average)	Polydispersity index	Zeta potential, mV	Entrapment efficiency%
A1	1 : 1	113.5 ± 7.7	0.19 ± 0.2	32.2 ± 1.8	50.0
A2	1 : 2	134.5 ± 3.4	0.75 ± 0.4	20.7 ± 1.8	69.9
A3	1 : 4	160.4 ± 2.7	0.56 ± 0.6	22.5 ± 1.6	73.6
A4	1 : 9	192.9 ± 8.2	0.67 ± 0.5	26.4 ± 1	72.5
B1	1 : 1	221.9 ± 6.4	0.41 ± 0.3	19.3 ± 1.6	61.0
B2	1 : 2	230.9 ± 2.2	0.26 ± 0.3	28.2 ± 1.5	75.5
B3	1 : 4	278.9 ± 5.1	0.70 ± 0.8	25.8 ± 2	78.5
B4	1 : 9	318.0 ± 3.7	0.42 ± 0.4	24.8 ± 3	77.4
C1	1 : 1	301.4 ± 1.9	0.49 ± 0.2	19.3 ± 1	73.0
C2	1 : 2	315.9 ± 8.7	0.38 ± 0.1	22.7 ± 12	89.5
C3	1 : 4	368.8 ± 2.9	0.49 ± 0.7	28.5 ± 6	92.5
C4	1 : 9	393.6 ± 7.5	1 ± 0.2	23.3 ± 1	83.6

Table 2: Properties of Dorzo- loaded RL nanoparticles. Stirring rate was 10,000 rpm, 20,000 rpm and 24,000 rpm, for batches A, B, and C, respectively

Batch code	Dorzo versus RL (w/w)	Mean size, nm (Z average)	Polydispersity index	Zeta potential, mV	Entrapment efficiency%
A5	1 : 1	64.6 ± 2.7	0.95 ± 0.20	22.9 ± 0.33	41.0
A6	1 : 2	79.0 ± 5.6	0.81 ± 0.25	30.5 ± 0.52	61.0
A7	1 : 4	95.0 ± 8.2	0.53 ± 0.22	35.3 ± 0.60	67.9
A8	1 : 9	120.3 ± 7.7	0.59 ± 0.12	40.9 ± 0.23	64.4
B5	1 : 1	166.1 ± 9.7	0.38 ± 0.10	34.4 ± 0.76	55.0
B6	1 : 2	172.8 ± 7.8	0.64 ± 0.28	31.1 ± 0.50	68.5
B7	1 : 4	190.0 ± 4.2	0.81 ± 0.28	40.2 ± 0.26	72.8
B8	1 : 9	211.1 ± 12.2	0.45 ± 0.50	41.7 ± 0.24	71.2
C5	1 : 1	210.0 ± 8.9	0.43 ± 0.18	26.3 ± 0.21	64.0
C6	1 : 2	226.0 ± 8.9	0.71 ± 0.30	38.3 ± 0.88	77.0
C7	1 : 4	246.0 ± 9.6	0.48 ± 0.81	37.5 ± 0.66	82.0
C8	1 : 9	277.0 ± 2.4	0.53 ± 0.34	42.4 ± 0.70	80.7

in situ precipitation of the polymer with the formation of matrix- type nanoparticles. Ethanol residues evaporated off during a further slow stirring at 200 rpm for 24 h of the nanosuspension at room temperature.[17]

Evaluation of nanoparticles

Particle size and zeta potential

Nanoparticle size distribution and zeta potential were determined using photon correlation spectroscopy (Zetasizer Nano ZS, ZEN3600; Malvern Instruments, Malvern, UK). The size distribution analysis was performed at a scattering angle of 90 degrees and at a temperature of 25°C, whereas zeta potential was measured using a disposable zeta cuvette. For each sample, the mean diameter/zeta potential ± standard deviation of six determinations was calculated applying multimodal analysis.

Entrapment efficiency

Two-ml aliquots of the freshly prepared nanosuspensions were centrifuged at 11,000 rpm for 15 min, and the amount of unincorporated drug was measured by UV analysis of the supernatant. Some samples were submitted to a second centrifugation cycle.[17] The pellets obtained after centrifugation was then re-suspended and further dialysis process was used to measure any un-entrapped Dorzo might be precipitated in the system.

$$\text{Entrapment efficiency}\,\% = \frac{\text{Amount of Dorzo actually present in nanoparticles}}{\text{Amount of Dorzo actually used}} \times 100$$

Where,

Amount of Dorzo actually present in nanoparticles = (Amount of Dorzo actually used - Amount of Dorzo present in supernatant)

The determinations were performed in triplicate.

Morphology

The morphology of the Dorzo-Eudragit nanoparticles (A2 and A5) was analyzed using a transmission electron microscope (TEM). Initially, carbon-coated grids were floated on a droplet of the nanoparticles suspension on parafilm, to permit the adsorption of the nanoparticles onto the grid. After blotting the grid with a filter paper and air drying for 5 minutes, the grid was transferred onto a drop of the negative stain. Following this, the grid was blotted with a filter paper and air dried for 5 minutes. Uranyl acetate (0.5%) was used as a negative stain in these experiments. Finally, the samples were examined with a Jeol-JEM-1011 electron microscope.[25]

In vitro drug release profile

In vitro release pattern of the selected nanoparticle formulations was studied over 48 hr. The *in vitro* release study was carried out in phosphate buffer saline (PBS) at pH 7.4 using a dialysis system. Nanoparticle formulation was taken in a dialysis bag (with a molecular weight cut-off 12,000 to 14,000 Da). The bag was placed into a beaker containing 100 ml of PBS. The beaker was placed over a magnetic stirrer. The temperature was maintained at 37 ± 1°C. Four milliliter of sample was withdrawn periodically and equal amounts of fresh PBS were replaced. The withdrawn samples were then analyzed for drug content spectrophotometrically at 252.6 nm.[15] Experiments were run in triplicates.

To check the eventual limiting effects of dialysis membrane on drug release, separate experiments were run with a solution of free Dorzo solution in the same PBS.

RESULTS AND DISCUSSION

The prepared nanoparticles were evaluated and effect of variables like drug to polymer ratio, type of the polymer

and stirring rate on particle size, zeta potential, entrapment efficiency, and release rate were studied.

Preparation of dorzo-loaded nanoparticles

The main advantages of the used QESD technique are avoiding toxic organic solvents,[22] possibility to modify particle morphology by changing preparation parameters,[17] avoiding nanoparticle swelling during preparation due to the presence of quaternary ammonium groups,[24] and the pH value, which is always close to that of pure water (5.5-6.3). These are of particular significance in a case of ophthalmic application.[15] Formation of a colloidal nanodispersion can be visualized by the bluish opalescence [Figure 1].

Evaluation of nanoparticles

Particle size, zeta potential and entrapment efficiency

All batches [Tables 1 and 2] showed a small mean size, well suitable for possible ocular administration.[26] The mean particle size for drug-loaded nanoparticles varied from 114 nm Figure 2 to 395 nm for RS and 65 nm to 277 nm for RL. The drug entrapment efficiency varied from 50% to 92% for RS formulations and from 41% to 82% for RL formulations.

Mean particle size of blank nanoparticles was found to be almost similar to that of drug-loaded nanoparticles (data not shown). The drug: polymer ratio has an enormous effect on particle size and entrapment efficiency. Increase in polymer concentration in batches having drug: polymer ratio ranges from 1:1 to 1:9, increased particle size proportionately. Thus, larger particle size was obtained for formulations containing more polymers, which produced a significant increase in the viscosity, leading to the formation of larger size emulsion droplets and finally a higher size of nanospheres. Entrapment efficiency was found to be particle size dependent so more entrapment efficiency was obtained with higher polymer concentration. These results are in agreement with that of previous researches.[8,21,27-36] Nevertheless, up to particular concentration (1:4), further increase in polymer ratio showed insignificant change in the drug entrapment efficiency due to the saturation capacity of polymer. This finding had been reported by several authors.[37,38]

It was found that nanoparticles prepared using RL showed a smaller mean size compared to RS nanosuspension in all batches, and hence, entrapment efficiency of RL nanoparticles was lower than that of RS nanoparticles. This could be explained by the fact of higher amount of quaternary ammonium groups in RL, which showed a smaller mean size compared to RS nanosuspension. [17,39]

As regard to the stirring rate, all the nanoparticles obtained at 10,000 rpm (batches A) showed smaller mean sizes than those obtained at 20,000 pm and 24 000 rpm, respectively (batches B and C, respectively). Hence, all the nanoparticles obtained at 10,000 rpm (batches A) showed lower entrapment

Figure 1: Appearance of Dorzo-loaded nanosuspensions

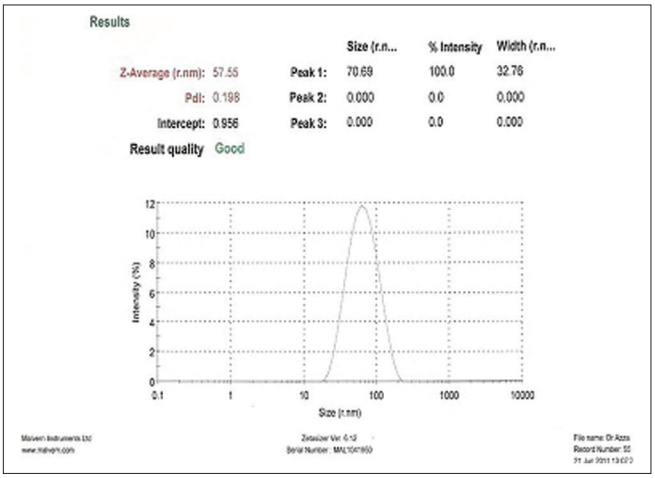

Figure 2: Particle size distribution of A1 (Dorzo: RS/1:1, stirring rate 10,000 rpm) nanoparticles

efficiency than those obtained at 20,000 pm and 24,000 rpm, respectively (batches B and C, respectively). These findings are typically unusual and may be attributed to higher stirring rate, which produced a greater foaming and rapid evaporation of the solvent occurred in the mixture. This caused an earlier separation of solid nanoparticles from the aqueous medium that limited the size reduction effect induced by stirring. These results are matching with that of Pignatello *et al.*[18]

All the formulations Tables 1 and 2 showed positive z -potential values in the range of +19 to +32 mV for RS and +23 to +42 mV for RL. These were similar to the values obtained for plain RS and RL nanoparticles (data not shown). These data indicate the charges of native polymers, the polycationic RS and RL bearing positive charges, which were not affected by the drug entrapped. This positive zeta potential value has a great effect on enhancing an effective adhesion to the corneal surface and a strong interaction with the negatively charged mucosa of the conjunctiva and anionic mucin present in the tear film, extending the residence time of the nanoparticles preparations, increasing

the drug's availability within the eye.[21,30,31] Acceptable values for polydispersity index were obtained for most preparations.

Morphology

TEM of the Dorzo-loaded Eudragit nanoparticles (batches A2 ad A5) showed that nanoparticles have solid dense structures and round shapes [Figures 3 and 4, respectively]. Particle size was further confirmed by TEM studies.

In vitro release profile

Figures 5-7 showed that Eudragit nanoparticles slow down the rate of Dorzo released compared with the free drug thus there is an immense effect of the polymer in slowing down the rate of drug released. It is clear that the efflux of Dorzo from nanoparticles was biphasic, with an initial faster release for the first 3 hours, followed by a period of slow but sustained release. The initial fast release phase may be due to the rapid dissolution of the Dorzo nanocrystals adsorbed onto the surface of the Eudragit nanoparticles. Then, the dispersion of Dorzo in the polymer matrices led to a gradual dissolution and release of the drug.[15,17,40] The principal requirement of any controlled release system is that the release profile and rate are controlled.[41]

The effect of drug to polymer ratio on release rate of Dorzo from nanoparticles [Figure 5] showed that A1, A2, A3, and A4 nanoparticle formulations. At 12 hours released 60.11%, 51.29%, 49.50% and 41.47% Dorzo, respectively. Thus, release rate was related to the polymer concentration. The A4 batch (Dorzo/RS 1:9 system) showed the slowest release rate. Such a behavior would suggest that a diffusive (matrical-type) pattern of release also took place, and increasing the amount of polymer seemed to hinder the penetration of the dissolution medium into the nanoparticles and the subsequent drug dissolution and diffusion.[28,29,32] So, it was evident that the drug release is always the result of both dissolutive and diffusional phenomena.[42,43]

The effect of type of polymer on release rate of Dorzo from nanoparticles [Figure 6] were studied in 5 batches A4, A9, A10, A11 and A8 consisting of drug: RS: RL in different ratios, 1:9:0, 1:7:2, 1:5:4, 1:2:7, and 1:0:9, respectively. It was evident that increasing RL content (from 0 in batch A4 to 9 in batch A8) led to an increase in the amount of Dorzo released after 12 hours from 41.47% to 84.23%. This could be attributed to the less permeability of RS compared to RL due to its lower content in quaternary ammonium groups (RS 1/40 ammonium / ester; RL 1/20 ammonium / ester).[17]

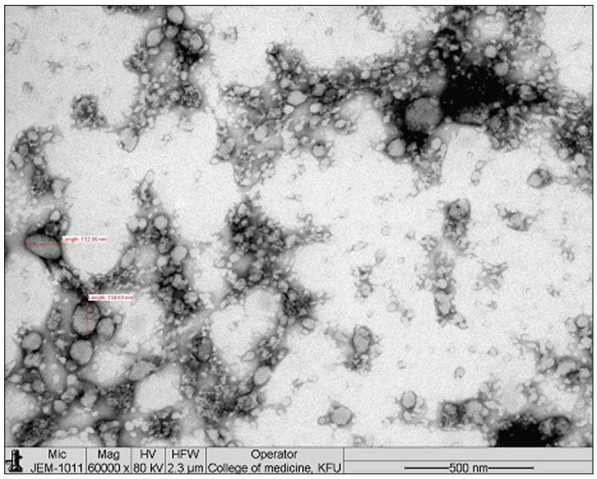

Figure 3: Transmission electron micrograph of A2 (Dorzo: RS/1:2, stirring rate 10,000 rpm)

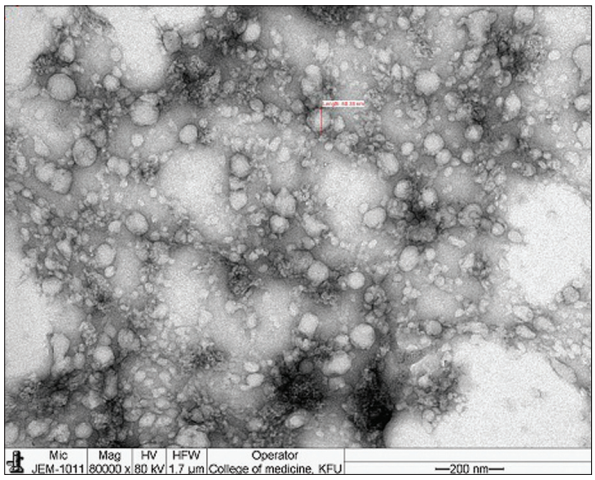

Figure 4: Transmission electron micrograph of A5 (Dorzo: RL/1:1, stirring rate 10,000 rpm)

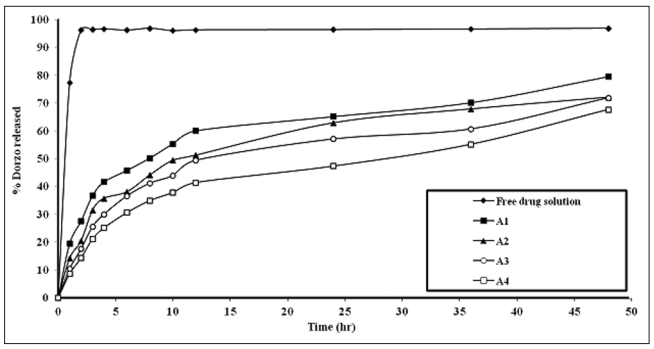

Figure 5: Effect of drug to polymer ratio on release rate of Dorzo from nanoparticles at pH 7.4

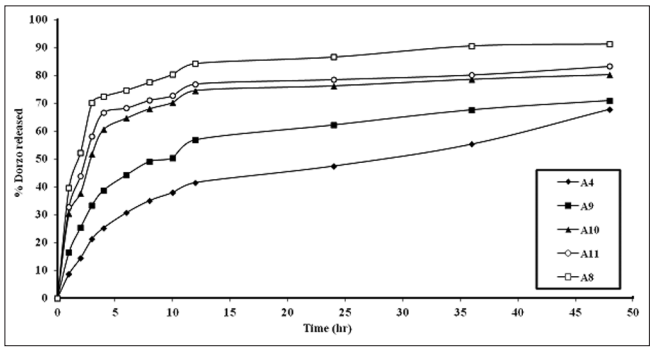

Figure 6: Effect of type of polymer on release rate of Dorzo from nanoparticles at pH 7.4

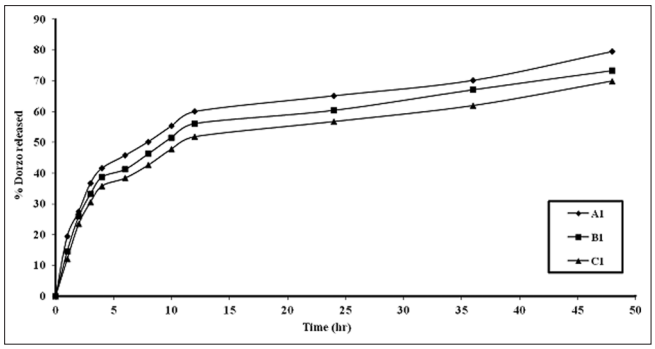

Figure 7: Effect of stirring rate on release rate of Dorzo from nanoparticles at pH 7.4

The effect of stirring rate during preparation of Dorzo-Eudragit RS nanoparticles on the *in vitro* drug release Figure 7 was studied. It can be observed that the drug release pattern obtained from Dorzo nanoparticles prepared using stirring rate of 24,000 rpm (batch C1) and 20,000 rpm (batch B1) was slower than that prepared at 10,000 rpm (batch A1). Since it was confirmed that increasing the stirring rate is accompanied by an increase in the particle size of the nanoparticles, and hence, a decrease in the surface area. Also, the higher structural homogeneity of this polymeric matrix, and the uniform distribution of the drug-loaded nanoparticles obtained at the higher speed caused a slower and more gradual release.[15]

CONCLUSION

Dorzolamide hydrochloride (Dorzo) is used in topical therapy for glaucoma. In order to increase the ocular absorption of Dorzo, this anti-glaucomatous drug was incorporated into series of polymeric nanoparticle systems. Indeed, the research and development of new ocular polymeric ophthalmic drug delivery systems is advantageous, as it shows interesting low mean size and positive zeta potential for ophthalmic application. Drug release profiles demonstrated the efficacy of nanoparticles in extending the Dorzo release so, this give a promising improvement in drug bioavailability and decreasing side effects, with respect to Dorzo solution. Further ocular bioavailability studies for these formulations including IOP lowering effect, measuring concentration of Dorzo in aqueous humor and ocular tolerability for the formulations were recommended.

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