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RESEARCH PAPER

Preparation, characterization and preliminary *in vivo* safety evaluation of cationic nano-emulsions containing α-lipoic acid after ocular administration in NZW rabbits

Asma Mahmoudi [1], Mahmoud Reza Jaafari [1,2], Bizhan Malaekeh-Nikouei [2,3]

[1]Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

[2]Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

[3]Department of Pharmaceutics, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

ABSTRACT

Objective(s): Oxidative stress has a considerable role in prevalence probability of many widely common eye problems including cataract, diabetic retinopathy and age-related macular degeneration. It has been revealed that using oral antioxidants could prevent or delay the incidence of these problems. α-Lipoic acid (ALA) is an endogenous molecule with an excellent antioxidant properties which makes its oral and topical usage suitable as supplement. The special characteristics of cationic nano-emulsions (NEs) makes them an optimum carrier for ocular drug delivery. These nanoparticles provide a high drug loading efficiency for water insoluble substances like ALA and improves the penetration through electrostatic interactions with negatively charged ocular surface.

Materials and Methods: In this study, ALA loaded cationic NEs were prepared and characterized by size, release profile, loading efficiency and their physicochemical properties. After thermodynamic stability evaluations, the animal studies conducted to examine the safety of final preparation in rabbit.

Results: Results demonstrated a drug loading efficiency of 61% for ALA and the size of cationic NEs increased from 132 nm to 289 nm after ALA entrapment. The prepared nanoparticles showed acceptable physicochemical properties and released up to 10% of loaded ALA during 6 h. the final preparation passed thermodynamic stability tests and was safe in ocular irritancy studies.

Conclusion: In this study the developed cationic NE formulation of ALA demonstrated to be useful for further evaluations in future.

Keywords: *α-Lipoic acid (ALA), Cationic nano-emulsion, Diabetes, Eye drop formulation, Ophthalmic preparation*

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INTRODUCTION

Cataracts, diabetic retinopathy and age-related macular degeneration are included in the most important eye diseases in which oxidative stress is considerably involved. These problems are bothering many people all around the world and may cause visual impairment or even blindness [1]. Previous studies demonstrated that the oral administration of antioxidants (e.g. flavonoids, ascorbate, vitamin E, etc.) can lead to delay in incidence or rebate the signs of these diseases [2]. α-Lipoic acid (ALA) as a powerful antioxidant is able to play this important role in both hydrophilic and lipophilic environment [3]. The excellent antioxidant properties of ALA leads to its wide usage as food supplement [4]. ALA derivatives from octanoic acid and contains intramolecular disulfide bonds. Also it has been revealed that this substance as an endogenous molecule has an excellent safety profile [5]. ALA acts as scavenger and plays its role through neutralizing reactive oxygen species (ROS), chelating metallic ions, preventing lipid peroxidation and helping

* *Corresponding author: Email: malaekehb@mums.ac.ir*

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to regeneration of other antioxidants such as vitamin C and E and intracellular glutathione [1, 5]. ALA on ocular surface reduces the effects of oxidative stress [6] and causes corneal epithelium reparation [5] and capillary injuries relief [7] and also lessens vascular proliferation by controlling the level of vascular endothelial growth factor and provides the efficient function of nerve endings through inhibiting lipid peroxidation [8].

Currently ALA is orally using for treatment of neuropathy or other ophthalmic complications in diabetic patients [9]. Regarding the fact that prolonged oral administration may cause some adverse effects on cardiac function [10], using the eye drop formulation for topical use on ocular surface can be preferable in prevention or treatment supplementation of described ophthalmic disorders. From disadvantages of conventional aqueous ophthalmic solutions we could mention to their limited application for water soluble molecules and rapid elimination through nasolacrimal drainage system (over 80% within two minutes) which restricts ocular penetration. Therefore, pharmaceutical companies are trying to develop ocular formulations which could overcome these enumerated concerns [11].

Despite challenges for developing an ophthalmic formulation of ALA (including low solubility in water, instability in case of light exposure and reactivity to other components), some commercial products (e.g., Tioretin® A [12]) are available in European countries. ALA can permeate through the cornea to access the behind tissues, although it's ocular bioavailability is low due to defensive mechanisms in the eye and low drug concentration gradient across the cornea [1].

Nanotechnology is currently considered one of the best approaches to overcome the ophthalmic drug delivery challenges but the number of nanotechnology based products reached to market is still very low because of more complicated scale up process and manufacturing requirements [11]. Cationic nano-emulsions (NEs) are from promising nano-systems for ocular drug delivery due to their bioadhesive properties. Cationic NEs as a kind of cationic carrier interacts electrostatically to the negatively charged cell membrane on ocular surface which leads to prolonged residence time and better penetration efficiency [13].

In the present study, we aimed to prepare an efficient ophthalmic formulation of ALA based on pharmaceutical nanotechnology knowledge. Cationic NE was chosen as drug carrier and we tried to improve drug loading efficiency along with reduce the particle size. The resultant nanoparticles characterized and evaluated for their physicochemical properties. Finally, the safety of final product was investigated in rabbit in order to get the confirmation for planning further investigations in clinical study.

MATERIALS AND METHODS

Materials

Poloxamer 188, cetalkonium chloride (CTK), α-lipoic acid (ALA), soybean oil, glycerin and ammonium acetate were purchased from sigma-aldrich (Germany). Acetonitrile HPLC-grade was from DAEJUNG Company Ltd. (Korea) and methanol HPLC-grade was provided from *Scharlau Chemie S.A.* (Spain). Ethanol was supplied by Dr. Mojallali laboratory (Iran) and chloroform was obtained from Merck Company (Germany). Phosphate buffered saline tablets were prepared from Tetrachem® (Iran). All other chemicals were of analytical grade. Male New Zealand white rabbits were supplied by Razi Vaccine and Serum Research Institute (Mashhad, Iran), and housed under standard conditions.

Preparation of cationic nano-emulsion containing ALA

Table 1 describes different compositions in water and oil phase of formulation. Poloxamer 188 and glycerin as water phase composition were dissolved in water and placed in 80 ºC water bath. For preparing the oil phase, CTK and ALA were dissolved in soybean oil and ethanol 96 separately at 80 °C. ALA solution was added to the mixture of CTK and soybean oil keeping the temperature at 80 °C using water bath. The oil phase was then gradually added to the water phase on magnetic stirrer at 80 °C during 5 min and then homogenized through Ultraturax homogenizer (10 min, 28000 rpm) in 80 °C water bath to reach pre-emulsion. Then probe sonication (6 cycle, 60s on and 40s off) was used to decrease the size to nanometers range. The pH of final preparation was adjusted

Table 1. Composition of water and oil phases for preparation of nano-emulsion containing ALA

Water phase		Oil phase	
CTK[a]	4 mg	Poloxamer 188	100 mg
Soybean oil	392 µl	Glycerin	80 µl
ALA[b]	40 mg	NaOH	As needed
Ethanol 96	100 µl	DW up to	4 cc

[a] Cetalkonium chloride; [b] α-Lipoic acid

to 6.5±0.1 using NaOH 0.1N after cooling the NEs in room temperature. The final formulation refrigerated under inert gas.

Nanoparticle characterization

Size and zeta potential

Particle size analysis (through dynamic light scattering method) and zeta potential of nanoparticles were determined using Zetasizer Nano ZS (Malvern, UK). Samples were prepared by adding 10 µL cationic NE preparation to 990 µL deionized water.

TEM electron microscopy

The morphology of nanoparticles in cationic NE formulation was examined through transmission electron microscopy (TEM). For this purpose a drop of diluted sample (1:1000 in deionized water) were placed onto a copper grid and air-dried. This step followed by negative staining with a drop of 1% uranyl acetate in aqueous solution for contrast enhancement. Grids were then dried keeping at room temperature and evaluated through TEM.

Determination of Incorporated ALA

Calibration curve was obtained from sample chromatography through a 4.6×250 mm^2 reverse-phase stainless steel C18 column in concentration range of 125-1000 µg/mL measuring at 210 nm by HPLC method. Mobile phase consisted of acetonitrile:ammonium acetate 20 mM (pH 4.6) (50:50, v/v) with 0.8 ml/min flow rate at room temperature. Samples were properly diluted in methanol:chloroform (50:50) to obtain a clear solution. The volume of each injection was 20 µL.

Physicochemical characterization of cationic nano-emulsions

Evaluation of rheological properties

Rheological measurements were performed at 34 ± 0.5 °C (the ocular surface temperature) using Brookfield R/S^+ Rheometer (Germany). Continuous variation of shear rate (γ) in the range of (0 - 20 S^{-1}) was applied through 10 cycle of 1s and the resulting shear stress (σ) was measured. Finally viscosity (η) of cationic NE preparation with Newtonian flow properties was calculated from the following equation: $\eta=\sigma/\gamma$

pH measurements

pH of the cationic NE formulation was determined by pH meter (mi 151, MARTINI instruments, USA).

Refractive index measurements

Refractive index determination was carried out using refractometer (BPTR50, PrismaTech, Iran) at 25 °C.

Surface tension measurements

Surface tension evaluations were performed at room temperature using a SIGMA 703D tensiometer (Biolin Scientific, Sweden) provided with a Du Nouy ring (ring radius 9.545 mm, wire diameter 0.37 mm).

Osmolality determination

Osmolality was measured through Micro Osmometer (K-7400S, KNAUER, Germany).

Release test

500 µl of formulation diluted in 1.5 ml PBS pH 7.4 and the microtube containing the solution fixed on tube rotator (LD79 Digital Test-Tube Rotator, LABINCO, Netherlands) (50 rpm, 34 ± 0.5 ºC, 6 h). 1, 2, 3, 4, 5 and 6 h afterwards samples were centrifuged in microtube (14000 rpm, 5 min) and 100 µl of supernatant removed for ALA concentration determination and replaced with PBS pH 7.4. After each sampling, bath sonication was used to disperse the sediment and microtube fixed again on tube rotator for rest of study. The amount of released ALA determined through HPLC method described above.

Accelerated thermodynamic stability studies

Prepared formulation was evaluated in three phases for thermodynamic stability studies according to this protocol [14, 15]:

- Six cycle of heating at 45 °C and cooling at 4 °C (48 h storage at each temperature for each cycle).
- Then samples were centrifuged at 3500 rpm for 30 min.
- In the last phase samples exposed to three cycle of freezing at -20 °C and thawing at 25 °C (48 h storage at each temperature for each cycle).

Ocular irritancy studies

Eight New Zealand rabbits weighing 2-5 Kg were kept in air-conditioned room at 25 °C. They received standard diet and artificial light was provided to induce a cycle of 12 h day and 12 h night. All animal procedures were organized according to guidelines for animal

Table 2. The modified version of Draize grading scale for clinical evaluation of ocular irritation (14)

Clinical parameter	Observation	Score
Conjunctival discharge	Normal	0
	Slight discharge	1
	Severe discharge covering a small area around the cornea	2
	Severe discharge covering a large area around the cornea	3
Conjunctival chemosis	Normal	0
	Slight chemosis including nictitating membrane	1
	Severe chemosis with eye partially closed	2
	Severe chemosis with eye closed	3
Conjunctival redness	Blood vessels normal	0
	Some blood vessels definitely hyperaemic	1
	Diffuse color, individual vessels not easily discernible	2
	Diffuse beefy red	3

experimentation of the Institutional Animal Care and approved by ethical research committee in Mashhad University of Medical sciences (code number: IR.MUMS.PHARMACY.REC.1398.112) and it adhered to the Association for Research in Vision and Ophthalmology (ARVO). The formulation was sterilized by filtration through 0.45 µm membrane filter before instillation into the eyes.

A group of six rabbits received 50 µl of cationic NE containing ALA in right eyes and 50 µl of cationic NE without ALA in left eyes every 12 hours for 10 days. Simultaneously a group of two rabbits received 50 µl of normal saline in both eyes as control. At the day 11, all rabbits were examined after 1 and 24 h from last instillation under general anesthesia (35 mg/kg ketamine and 5 mg/kg xylazine) through a modified version of Draize technique by Morsi and her colleagues [14, 16] for inflammation or toxic reactions in eyelids, cornea, iris, conjunctiva and anterior chamber of the eyes. Each animal was monitored to observe any sign of ocular reactions including redness, discharge, conjunctival chemosis, iris and corneal lesions. The ocular irritancy test was carried out by considering 0 (absence) to 3 (highest) grades for any clinical reaction (according to Table 2). Ocular irritation index (Iirr) was calculated via summing up the specified clinical scores. A score of 2 or 3 in any category or Iirr more than 4 was considered as an indicator of clinically significant irritation [14].

After medical examination randomly cornea of three eyes among six eyes received ALA containing formulation, two eyes among six eyes received formulation without ALA and one eye from the group received normal saline were separated, washed with PBS (pH 7.4) and fixed immediately in Bouin's solution. Cross-sections were evaluated under optical microscope after hematoxylin & eosin staining by pathologist for any lesion.

Table 3. Size and zeta potential of cationic NEs in deionized water before and after being loaded with ALA (mean ± SD)

Formulation	Z-average (nm)	Zeta potential (mV)
Cationic NE[a] containing ALA[b]	289.13 ± 4.55	40.5 ± 1.10
Cationic NE without ALA	132.4 ± 0.404	41.2 ± 4.82

[a] Nano-emulsion; [b] α-Lipoic acid

RESULTS & DISCUSSION

Nanoparticle characterization

Size and zeta potential

Results of size and zeta potential evaluation for ALA containing cationic NEs and drug free vectors are presented in Table 3. Loading the ALA in nanoparticles increased the size about 150 nm as a result of entrapment in nano-droplets but did not induce any alteration in the surface charge. As it was expected the surface charge of nanoparticles was cationic due to the presence of CTK as preservative and cationic agent.

TEM electron microscopy

TEM result (Fig. 1) revealed that the nanoparticles are almost spherical in morphology and also confirmed the result of size evaluation by DLS method using Malvern ZS Nanosizer device.

Determination of Incorporated ALA

A linear HPLC standard curve was obtained

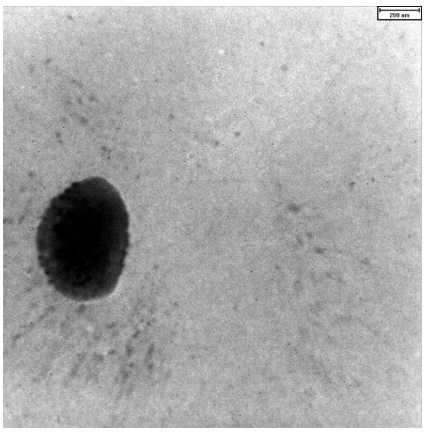

Fig. 1. Transmission electron microscopy (TEM) image of cationic NE containing ALA

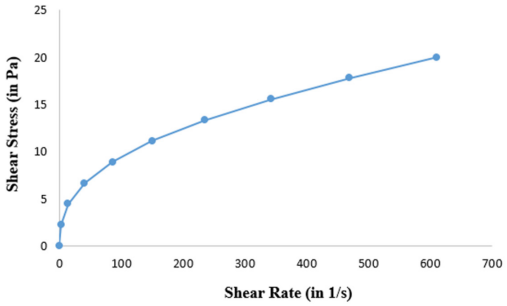

Fig. 2. Shear stress as a function of shear rate for ALA loaded cationic NEs within 10 s by Rotation Stairs viscometer

over the evaluated concentration range (r^2=0.9998) and the loading efficiency of ALA in cationic NE formulation was 61% (6.1 mg/ml) ±5.82. Regarding the water solubility of 0.87 g/L which is reported for ALA in sigma specification sheet, using cationic NE formulation increased this solubility to 7 times higher.

Physicochemical characterization of cationic nano-emulsions

Evaluation of rheological properties

It is expected that the administration of an ophthalmic preparation would influence on the normal behavior of tears as little as possible. Usually the low viscosity formulations induce good tolerance with minimal discomfort. On the other hand, enhanced viscosity formulations, although may cause less tolerant, improve bioavailability by reducing the drainage rate and increasing ocular contact time [17].

The whole tear film displays non-Newtonian shear-thinning behavior and this means applying shear rate during eyelid movement decreases the tear viscosity [18]. Overall, ocular surface temperature in healthy eyes ranges from 34–35 °C [19]. ALA cationic NEs like the human tear exhibited a non-Newtonian shear-thinning behavior (Fig. 2). The viscosity values of studied ALA cationic NEs at 34 ± 0.5 °C and shear rate range of (0 - 20 S^{-1}) were shown in Table 4.

pH measurements

The pH range of 7.2±0.2 (near the tear fluid pH) is ideal to provide the maximum comfort when instilling an ophthalmic preparation [20, 21]. The pH value of our prepared formulation was about 5 and this was expected because of acidic nature of ALA molecule, therefore to reduce the acidity NaOH 0.1 N was added and the final pH adjust on 6.5±0.1. Sometimes using an ophthalmic solution with a pH different from tears induces irritation and discomfort, but that depends on the composition of preparation, its buffering capacity, the instilled volume and the contact time on ocular surface [20]. If the ophthalmic preparation is not buffered, the limited buffering capacity of tears would be able to adjust the different pH values to physiologic level soon after instillation and therefore the pH could be tolerated with minimum discomfort [21, 22]. Hence the pH of therapeutic eye drop formulations can vary from 3.5 to 8.5 [20]. Regarding these explanation, the pH value of 6.5 would be acceptable for our ophthalmic preparation which is not buffered.

Refractive index measurements

Measurements revealed that the refractive index of ALA loaded and drug-free cationic NEs were 1.3452±0.00013 and 1.3447±0.00004 respectively. These values are close to refractive index of water (1.33) and the cornea and lachrymal fluid (1.34-1.36) [22], thus we do not expect any visual impairment or discomfort after administration. The refractive index values of greater than 1.476 is not recommended for eye

Table 4. Viscosity evaluation of ALA loaded cationic NEs within 10 s by Rotation Stairs viscometer

Time (in s)	Shear rate (in 1/s)	Shear stress (in Pa)	Viscosity (in Pa·s)
1	0	0	0
2	2.252795	2.22651	0.988332271689168
3	13.476749	4.441602	0.329575181670297
4	40.816256	6.668112	0.163369026301677
5	85.768876	8.883204	0.103571416745627
6	149.900592	11.109714	0.0741138767483987
7	234.955545	13.336224	0.0567606267815471
8	342.923166	15.551316	0.0453492722040249
9	469.486351	17.777826	0.0378665449211323
10	610.739825	20.004336	0.0327542681533827

drop formulations [14, 20].

Surface tension measurements

The surface tension value obtained for the ALA loaded cationic NEs was 48.7 mN/m which is in the range of the lachrymal fluid surface tension (40 to 50 mN/m) [22]. This surface tensions provides an acceptable spreading effects similar to the tear film. Good spreading improves the contact to ocular surface, and electrostatic interactions between cationic NEs and negatively charged corneal epithelium increases this contact time. Indeed, the prolonged exposure time helps to better drug absorption.

Osmolality determination

The osmolality of lacrimal fluid is in the range of 280-293 mOsm/kg on waking times. This range could be changed to 231-446 mOsm/kg regarding the evaporation when the eyes are open [23]. An osmolality of less than 100 mOsm/kg or more than 640 mOsm/kg for non-isotonic ophthalmic solutions would be irritant, but depending on their droplet size the original osmolality will be restored within 1 or 2 min [24]. The osmolality of our prepared formulation was 595.66 ± 2.30 mOsm/kg which is not in irritant range and would be adaptable.

Release test

Release study was conducted at 34 ± 0.5 °C (ocular surface temperature) and up to 10% of entrapped ALA released during this period while after 6 h reached to plateau (Fig. 3). When the formulation is going to use as an ophthalmic preparation, the exposure time would be low. We followed the release profile up to 6 h. Our evaluation demonstrated up to 10% drug release during this time period. Because of very low solubility of ALA in water based environments [25], the NEs acts as reservoir and provides a sustained release pattern [26]. Although, ALA molecules which did not release, have the chance to be absorbed through the corneal epithelium via the advanced characteristics of their nanoparticulate vectors.

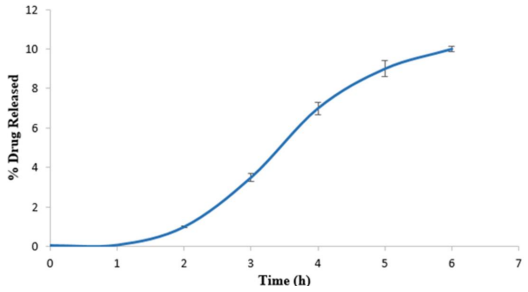

Fig. 3. Release of ALA from different formulations

Accelerated thermodynamic stability studies

The stability of prepared nanoparticulate formulation was evaluated through heat–cool cycles, centrifugation, and then alternative freeze–thaw cycles respectively. After 6 cycles of heat and cool we had no cracking, creaming or color change and the formulation retained uniformity. After 30 min centrifugation as the next step we had no phase separation and the nanoparticle system maintained uniform. Finally in the last stage; although the texture was opaque and cloudy upon freezing, but recovered its uniformity through thawing process. Previous studies with similar experiences [26, 27] explained this transient instability could be related to the internal phase coagulation at freezing temperature or the exerted pressure induced by ice crystals. The result of thermodynamic stability evaluation was promising and the formulation passed the test.

Ocular irritancy studies

Before animal grouping, the ophthalmologist confirmed all animals are healthy and no sign of inflammation observed in eyes including eyelids, cornea, iris, conjunctiva and anterior chamber of the eyes. On the day 11th, 1 h and 24 h after the last instillation according to the scores described in Table 2, the obtained data revealed that ALA loaded cationic NEs and drug free vectors were nonirritant and tolerated very well by the rabbit eye (average Iirr score = 0). But one rabbit in control group (received normal saline) revealed inflammation and redness in both eyes (Iirr score = 3) which could be accidental due to possible contaminants in environment.

After light microscopic observation, cross sections from the corneas of three eyes received

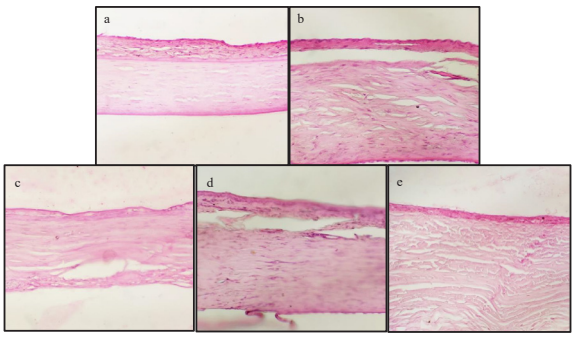

Fig. 4. Light microscopic pictures from cornea cross sections. ALA containing cationic NEs (a, b, c) and drug free cationic NEs (d, e). (Hematoxylin and Eosin, ×400)

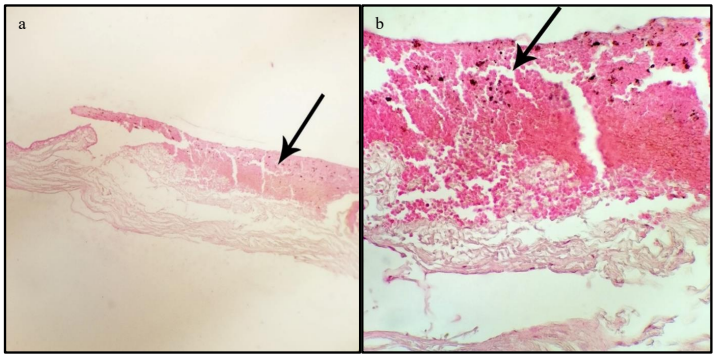

Fig. 5. Light microscopic pictures from cornea cross section of the rabbit received NS as control. Presence of hemorrhage in corneal sub- epithelium (arrows). Hematoxylin and Eosin, ×100 (a), ×400 (b)

ALA containing formulation and two eyes received formulation without ALA (Fig. 4); showed that the corneal epithelium, endothelium and stroma were arranged in order and no inflammatory cells and edema were observed. Light microscopic observation of cross sections from the eye with inflammation in normal saline group (Fig. 5) showed corneal sub-epithelium hemorrhage but we do not have any explanation for this event because this hemorrhage could not be due to the normal procedure of study. Regarding the fact that the rabbit eye is more susceptible to irritant substances compared to human eye [26, 28], these obtained results would be considered very promising.

CONCLUSION

In the present study, cationic NE eye drop formulation for ALA was prepared and characterized by thermodynamic stability, acceptable physicochemical properties, size and ability to retain the drug. Our ocular irritancy study revealed that the prepared ALA loaded cationic NEs were safe and can be useful for future studies because of the expected potential advantages including sustained drug release and better ocular surface interaction and penetration.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

REFFERENCES

1. Cagini C, Leontiadis A, Ricci MA, Bartolini A, Dragoni A and Pellegrino RM. Study of alpha-lipoic acid penetration in the human aqueous after topical administration. Clin Experiment Ophthalmol 2010;38(6):572-576.
2. Varma S, Hegde K, Kovtun S. Attenuation and delay of diabetic cataracts by antioxidants: effectiveness of pyruvate after onset of cataract. Ophthalmologica 2005;219(5):309-315.
3. Packer L, Kraemer K, Rimbach G. Molecular aspects of lipoic acid in the prevention of diabetes complications. Nutrition 2001;17(10):888-895.
4. Kowluru RA, Zhong Q, Santos JM, Thandampallayam M, Putt D and Gierhart DL. Beneficial effects of the nutritional supplements on the development of diabetic retinopathy. Nutr Metab 2014;11(1):1-10.
5. Alvarez-Rivera F, Fernández-Villanueva D, Concheiro A and Alvarez-Lorenzo C. α-Lipoic acid in Soluplus® polymeric nanomicelles for ocular treatment of diabetes-associated corneal diseases. J Pharm Sci 2016;105(9):2855-2863.
6. Berkowitz BA, Grady EM, Khetarpal N, Patel A and Roberts R. Oxidative stress and light-evoked responses of the posterior segment in a mouse model of diabetic retinopathy. Investig Ophthalmol Vis Sci 2015;56(1):606-615.
7. Chen C-L, Cheng W-S, Chen J-L and Chiang C-H. Potential of Nonoral a-Lipoic Acid Aqueous Formulations to Reduce Ocular Microvascular Complications in a Streptozotocin-Induced Diabetic Rat Model. J Ocul Pharmacol Ther 2013;29(8):738-745.
8. Obrosova I, Minchenko A, Marinescu V, Fathallah L, Kennedy A, Stockert C, et al. Antioxidants attenuate early up regulation of retinal vascular endothelial growth factor in streptozotocin-diabetic rats. Diabetologia 2001;44(9):1102-1110.
9. Gomes MB, Negrato CA. Alpha-lipoic acid as a pleiotropic compound with potential therapeutic use in diabetes and other chronic diseases. Diabetol Metab Syndr 2014;6(1):1-18.
10. Al-Rasheed NM, Al-Rasheed NM, Attia HA, Hasan IH, Al-Amin M, Al-Ajmi H, et al. Adverse cardiac responses to alpha-lipoic acid in a rat-diabetic model: possible mechanisms? J Physiol Biochem 2013;69(4):761-778.
11. Lallemand F, Daull P, Benita S, Buggage R and Garrigue J-S. Successfully improving ocular drug delivery using the cationic nanoemulsion, novasorb. J Drug Deliv 2012;2012.
12. Roszkowska AM, Spinella R, Oliverio GW, Postorino EI, Signorino GA, Rusciano D, et al. Effects of the Topical Use of the Natural Antioxidant Alpha-Lipoic Acid on the Ocular Surface of Diabetic Patients with Dry Eye Symptoms. Front Biosci (Landmark Ed) 2022;27(7):202.
13. du Toit LC, Pillay V, Choonara YE, Govender T and Carmichael T. Ocular drug delivery–a look towards nanobioadhesives. Expert Opin Drug deliv 2011;8(1):71-94.
14. Morsi N, Mohamed M, Refai H and El Sorogy H.

Nanoemulsion as a novel ophthalmic delivery system for acetazolamide. Int J Pharm Pharm Sci 2014;6(11):227-236.
15. Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK and Ali M. Development and bioavailability assessment of ramipril nanoemulsion formulation. Eur J Pharm Biopharm 2007;66(2):227-243.
16. Draize J, Woodard G, Calvery H. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. J Pharmacol Exp Ther 1944;82(3):377-390.
17. Zignani M, Tabatabay C, Gurny R. Topical semi-solid drug delivery: kinetics and tolerance of ophthalmic hydrogels. Adv Drug Deliv Rev 1995;16(1):51-60.
18. Recchioni A, Mocciardini E, Ponzini E and Tavazzi S. Viscoelastic properties of the human tear film. Exp Eye Res 2022;109083.
19. Shah AM, Galor A. Impact of ocular surface temperature on tear characteristics: current insights. Clin Optom 2021;13:51-62.
20. Ammar H, Salama H, Ghorab M and Mahmoud A. Development of dorzolamide hydrochloride in situ gel nanoemulsion for ocular delivery. Drug Dev Ind Pharm 2010;36(11):1330-1339.
21. Najafi Bi R, Samani SM, Pishva N and Moheimani F. Formulation and clinical evaluation of povidone-iodine ophthalmic drop. Iran J Pharm Res 2010;2(3):157-160.
22. Fialho SL, Da Silva-Cunha A. New vehicle based on a microemulsion for topical ocular administration of dexamethasone. Clin Experiment Ophthalmol 2004;32(6):626-632.
23. Terry JE, Hill RM. Human tear osmotic pressure: diurnal variations and the closed eye. Arch Ophthalmol 1978;96(1):120-122.
24. Edman P. *Biopharmaceutics of ocular drug delivery*. Boca Raton: CRC Press; 1993.
25. Takahashi H, Bungo Y, Mikuni K. The aqueous solubility and thermal stability of α-lipoic acid are enhanced by cyclodextrin. Biosci Biotechnol Biochem 2011;75(4):633-637.
26. Ammar HO, Salama H, Ghorab M and Mahmoud A. Nanoemulsion as a potential ophthalmic delivery system for dorzolamide hydrochloride. AAPS PharmSciTech 2009;10(3):808-819.
27. Tayel SA, El-Nabarawi MA, Tadros MI and Abd-Elsalam WH. Promising ion-sensitive in situ ocular nanoemulsion gels of terbinafine hydrochloride: design, in vitro characterization and in vivo estimation of the ocular irritation and drug pharmacokinetics in the aqueous humor of rabbits. Int J Pharm 2013;443(1-2):293-305.
28. Roggeband R, York M, Pericoi M and Braun W. Eye irritation responses in rabbit and man after single applications of equal volumes of undiluted model liquid detergent products. Food Chemical Toxicol 2000;38(8):727-734.