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Ocular delivery of ketorolac tromethamine using microemulsion as a vehicle: Design, evaluation, and transcorneal permeation

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ABSTRACT: Ketorolac is a nonsteroidal anti-inflammatory drug with analgesic properties. Different clinical studies have established the safeness and efficacy of using 0.5% ketorolac formulations for alleviating ocular inflammation and pain. Ketorolac's eye drops have a short period of action because of its solubility in tear fluids, which resulting to its quick drainage from the eye, meaning that the patient has to administer it frequently. The present study describes the design of an microemulsion vehicle to be used for the ocular delivery of ketorolac. Ketorolac-loaded microemulsion (ME) was supplied using oleic acid-Transcutol P (oily phase), Tween 80, Span 20 (surfactant), and propylene glycol (co-surfactant). The physicochemical properties of the prepared MEs were evaluated according to their viscosities, pH, droplet sizes, surface tension, physical and chemical stability, drug release, and transcorneal rabbit permeation. The drug release profile revealed that 23.65-38.64% of the drug was released during the 24-hour experiment. The maximum permeated drug percentage was observed for ME-K-1 (9.041%). The whole of prepared MEs with various components and properties significantly increased the cornea permeation rate and permeation percentage after 6 hours (%P6h) from the rabbit cornea. The flux and diffusivity coefficient in ME-K-2 formulation were obtained 0.125 mg/cm^2/h and 0.0126 cm2/h, which are 3.49 and 6.464 times higher, respectively, then the values for ketorolac drops (KT 0.5%). The MEs developed in the present work were within the range of acceptable droplet sizes for ocular use and possessed physical and chemical stability. Furthermore, the values recorded for the physicochemical parameters support their suitability for ophthalmic use.

KEYWORDS: Transcorneal permeation; microemulsion; ketorolac; rabbit; release.

1. INTRODUCTION

Ocular delivery is one of the most important drug delivery routes. The eye has unique physiological structures that make this organ resistance to permeation of drugs. For a treatment to be effective, sufficient quantities of active ingredients need to be delivered and retained within the eye. The eye drops are the most usual dosage forms that have not suitable bioavailability because of, after an eye drop is administered, approximately 70% may be waste, thus resulting in poor ocular bioavailability[1]. The poor ocular bioavailability of drugs because of physiological and anatomical barriers, including low precorneal residence time, and low corneal permeability, are the most notable problems faced when delivering drugs to the eye using a conventional method[2].

The cornea is a physical and chemical barrier, the primary purpose of which is to keep the intraocular tissues of the eye safe. The corneal membrane has a heterogeneous texture consist epithelium, stroma and endothelium that any layer has different physicochemical characteristics. Among these layers, stroma and epithelium are the most important ways to drug absorption. Drug compounds with high hydrophilic or lipophilic effects do not have a passive transport method into the cornea. The stroma layer is a rate-limiting membrane for water-hating drugs, whereas the epithelium layer is a rate-limiting membrane for water-loving drugs [3-5].

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The compactness of the corneal structure and the rapid waste of drug solutions applied to the precorneal area decrease the effect of drugs. The corneal permeability of drugs administered using ophthalmic drops is weak because of short survival time and the impenetrability of the corneal epithelium layer [6] Ocular nano-carriers can be classified as nanosuspensions, liposomes, dendrimers, micelles, solid lipid nanoparticles, and microemulsions (MEs) [7-10].

Ketorolac is a drug with analgesic and anti-inflammatory effects. Different clinical researches have shown the safety and efficacy of 0.5% ketorolac formulations for alleviating ocular inflammation and pain[11]. Ketorolac's anti-inflammatory and analgesic effects are due to the inhibition of prostaglandin synthesis, which is accomplished through the competitive blocking of the enzyme cyclooxygenase[12].

Microemulsions are transparent, thermodynamically stable, isotropic liquid mixtures of oil, water and surfactant. They have nano-size range 10-100 nm and very low surface tension. Due to nano-size structures of MEs, they are often used specific vehicles for ocular delivery that improved drug absorption and penetration through cornea[13-15]. The attendance of surfactants and co-surfactants in ME vehicles enhances drug penetration and absorption through the corneal membrane. Hence, they can be used ocular penetration enhancers. In addition, because of their low surface tension, it is easy to spread MEs on the cornea, thereby increasing the contact area between the drug and the corneal epithelial surface[16]. Furthermore, MEs enable the extended release of any drug indicated to the cornea and increase drug permeation into the different layers of the cornea.

The present study describes the design of an ME vehicle to be used for the ocular delivery of ketorolac. Their physicochemical properties and permeation through rabbit corneal were also investigated to determine whether the drug permeation percent could be improved.

2. RESULTS AND DISCUSSION

2.1. Ketorolac solubility

The solubility of ketorolac tromethamine is presented in Table 1. ME formulations were developed and designed by selecting a proper oil, which was done by determining the amount of ketorolac that could be dissolved in the oil. According to the experiments that tested the solubility of ketorolac in oil phase, surfactant and co-surfactant phase, it was calculated that oleic acid:Transcutol P (10:1) (as oily phase), Tween 80, Span 20(as surfactant phase), and PG (as Co-surfactant phase) was the most suitable ME combination.

Table 1. Solubility of ketorolac in oil, surfactants and co-surfactant (Mean ± SD, n = 3).

Phase type	Component	Solubility(mg/mL)
Oil	Oleic Acid	7.3±0.25
	Transcutol p	9.5±0.75
	Oleic Acid+ Transcutol p(10:1)	8.7±0.1
Surfactants	Tween 80	18.4±0.25
	Span 20	6.1±0.5
Co Surfactant	Propylene Glycol	18.8±0.1

2.2. Phase diagrams behavior

Figure 1 shows the Pseudo ternary phase diagrams of oleic acid:Transcutol P/Tween 80: Span 20/Propylene glycol/Water.

The mass ratios of Surfactant: co-surfactant amounts are important parameters that affected on phase diagrams behavior of MEs. The increase in the area of ME region with increasing the relative quantity of surfactant component was established in former studies[16]. The ME boundaries were extended by increasing of S:Co weight ratio (km = 1-3).

2.3. Physicochemical properties of the ketorolac MEs

Eight MEs with 1:1 and 3:1 s:co mass ratios were selected from the phase diagrams. Table 2 shows the compositions of the selected MEs. Table 3 shows the viscosities, mean droplet sizes, pH, and surface tensions of the MEs.

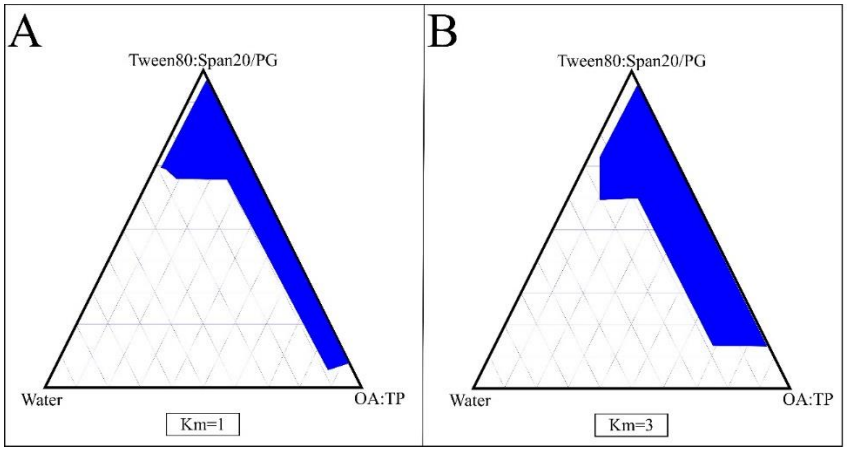

Figure 1. The Pseudo Ternary Phase Diagrams of the Oil: surfactant/Co-surfactant mixture: water System at the 1:1 and 3:1 Weight Ratio of Tween 80/Span 20/PG at Ambient Temperature, Dark Area Show MEs boundary.

Table 2. The composition of prepared microemulsions of ketorolac.

Formulation	Factorial Design	(S:C)	% Oil	%S+C	%Water
ME-K-1	+++	3:1	50	40	10
ME-K-2	++-	3:1	50	45	5
ME-K-3	+-+	3:1	5	85	10
ME-K-4	+--	3:1	5	90	5
ME-K-5	--+	1:1	5	85	10
ME-K-6	---	1:1	5	90	5
ME-K-7	-+-	1:1	50	45	5
ME-K-8	-++	1:1	50	40	10

Table 3. pH, Viscosity, Mean droplet sizes (in the beginning and 3 months after experiment), Polydispersity Index and Surface Tension of the prepared Ketorolac MEs (Mean±SD, n=3).

Formulation	pH	Viscosity (cps)	Surface Tension (dyne/cm)	Mean droplet Size (nm)	Mean droplet Size after 3 month (nm)	Polydispersity Index
ME-K-1	5.41±0.02	142±1.1	34±0.1	6.64±1.1	7.1±0.1	0.444±0.01
ME-K-2	5.19±0.02	153±1.3	31±0.3	16.33±1.1	16.6±0.5	0.411±0.01
ME-K-3	5.31±0.02	224±1.5	32±0.2	11.1±0.84	11.5±0.3	0.470±0.01
ME-K-4	5.38±0.02	245±1.4	37±0.1	10.58±1.57	11±0.75	0.388±0.02
ME-K-5	5.44±0.02	137±1.3	31±0.2	8.49±0.90	9.05±0.2	0.470±0.01
ME-K-6	5.92±0.02	196±1.5	35±0.1	7.66±1.50	7.91±0.8	0.470±0.01
ME-K-7	6.16±0.02	128±0.98	37±0.1	13.1±1.45	13.3±0.9	0.444±0.02
ME-K-8	6.22±0.02	118±0.78	38±0.1	5.83±0.75	6.1±0.4	0.437±0.02

The selected MEs had a droplet size of 5.83 - 16.33 nm. The relationship between mean droplet size amounts and the independent variable (%Oil) was significant. The mean droplet size values decreased as the percentage of oil in the ME samples increased. Meanwhile, decreases in particle size were associated with significant increases in surface area, thus leading to improved bioavailability[17]. In the present research, the droplets of all ME samples were smaller than 30 nm. The polydispersity index indicated the uniformity amount of the size of droplets. Also, all of polydispersity amounts were below 0.470. According to the results, the size of droplets was narrowly distributed in selected ketorolac MEs.

The ME samples in this study had pH values of 5.19 to 6.22, and the significant relationship was showed between pH and the oil percentage variable. Specifically, pH increased in some ketorolac MEs when the amount of oil was reduced. This data is not agreement with previous studies[18]. The selected MEs samples are showed an average viscosity range of 118-245 cps.

The statistic results highlighted that viscosity's correlation with the %Water, %Oil, and S/Co was significant. Viscosity increased at low percentages of water, low S/C ratios, and high percentages of oil in ketorolac MEs. These findings are consistent with the previous report of Tiffany J Et al.[19]. Increased viscosity may be help to improve the preocular keeping time and, so, the quantity of drug penetrated into the cornea. All of the prepared MEs were more viscose than the ophthalmic drop (containing 35 cps viscosity). The obtained viscosities data of MEs displayed Newtonian flow characteristics for all of the ME systems. Then, the rheogram for viscosity (cps) versus the shear rate of MEs was plotted (Figure 2).

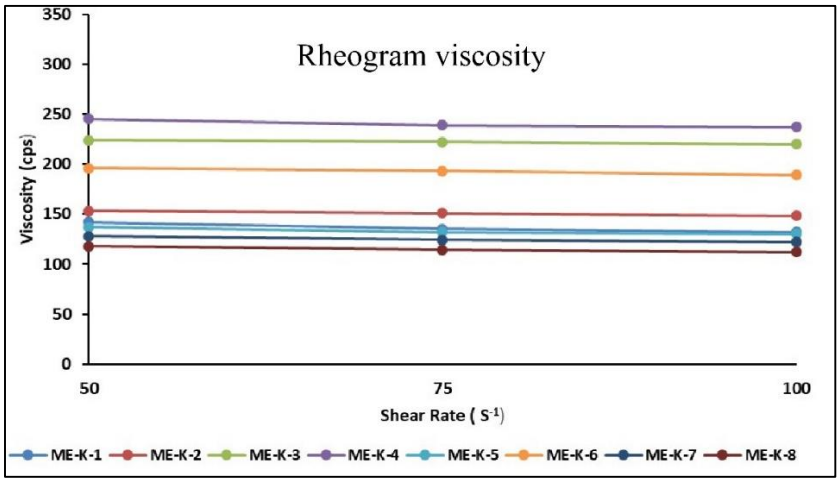

Figure 2. Rheogram viscosity (cps) versus shear rate of prepared MEs tested at 25◦C (n=3, Mean±SD).

The tear fluids have surface tension about approximately 44-50 dyne/cm at an eye surface temperature of 33^{0}C. The administration of any vehicle containing ingredients can lower surface tension, thereby disrupting the outer lipid layer of the tear film[19].

When the surface tension of ophthalmic solutions is much lower than the surface tension of the lachrymal fluid, tear film is destabilized. The surface tension of the ketorolac MEs was ranged from 31 to 38 dyne/cm. The low surface tension of MEs can be causes a suitable spreading on corneal surface and appropriate blending with the film of precorneal elements, thus probably correcting the contact of drug substances and the corneal epithelium layer. Such findings corroborate the previous report of Nair et al.[20].

Drug release profile revealed that a range of 23.65 - 38.641% of the ketorolac was released from prepared MEs in the 24 hours. The drug release percent and the kinetic of the release in the prepared formulations are displayed in Table 4. The prepared MEs follow the Higuchi and first release models.

Data analysis revealed that the relationship between released drug percent in the first 2 hours of experiment (R2h) with oil percentage is significant, it is also show that by increasing of the oil percent in ketorolac microemulsions, R2h increases.

Table 4. The kinetic of release and ketorolac release percent of the prepared MEs (Mean±SD, n=3).

Formulation	% Release(2h)	%Release(24h)	Kinetic of Release	%Water	r^2
ME-K-1	4.552±0.1	35.427±0.343	Higuchi	10	0.8474
ME-K-2	4.430±0.073	38.641±0.295	Higuchi	5	0.8054
ME-K-3	3.081±0.040	25.804±2.61	Higuchi	10	0.9161
ME-K-4	4.715±0.126	23.656±0.249	Higuchi	5	0.8670
ME-K-5	5.068±0.040	34.172±0.304	First	10	0.9390
ME-K-6	5.231±0.006	31.987±0.1	Higuchi	5	0.9475
ME-K-7	4.619±0.112	31.258±0.641	First	5	0.9552
ME-K-8	4.925±0.075	25.618±1.936	Higuchi	10	0.9836

2.4. Stability testing

Following the stability testing, we found that the whole of the MEs have suitable properties concerning their uniformity in droplet sizes and stable after 3 months' period. There was no significant correlation between size of droplet, viscosity and pH at the beginning and three-month after production of the MEs. There was no phase separation and precipitation on visual observation, also no change in clarity was observed. In the physical stability experiments, narrow polydispersity index amount was seen for MEs. In the previous studies, it was demonstrated that the physical stability of MEs was related to zero surface tension and thermodynamic law [21].

2.5. Transcorneal permeation

The corneal permeation parameters of prepared ketorolac MEs are shown in Table 5. In corneal permeation studies, the correlations of steady-state flux (Jss), lag time (Tlag), apparent corneal diffusivity coefficients (Dapp), and corneal permeability coefficients (P) with %Oil, %Water, and S/Co ratio were not significant.

Table 5. *Ex-vivo* permeation parameters of ketorolac tromethamine MEs through rabbit cornea (n=3, Mean±SD).

Formulation	Jss (mg/cm2 h)	TLag(h)	Dapp (cm^2/h)	P (cm/h)	ERflux	ERD	%P(6h)
Ketorolac Drop	0.035± 0.001	0.856± 0.023	0.001± 5.235	0.014± 0.0005	-	-	3.613± 0.134
ME-K-1	0.109± 0.002	0.350± 0.085	0.004± 0.001	0.043± 0.0009	3.042± 0.158	2.535± 0.547	9.041± 0.124
ME-K-2	0.125± 0.0002	0.134± 0.024	0.0126± 0.002	0.050± 0.0001	3.490± 0.124	6.464± 0.944	7.570± 0.020
ME-K-3	0.068± 0.010	2.452± 0.737	0.0007± 0.0002	0.027± 0.004	1.917± 0.329	0.371± 0.115	8.326± 0.042
ME-K-4	0.047± 0.002	4.844± 0.394	0.0003± 0.00002	0.019± 0.0009	1.33± 0.017	0.177± 0.013	8.094± 0.031
ME-K-5	0.099± 0.005	0.946± 0.129	0.001± 0.0002	0.039± 0.002	2.784± 0.237	0.915± 0.115	7.067± 0.083
ME-K-6	0.096± 0.006	0.297± 0.185	0.009± 0.008	0.038± 0.002	2.677± 0.118	4.650± 0.006	6.878± 0.117
ME-K-7	0.086± 0.004	1.466± 0.271	0.001± 0.0001	0.034± 0.001	2.406± 0.161	0.597± 0.110	7.897± 0.129
ME-K-8	0.051± 0.003	3.187± 0.514	0.0005± 0.00009	0.020± 0.001	1.419± 0.049	0.273± 0.047	7.230± 0.009

All of MEs increased in Jss parameters and %P6h. The amount of Jss in the ME-KT-2 formulation was 0.125 mg cm-2h-1, which is 3.49 times higher than that of ketorolac drops (KT 0.5%). The Dapp of ketorolac from ME-KT-2 was 0.0126 cm2h-1, which is 6.464 times greater than that of ketorolac drops.

Table 5 shows the accumulated permeat percentages of ketorolac through rabbit cornea over 6 hours for different MEs. The percent of drug permeated over 6 hours of permeability experiment (%P6h) was significant with oil percent variable. Therefore, it was concluded that %P6h increases when the oil phase percentage increases. The minimum and maximum %P6h values were obtained for ME-K-6 (6.878%) and ME-K-1 (9.041%).

The staining tests of ketorolac MEs were shown water-in-oil ME structures. Previous reports have showed that the oil in water microemulsions might be useful for increasing barrier permeability due to the attendance of surfactant and co-surfactant components [16]. In the present study, ketorolac ME formulations could have acted as penetration enhancers, thereby improving drug permeate through cornea. The obtained results are agreeing with those reported by U¨ stu¨ ndag-Okur et al.[21] who revealed that a special oil in water microemulsion increased the corneal absorption of ofloxacin.

In the present study, we used the rabbit cornea was used because of the its structural similarity between rabbit and human corneas[22]. The oleic acid oil phase in MEs can be enhanced in terms of P, Jss, Dapp, and drug permeated percentage in 6 hours (%P6h) in ME samples. Our findings support the previous work of Xiang-Chun Gao et al.[5]. Also, A study showed that oleic acid concentration in MEs is affected on hydrophilic and lipophilic drug molecules permeation through[23]. Also, oleic acid can cause microstructural changes in bio-barriers by forming diffusion routes for the drug molecules by extracting and disordering lipid bilayers[24, 25].

Surfactant (Tween 80 and Span 20) phases could enhance the permeation parameters and drug permeated percentage of ketorolac MEs. Alternatively, when permeability enhancement is desired, surfactant materials alter membrane properties by negating the protective properties of tear film and mucin, thus disordering the entirety of the epithelial layer by loosening tight junctions or epithelial cell membrane modification[25, 26].

The presence of Transcutol P (TP) in ketorolac MEs can increase the corneal permeation of drugs by altering the function of corneal barrier. Kuar and Smitha have indicated that an outer cell membrane composed of a phospholipid bilayer and a lipid membrane containing protein molecules are surrounded corneal epithelial cells [27]. TP is a surfactant substance, and the micelles it produces in the epithelial lipid bilayer might affect drug corneal permeation. Specifically, the micelles produced by TP can remove phospholipids from epithelial cell membranes, thereby increasing the transcorneal passage of drugs. High concentrations of TP improves the hydrophilic molecule permeation due to losing the epithelium structure membrane. However, TP might also hinder lipophilic molecule movement by creating a hydration barrier[28].

3. CONCLUSION

Delivering drugs to the eye is associated with several problems, such as lachrymal drainage and blockage by the corneal barrier. The MEs developed in the present work were within the range of acceptable droplet sizes for ocular use and possessed stability. Furthermore, the values recorded for the physicochemical parameters support their suitability for ophthalmic use. Additionally, the drug released percentage and transcorneal permeation of drugs from the prepared microemulsion were beneficial. Overall, the results of the present study indicate that MEs have favorable drug corneal permeability characteristics.

4. MATERIALS AND METHODS

4.1. Materials

Ketorolac tromethamine was gifted from Ramopharmin Company (IR Iran). Tween 80, span 20 and oleic acid were obtained from Merck (Germany). Transcutol P was provided from Gattefosse Company (France).

4.2. Animals

Male New Zealand white rabbits weighing 2.5-3.0 kg were used, which was approved by the Animal Ethical Committee, Ahvaz Jundishapur University of Medical Sciences (permit no: IR.AJUMS.ABHC.RES.1398.017).

4.3. Solubility of ketorolac

Solubility of Ketorolac was measured by dissolving an excess amount of Ketorolac in 5 mL of each oil, surfactant and co-surfactant components using a heater stirrer at 37˚C ± 0.5 for 24 h. Then, the equilibrated samples were then centrifuged at 10000 rpm for 30 min to emit the precipitated drug. Following that, the clear supernatants were filtered and the obtained solutions were analyzed using a UV spectrophotometer at 324 nm[29].

4.4. Phase diagram construction

Phase diagrams of free drug MEs were supplied to define the range of concentration of the substances for the existing zone of MEs and the two phase diagrams were constructed with the 1:1 and 3:1 mass ratios of Tween80:Span 20: Propylene glycol respectively. For preparing of each phase diagram, the surfactant/co-surfactant blended were added into the oil phase (Oleic acid:Transcutol-P) (10:1) at the mass ratios of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1. The prepared mixtures were vigorously mixed using a magnetic stirrer and then diluted drop by drop with double distilled water at 25^0C. The production of a clear liquid appearance was considered as the basis for creating microemulsion[30].

4.5. Ketorolac MEs preparation

After the ME boundary in the phase diagrams was defined, full factorial design was utilized regarding the 3 variables at 2 levels for preparing eight ME formulations. The main variables taken into account to determine the ME components quantities contain surfactant/co-surfactant ratio (S/Co), oil percentage (%Oil), and water percentage (%W). Eight ME samples with low and high levels of oil (5% and 50%), water (5% and 10%), and S/Co ratio (1:1 and 3:1) were determined for preparing MEs. Various MEs were prepared from the pseudo-ternary phase diagram with 1:1 and 3:1 mass ratio of Tween 80:Span 20/propylene glycol. (Table 2). Ketorolac (0.5%) was added to appropriate quantity of double distilled water and mixed as drop by drop to oily phase and S/Co blended by vigorously stirring the mixtures at ambient temperature until a clear liquid was formed[31].

4.6. Droplet size measurement

The range size of MEs was measured at 25^0C with a Scatter Scope 1 Quidix apparatus[32].

4.7. Viscosity determination

A Brookfield rheometer was utilized for measuring the viscosities of MEs at 25˚C[32].

4.8. Surface tension measurement

A torsion balance (White Elec Model NO. 83944E) was used for measuring the surface tensions of samples at 30˚C[32].

4.9. Stability experiments

The stability experiments were studied using a centrifuge stress test and temperature stability. MEs were stored in different temperatures (4˚C, 25˚C, 37˚C and 75% ± 5% relative humidity) according to the ICH protocols for three months and then assessed by monitoring time- and temperature-dependent variations of the physicochemical properties, such as phase separation, viscosity, precipitation, droplet size, pH and polydispersity index. In addition, the ME formulations were centrifuged at 15000 rpm for 30 minutes at 25˚C. After centrifugation, the instability of the ME samples was visually evaluated using the degree of phase separation[33].

4.10. Drug release

Vertical Franz diffusion cells (effective diffusion area 0.348 cm^2) with a cellulose membrane were utilized to measure the drug release amount of ketorolac from various MEs. Cellulose membrane was fixed between donor and receptor chambers. Ketorolac MEs (0.5g) were weighed and transferred on the membrane. Each receptor medium was filled with 10 mL of simulated tear fluid (STF, comprised of NaCl (0.67g), $NaHCO_3$ (0.2g), $CaCl_2$ (0.008g) and deionized water (100g). (pH =7.4), Externally driven magnetic beads were utilized for continuous stirring of the receptor medium. At predetermined time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h), a 1 mL sample was withdrawn from receptor chambers and then replaced immediately with an equal volume of fresh receptor fluid until to keep sink condition in the receptor chamber during studies (In the sink condition, the concentration of the drug in the receptor is less than 10% of the saturation concentration of the drug, and thus this condition is established) and analyzed using a UV spectrophotometer at 324 nm. Then, the percentages of drug release in different time intervals were plotted and behaviors were determined by fitting on various kinetic models[18].

4.11. The corneal permeability experiments

The eye cornea with a sclera ring was separated from a newly sacrificed male New Zealand Albino rabbits. The intact subcutaneous tissue was completely removed using scissor and scalpel. The prepared samples were kept in a DexSol solution (chondroitin-sulphate-based, commercial storage media) [34, 35]. The corneal permeability experiments were carried out using fabricated franz diffusion cells with a contact area of approximately 0.348 cm^2. The excised rabbit corneas were kept between the donor and receptor chambers so that they could face the receptor section without suffering any damage. The receiving phase consisted of 10 mL of simulated tear fluid (pH = 7.4), which was thermostated at 37±0.5°C and magnetically stirred at 200 rpm throughout the experiment. Then, 0.5 g ketorolac ME formulations (containing 0.5% of the drug) were accurately weighed and placed on the surfaces of corneas. The corneal permeability studies were carried out under non-occlusive conditions to allow air to permeate the corneal tissues. At predetermined interval times

(0.5, 1, 2, 3, 4, 5, and 6 h), a 1 mL sample was exited from the receptor medium for UV spectrophotometric analysis at 324 nm. The extracted samples were immediately replaced with an equivalent volume of fresh simulated tear fluid until to keep sink condition in the receptor chamber during corneal permeability studies. The free drug MEs were used as blanks. The same test for the ketorolac ophthalmic drop (0.5%) was carried out by an Iranian manufacturing company. Finally, the results were plotted in the form of cumulative permeated drug percent versus time[36-38].

4.12. Calculation of permeation parameters

The permeation parameters of the corneas were determined using obtained corneal permeation data, including flux (cornea permeation rate at steady state, Jss), corneal permeability coefficient (P), lag time (T_{lag}), and apparent diffusivity coefficient (Dapp). Flux was determined from the linear section of the slope of the permeability curve. The permeability coefficient (P, cm/s) was presented based on the equation P = Jss/CV, where Jss and Cv define the permeation rate at steady state and initial ketorolac concentration in the donor chamber, respectively. Meanwhile, the apparent diffusivity coefficient was determined using the equation Dapp = $h^2/6\ T_{lag}$. The lag time parameter was calculated by extrapolating the line of steady-state onto the time axis.

The enhancement ratio (ER) was determined to explain the relative improvement of the permeation parameters in ketorolac microemulsion samples concerning the ketorolac ophthalmic drop (0.5% drug) parameters. ER was measured by dividing the amount of permeation in the ME formulation by the amount of permeation in the ketorolac drop.

4.13. Statistics

For the design of experiments and the impact of variable parameters on the responses, Minitab 17 soft ware was utilized. . $P < 0.05$ indicated a significant diff erence.

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Conflict of interest statement: The authors declared no conflict of interest in the manuscript.

Ethics committee approval: All experiments conducted in this study were approved by he ethics committee of Jundishapur University of Medical Sciences with the approval number of IR.AJUMS.ABHC.REC.1398.017 on 2019-05-07.

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