Journal of Research in Pharmacy

Research Article

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Preparation and evaluation of levofloxacin microemulsion for ocular drug delivery

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Received: 3 January 2024 / Revised: 31 March 2024 / Accepted: 03 April 2024

ABSTRACT: Levofloxacin is a broad-spectrum antibacterial drug belonging to the third generation of fluoroquinolones. The project aims to develop a novel microemulsion system for effectively delivering levofloxacin to the eye. In addition, this study assessed the physical and chemical characteristics of a substance and its ability to permeate through the eyes of rabbits. The microemulsions (MEs) were evaluated for stability, drug release, viscosity, pH, particle size, and cornea permeability in rabbits. This study employed a three-variable design with two levels to prepare eight samples and analyze data. Based on research results, ME formulations had an average size ranging between 8.52 and 25.2 nm and a pH range of 4.45 to 6.01. Content viscosity ranged from 170-400 cps, and based on the drug release curve, 89.49% of the medicinal product was released within the first day of the trial. Drug sensitivity in rabbit cornea was highest in ME-LEV-5 (50.89%) and lowest in ME-LEV-7 (23.78%). This study demonstrates how the physical properties and permeability of the drug during drug penetration of ME formulations can be modified by changes in the quantity and quantity of ME. This phenomenon may be due to changes in the corneal structure caused by different ME components.

KEYWORDS: Corneal Permeability; Microemulsion; Levofloxacin; Rabbit, drug delivery.

1. INTRODUCTION

The human eye's complex anatomy and biology make external substances such as medications impervious. Due to several clearance pathways in the precorneal area, drug delivery to the eye is challenging and often results in limited medication efficacy. Despite numerous scientific advancements, pharmaceutical researchers still require assistance in developing effective methods for delivering drugs to the eyes [1-3].

Hoar and Schulman described MEs as colloidal dispersions that require surfactant and co-surfactant compounds to provide stability at the interfacial area between aqueous and oil phases [4]. The droplet diameter of these materials typically ranges from 10 to 100 nm. They are thermodynamically stable and optically isotropic. MEs essentially comprise four distinct phases: the oil phase, the aqueous phase, the surfactant, and the cosurfactant. ME is easily prepared, low in cost, and thermodynamically stable. Its small size and low surfactant tension facilitate absorption [5].

MEs have unique characteristics that make them suitable for ocular medication administration. MEs can accommodate both hydrophilic and lipophilic compounds. MEs are relatively stable and simple to manufacture and sterilize [5, 6]. Adding surfactant and co-surfactant to o/w MEs improves drug penetration and uptake through the biomembrane [7, 8]. MEs, characterized by their low viscosity and thermodynamic stability, consist of a harmonious blend of oil and water, often partnered with a cosurfactant for stabilization purposes. The utilization of MEs in drug delivery has been found to offer numerous benefits, such as facile manufacturing, robust stability, enhanced solubility of drugs, regulated drug release, and improved bioavailability of both hydrophilic and lipophilic agents across diverse delivery modalities [9].

In the 1950s, Schulman and Winsor were the first to observe MEs [10]. "Microemulsions" refers to a combination of water-based, non-polar, surfactant, and cosurfactant components. The three main types of conventional MEs are oil-in-water, water-in-oil, and bicontinuous phase [11]. In addition to improving the

How to cite this article: Koushki E, Salimi A, Loveimirad K. Preparation and evaluation of levofloxacin microemulsion for ocular drug delivery. J Res Pharm. 2025; 29(1): 407-417.

ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1644619

solubility of poorly soluble medications, MEs also increase bioavailability, protect unstable drugs from environmental factors, and have an extended shelf life [5].

The intraocular tissues of the eye are mainly protected by the cornea, which acts as a mechanical and chemical barrier. Both hydrophilic and lipophilic features distinguish the cornea, which acts as a powerful barrier against the absorption of both types of chemicals[12, 13]. Bioavailability decreases due to the strong barrier of the cornea and the rapid expulsion of medication solution injected into the precorneal region. Factors like tear generation, ineffective absorption, short residence time, and corneal epithelial impermeability limit the bioavailability of medications from ocular dosage forms. The bioavailability of lipophilic molecules in the cornea is predicted to range from 1% to 5%, while that of hydrophilic substances is expected to be less than 0.5% [14-17].

The most commonly prescribed antibiotic for ocular infections is levofloxacin, a broad-spectrum fluoroquinolone of the third generation that can penetrate deeply. The antibiotic under consideration exhibits a broad spectrum of activity, coupled with favorable pharmacokinetic properties such as efficient absorption into both aqueous and vitreous fluids and extended half-life. Notably, it is effective against anaerobic and aerobic Gram-positive and Gram-negative bacteria, including Chlamydia, Mycoplasma, Legionella, Staphylococcus aureus, and methicillin-sensitive streptococci. Its therapeutic utility extends beyond managing bacterial keratitis and conjunctivitis to encompass the area of surgical prophylaxis [18-20]. Levofloxacin operates by inhibiting the targeted bacterium's two critical enzymes, DNA gyrase and topoisomerase-IV. This action impedes bacterial DNA replication, ultimately leading to bacterial cell death [19-21].

The primary objective of the present study is to devise a novel technique for dispensing Levofloxacin (LEV) to the ocular region and subsequently investigate its physicochemical attributes and corneal permeability in rabbits, to optimize the drug's penetration. The method shall be evaluated based on its ability to enhance the distribution of LEV within the target area whilst minimizing any adverse effects that may arise from its application.

2. RESULTS AND DISCUSSION

2.1. Solubility of Levofloxacin

Table 1 presents the solubility of Levofloxacin. To develop and design ME formulations, it was necessary to identify a suitable oil that would allow the precise amount of Levofloxacin to dissolve. Through a series of solubility experiments, it was determined that the optimal combination for producing Levofloxacin ME consists of oleic acid-Transcutol P (in a 10:1 ratio), Tween 80, Span 20, and PG as surfactants and co-surfactants.

Table 1. Solubility of Levofloxacin in Various Oils, Surfactants, and Cosurfactants (Mean ± SD, n = 3)

Phase type	Excipient	Solubility(mg/ml)
Oil	Transcutol p	6.494±0.125
	Oleic Acid	2.01±0.001
	Oleic Acid+ TP (10:1)	6.9±0.1
Surfactants	Tween 80	1.15±0.3
Cosurfactant	Span 20	0.01±0.001
	Propylene Glycol	1.825±0.1

2.2. Phase diagrams behavior

The behavior of ME is influenced by the mass ratios of the surfactant and co-surfactant, as depicted in Figure 1. This figure illustrates the pseudo ternary phase diagrams of water, Tween 80, Span 20, propylene glycol, and Transistor P (10:1). It is important to note that the quantity of surfactant used is positively correlated with the size of the ME region as indicated by previous studies [22]. The phase diagrams show that the ME area (km = 1-2) extends considerably in the surfactant/co-surfactant weight ratio. The phase diagrams also reveal an increase in the ME area with the S/C ratio.

2.3. Characterization of the Levofloxacin MEs

A selection of eight specific Tween 80-Span 20/PG MEs featuring size ratios of 1:1 and 2:1 were carefully chosen from the pseudoternary phase diagram. The composition of the selected MEs has been

presented in Table 2, while Table 3 provides detailed information regarding the pH, particle size, viscosity index (PI), and viscosity of levofloxacin ME. The ME samples in this study boasted an average viscosity range of 170-400 cps, pH of 4.45-6.01, and particle size of 8.52-25.2 nm. The relationship between size and individual characteristics is insignificant if P > 0.05. According to the analysis of variance, the difference in S/C was significantly related to pH (P<0.05). Notably, in some MEs, the pH increases while the S/C level stabilizes.

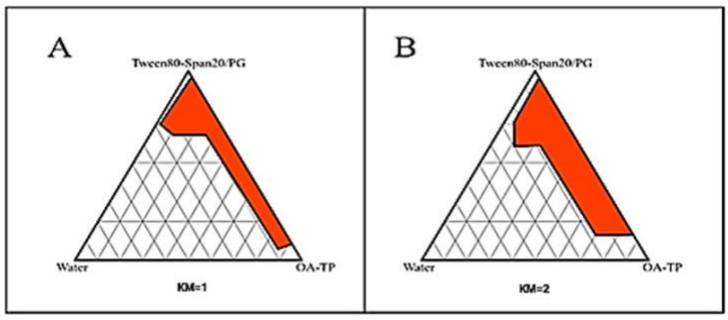

Figure 1. Pseudo-ternary phase diagram of the oil-surfactant/co-surfactant mixture-water system at 1:1 and 2:1 weight ratio or Tween 80/Span 20/PG at low temperature, with dark areas indicating the ME region.

2.4. Composition of selected MEs of Levofloxacin

The results of the ME formulations demonstrated that the average range of 170-400 cps, pH value of 4.45-6.01, and particle size of 8.52-25.2 nm were within the expected parameters. Our findings from the variance analysis indicate that there is no significant correlation between the independent variables and the mean particle sizes. It is noteworthy that particle size plays a crucial role in the nanoscale drug delivery systems, and reducing particle size can lead to a considerable increase in surface area, which can enhance bioavailability [23-26]**.** The current study employed ME formulations that exhibit droplet sizes of less than 30 nm. Notably, the droplet sizes of the prepared MEs are significantly smaller than the ten m-sized particles, which can potentially cause skin irritation [26-28]**.** Our research findings indicate that the homogeneity of droplet size in ME samples is defined by the polydispersity value, which was consistently below 0.5. This suggests that there is limited variation in droplet size in these samples. An analysis of variance revealed a significant association between the independent variable S/C and pH. Precisely, in some MEs, an increase in

Table 2. The composition of prepared MEs of Levofloxacin.

Formulation	Factorial Design	(S: C)	% Oil	%S+C	%Water	%Drug percent
ME-LEV-1	+ + +	2:1	40	49.5	10	0.5
ME-LEV-2	+ + -	2:1	45	49.5	5	0.5
ME-LEV-3	+ - +	2:1	85	4.5	10	0.5
ME-LEV-4	+ - -	2:1	90	4.5	5	0.5
ME-LEV-5	- - +	1:1	85	4.5	10	0.5
ME-LEV-6	- - -	1:1	90	4.5	5	0.5
ME-LEV-7	- + -	1:1	45	49.5	5	0.5
ME-LEV-8	- + +	1:1	40	49.5	10	0.5

pH corresponded with a decrease in the proportion of the S/C phase. These results are in alignment with earlier reports [29-31]. The pH of all ME formulations was observed to be approximately 6.5. The current study's analysis of variance on viscosity revealed a significant correlation between the independent variable (%Oil) and viscosity. It was noted that MEs with less oil phase of Levofloxacin tend to increase viscosity. These results are consistent with previous research findings by other experts in the field [32-35]. Our study indicates that the permeation of medicine through the cornea can be enhanced through increased viscosity by reducing the preocular retention time. Notably, all the MEs systems created for our study exhibited higher viscosity than an aqueous suspension [36-38].

Table 3. pH, Viscosity, Mean Particle size, PolydispersityIndex of selected Levofloxacin MEs (means, n=3)

Formulation	pH	Viscosity(cps)	Mean Particle Size(nm)	Mean droplet Size after 6 months(nm)	Polydispersity Index
ME-LEV-1	4.45 ± 0.1	243 ± 2.5	15.55 ± 2.1	16.1±0.15	0.416 ± 0.002
ME-LEV-2	4.58 ± 0.2	196 ± 1.5	18 ± 0.2	18.6±0.3	0.433 ± 0.003
ME-LEV-3	5.25 ± 0.1	388 ± 2	23.26 ± 1.63	24.1±1.5	0.416 ± 0.002
ME-LEV-4	5.66 ± 0.3	400 ± 1.6	20.07 ± 1	21±0.8	0.342 ± 0.001
ME-LEV-5	6.01 ± 0.2	327 ± 1.3	11.83 ± 0.1	12.1±0.3	0.408 ± 0.002
ME-LEV-6	6 ± 0.1	319 ± 1.5	25.2 ± 0.7	25.8±0.4	0.452 ± 0.004
ME-LEV-7	4.86 ± 0.1	170 ± 1.1	8.52 ± 0.1	9.1±0.2	0.418 ± 0.002
ME-LEV-8	4.51 ± 0.2	177 ± 0.9	11.25 ± 0.2	11.5±0.3	0.390 ± 0.001

The graphical representation of levofloxacin ME's release profile is depicted in Figure 2. As per the drug release profile analysis of MELEV-3, it was observed that 89.49% of the drug was released during the 24-hour testing period. The MEC-3 analysis presents the Higuchi dynamics. For a few ME samples, Table 4 provides information concerning their percentage drug release and release kinetics.

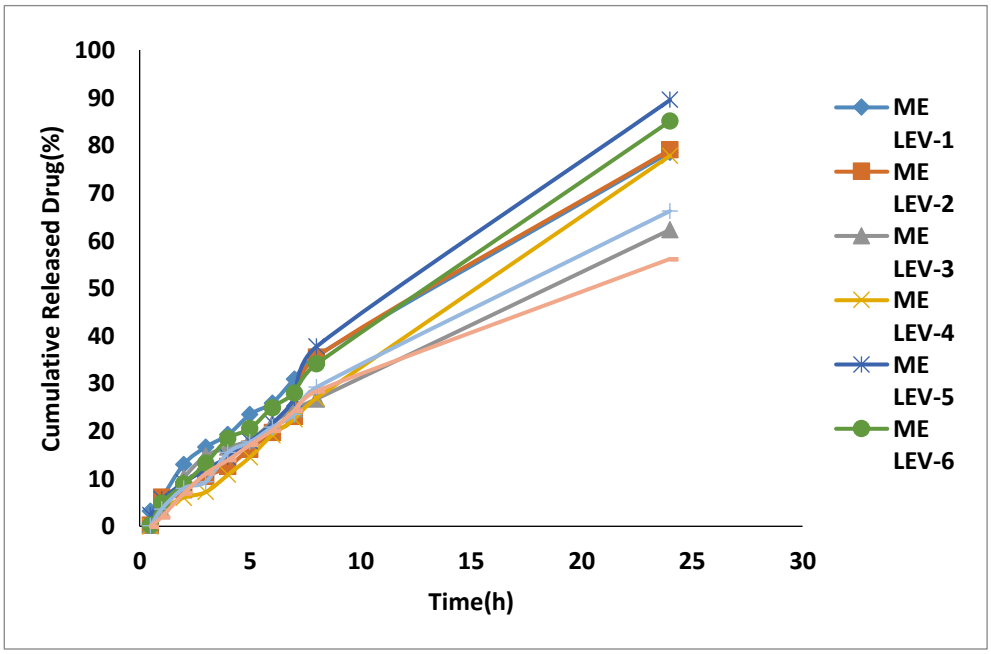

Figure 2. In vitro Release Profile of MEs Formulation of Levofloxacin.

Table 4. Percentage release and kinetic release of selected MEs (mean±SD, n=3).

Formulation	% Release(24h)	Kinetic of Release	R^2	%Release(2h)
ME-LEV-1	78.52 ± 0.53	Hixon	0.981	13.04 ± 0.29
ME-LEV-2	79.04 ± 1.72	3/2 root of mass	0.982	7.69 ± 0.19
ME-LEV-3	62.25 ± 2.03	First	0.972	10.21 ± 0.12
ME-LEV-4	77.79 ± 2.62	Zero	0.997	6.03 ± 0.21
ME-LEV-5	89.49 ± 3.37	3/2 root of mass	0.990	9.13 ± 0.33
ME-LEV-6	85.05 ± 3.92	3/2 root of mass	0.992	8.87 ± 0.49
ME-LEV-7	66.19 ± 1.49	Hixon	0.992	7.99 ± 0.56
ME-LEV-8	56.14 ± 1.33	Log Wagner	0.992	6.92 ± 0.39

The results of the analysis conducted on the levofloxacin samples have revealed a positive correlation between the drug release (R2h) and the percentage of fat during the first two hours, which consequently leads to an increase in R24h. Furthermore, it has been observed that the drug release at 24 hours (R24h) is related to another independent variable, namely the percentage of water. The findings of the study suggest a positive correlation between the percentage of R24h and the decreasing water level. It is important to note that the study utilized small-sized levofloxacin ME droplets that helped in their swift release [39-43].

The results of the permeability experiments revealed that there was no statistically significant relationship between Papp and the independent parameters (Table 5). However, the Jss of Levofloxacin from MEC-5 was found to be 1.0050.042mg cm-2h-1, which is 14.56 times greater than that of Control (Levofloxacin suspension, 0.5%). It was noted that Jss and the independent variables (%Oil) exhibit a significant connection (P 0.05). As such, any decrease in the proportion of the oil phase resulted in a considerable increase in the Jss parameters [44].

A decrease in the percentage of the water phase has been observed to result in a considerable increase in the Tlag parameter, as there is a significant correlation between Tlag and independent variables (%Water). However, there was no statistically significant relationship found between the apparent diffusivity coefficients (Dapp) and independent variables. Based on our findings, the Dapp and Papp parameters were found to be 7.23 times higher in MEC-3 and MEC-5 formulations, as compared to the Control (Levofloxacin suspension, 0.5%), at 0.0233 cm2h-1, 0.13cmh-1, and 46.62, respectively. It was also observed that a significant association exists between ERflux and the independent variables %Oil and s/c ratio, and any change in the oil phase percentage and S/C ratio leads to a significant increase in the ERflux parameter. However, there was no statistically significant connection found between ERp and ERD and independent factors [43-45].

The data presented in Figure 3 illustrates the percentage penetration of levofloxacin through rabbit cornea over a period of 7 hours, as observed with different MEs. It was observed that there existed a significant relationship between the drug penetration percentage and the independent variable, i.e. % oil, for the time interval between 2 hours (%P2h) and 7 hours (%P7h). Specifically, with an increase in the oil level, the percentage of drug penetration increased from P2h to P7h. The maximum and minimum values of P7h% were recorded with ME-LEV-7 (23.78%) and ME-LEV-5 (50.89%), respectively.

The results of our study indicate that not all MEs have a noticeable effect on drug diffusion through the skin. However, all MEs tested showed an increase in drug efflux through the rabbit cornea via diffusion. Moreover, we found that ME formulations with varying compositions and properties can increase the distribution coefficient, flow coefficient, and permeability coefficient of the rabbit cornea. Levofloxacin ME staining tests produced oil-in-water ME samples. Prior research has suggested that oil-in-water ME may be useful as the inclusion of surfactants and co-surfactants in the formula enhances barrier permeability [8, 46, 47].

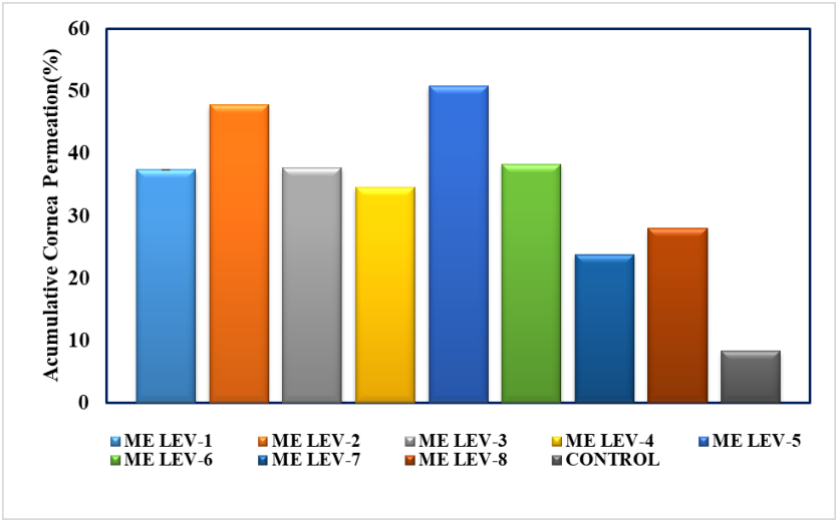

Figure 3. Levofloxacin Cornea permeation percent from various ME formulations after 7 hours (%P5h). (n=3, Mean±SD)

The present study evaluates the potential of Levofloxacin ME formulations as permeation enhancers to enhance corneal drug delivery. Our results are in alignment with prior research conducted by P Jain et al [47]. The study conducted shows that the application of a specific oil-in-water ME system enhances the absorption of pilocarpine in the cornea. In order to explore drug delivery methods, the rabbit cornea model was used due to its similarity to human corneas [48].

Table 5. Ex-vivo Permeability Parameters of Levofloxacin ME Formulations Through Rabbit Cornea (n=3, Mean±SD)

Formulation No	J_{ss}, mg/cm² h	T_{Lag}, h	D_{app}, cm2/h	P_{app} (cm/h)	ER_{flux}	ER_D	ER_P
Levofloxacin Drop	0.069±0.003	3.171±2.185	0.00101±0.00109	0.027±0.001	-	-	-
ME-LEV-1	0.106±0.065	2.904±0.674	0.00059±0.00016	0.042±0.026	1.50±0.85	1.18±0.84	1.50±0.84
ME-LEV-2	0.228±0.005	3.899±0.065	0.000428±0.0000071	0.091±0.002	3.32±0.22	0.81±0.55	3.32±0.22
ME-LEV-3	0.552±0.003	3.744±0.013	0.000445±0.0000015	0.220±0.001	8.01±0.41	0.84±0.58	8.01±0.41
ME-LEV-4	0.712±0.008	3.157±0.016	0.000528±0.0000027	0.284±0.003	10.34±0.55	1.01±0.69	10.34±0.55
ME-LEV-5	1.005±0.042	3.937±0.014	0.000423±0.0000015	0.402±0.017	14.59±0.72	0.80±0.55	14.59±0.72
ME-LEV-6	0.415±0.017	3.906±0.028	0.000427±0.0000031	0.166±0.006	6.03±0.4	0.81±0.55	6.03±0.4
ME-LEV-7	0.597±0.008	3.884±0.020	0.000429±0.0000022	0.238±0.003	8.66±0.29	0.81±0.56	8.66±0.29
ME-LEV-8	0.767±0.014	3.616±0.422	0.000465±0.000058	0.306±0.005	11.13±0.13	0.92±0.69	11.13±0.13

2.5. Stability testing

Upon conducting a stability analysis, it was determined that all MEs possessed the requisite water content and remained stable for a period of six months. Our findings indicate that there exists no correlation between water content, viscosity, and pH at the outset of ME production and three months thereafter. Furthermore, a visual inspection revealed no evidence of phase separation, precipitation, or change in

clarity. It is noteworthy to mention that the polydispersion indexes were observed to be narrow in the physical stability test. It is also worth noting that prior research has established a direct link between the physical stability of MEs and zero voltage as well as the principles of thermodynamics.

3. CONCLUSION

According to recent research, the physicochemical characteristics, chemical properties, and in vitro release of Levofloxacin into the rabbit cornea are significantly impacted by the overall composition of surfactant and co-surfactant (s+c), water, and oil in the ME. This finding highlights the importance of carefully considering the composition of MEs to optimize their properties for drug delivery applications.

4. MATERIALS AND METHODS

4.1.Materials

The levofloxacin powder was procured from the well-known IR Iranian firm, Abidi. We purchased Oleic acid, Tween 80, and Span 20 from Merck (Germany), while Transcutol P, also known as diethylene glycol monoethyl ether, was generously provided as a gift from the esteemed French company, GATTEFOSSE. All substances utilized for the experiments were strictly of analytical grade. Pure double distilled water was employed for the experiments. To facilitate the experiments, we procured a dialysis bag from Tuba Azma Co. in Tehran, Iran.

4.2.Animals

This research was conducted under the endorsement of the Animal Ethics Committee of the University of Medical Sciences, Jundishapur, Ahvaz, with the usage of male New Zealand white rabbits that weighed between 2-2.5 kg. The license number for this study is IR.AJUMF.REC.1395.131.

4.3.Levofloxacin Assay

The quantity of levofloxacin was measured using a UV spectrophotometer manufactured by WPA, a reputable English company, at a precise wavelength of 286 nm. Method validation is performed and LOQ was 0.001mg/ml.

4.4.Solubility of Levofloxacin

The objective of this study is to investigate the solubility of excessive levofloxacin in various oils, surfactants, and co-surfactants. Specifically, the oils include oleic acid, Transcutol P, and a combination of oleic acid and Transcutol P (10:1), while the surfactants used are Tween 80 and Span 20, and propylene glycol serves as the co-surfactant. The method entails mixing three milliliters of the oil and other ingredients using an IKA magnetic stirrer, and then stirring the mixture at 37°C for 0.5 to 72 hours. Afterward, the samples are centrifuged using an MPW centrifuge at 5000 rpm for 30 minutes to eliminate the remaining solution, and the supernatant is decanted. The solubility of levofloxacin was determined using reliable UV spectrophotometry, prepared by the esteemed English company WPA, at 286 nm.

4.5.Construction of Phase Diagram

In order to determine the concentration range of each of the current limits of the drug-free ME, it was necessary to plot the pseudo-ternary phase diagram of the surfactant, cosurfactant, and oil and water mixture titrimetrically at room temperature. Two graphs were plotted at ratios of 1:1 and 2:1, respectively, using Tween80-Span 20/propylene glycol. For each image phase, the oil phase (oleic acid-Transcutol-P) and surfactant mixture were combined in various ratios ranging from 1:9 to 9:1. The resulting mixture was diluted with double distilled water at 25 ± 1°C with moderate stirring. When the sample became a clear liquid, it was classified as a ME [49].

4.6.Preparation of MEs

A ME formula was developed for the selected drawing samples based on the composition outlined in Table 2. The ME quality was determined by important factors such as the surfactant/co-surfactant ratio (S/C), oil percentage (%Oil), and water percentage (%W). To generate the ME formulations, eight different ME formulations were created using a two-level three-variable full factorial design. These formulations consisted of low and high oil (5% and 50%), water (5%, 10%), and S/Co mixing ratios (1:1, 2:1). One specific formulation was selected for use. The oil phase was prepared by adding levofloxacin (0.5%), followed by the

addition of S/C, which was double-distilled water. The mixture was then rotated at room temperature until the components were fully blended [29, 49].

4.7.Droplet Size examination

The droplet size of MEs was determined using a particle size analyzer and dynamic light scattering technique at room temperature. The analysis was performed using SCATTER SCOPE 1 QUIDIX, South Korea.

4.8.Viscosity and pH Measures

The pH level of the ME present in the sample was promptly determined at room temperature with the aid of a digital pH meter, specifically the Mettler Toledo Seven Simple model from Switzerland. Additionally, the viscosity of the sample was measured via a No. 34 Brookfield viscometer (DV-II + Pro Brookfield, USA) at a temperature of 25°C.

4.9.Release of MEs

The objective of the present study was to determine the rate of levofloxacin release from different MEs using a Franz diffusion cell manufactured by the esteemed Iranian company Tuba Azma, with a cellulose membrane of 3.4618 cm^2 surface area. Prior to each experiment, the cellulose membrane underwent a 24-hour hydration process in double distilled water at 25°C before being placed between the donor and recipient compartments. A precisely measured 5g sample of levofloxacin ME was applied to the membrane, and a buffered phosphate solution (25 ml, pH = 7.3) was introduced into each diffusion cell. Throughout the test, an externally driven magnetic rod rotating at 200 rpm constantly agitated the receptor liquid. At specific time intervals (0.5, 1, 2, 3, 5, 6, 7, and 24 hours), 1 ml of the receptor medium was sampled for spectrophotometric analysis, and immediately replaced with half of the equal volume of fresh receptor medium to maintain the initial volume. The samples were analyzed using a UV spectrophotometer at 286 nm, and the percentage of drug released was plotted against time. The release rates of drugs over time were plotted and subjected to various kinetic models to explain their behavior. The model with the maximum r^2 value was considered indicative of the most likely release mechanism [49].

4.10.Physical Stability of MEs

The physical stability of each ME sample was evaluated using centrifugal pressure and temperature tests. As per the recommendations of International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), the MEs were subjected to storage at different temperatures (4°C, 25°C, 37°C, and 75% 5% RH) for six months. After the storage period, physical changes, including transparency, odor, color, phase separation, pH, were evaluated. It is worth noting that viscosity and particle size are temperature-dependent. The ME was centrifuged at 12000 rpm for 30 minutes at room temperature using a high-speed brushless centrifuge (MPV-350R, Poland). The level of phase separation after centrifugation was used as a measure of the physical stability of the sample [50, 51].

4.11.The Ex vivo cornea permeation experiments

The study involved the use of male New Zealand albino rabbits, whose corneas and sclera rings were excised and carefully prepared for analysis. The corneas were preserved in a DexSol solution, which is a chondroitin sulfate-based commercial storage media that provides protection for corneal epithelium. Modified Franz diffusion cells were employed for the ex vivo cornea permeation study, with an effective diffusion area of roughly 0.348 cm2. The excised rabbit corneas were placed between the donor and receptor compartments of the cell to prevent damage to the sclera ring constricted between two chambers and the cornea facing the receptor. Levofloxacin ME samples weighing precisely 0.5g and having 0.5% medication were applied to the corneas under non-occlusive conditions to allow air to reach the corneal tissues. A spectrophotometer was used to measure the amount of medicine that permeated the corneas at predetermined intervals, with a drug-free ME serving as the control. The results were plotted to show cumulative permeated drug percent with time [52, 53].

4.12.Calculation of permeation parameters

The evaluation of corneal penetration data involves the assessment of corneal flux (Jss), permeability coefficient (P), delay time (Tlag), and diffusion coefficient (D). In order to calculate steady-state bone

penetration (Jss, mg/cm2h), the linear combination of the slope of the penetration curve is utilized. It should be noted that the thickness (h) of the cornea does not provide an accurate indication of the drug's route of entry. As such, appearance D (Dapp) is defined as the diffusion coefficient. The formulas (Papp = Jss/C0) and (Dapp = h2/6 Tlag) are not applicable for determining the apparent diffusivity (Dappcm2/h) and apparent transmittance (Papp, cm/s), respectively. The time delay (tlag, hour) can be calculated by projecting the solid line onto the time axis.

4.13.Statistical analysis

The findings have been presented in terms of the mean value and standard deviation obtained after conducting three iterations of each experiment. The statistical significance level was set at P0.05 with a 95% confidence interval for the one-way analysis of variance (ANOVA), which was utilized to identify any significant changes that occurred.

Acknowledgements: This article was extracted from the Pharm.D thesis (koushki) and was sponsored by Ahwaz University of Medical Sciences, Jundishapur, Ahwaz.

Author contributions: Concept – A.S.; Design – A.S., E.K.; Supervision – A.S.; Resources – A.S., E.K.; Materials – A.S.; Data Collection and/or Processing – A.S., E.K.; Analysis and/or Interpretation – A.S.; Literature Search – A.S., K.L., E.K.; Writing –AS., K.L.; Critical Reviews – A.S., K.L.

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

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