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Original Article

Brinzolamide-Loaded Nanoemulsions: *In vitro* Release Evaluation

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Abstract

The aim of this investigation was to design and develop nanoemulsions (NEs) as novel ophthalmic delivery systems for brinzolamide (BZD). Phase behavior of quaternary systems composed of triacetin and Capryol™ 90 (selected oils, screened through the solubility studies), various surfactants (namely, Cremophor RH 40, Brij® 35, Labrasol® and tyloxapol), Transcutol® P (as co-surfactant) and water at different surfactant/co-surfactant weight ratios (R_{sm}) was investigated by the construction of phase diagrams. Formulations were taken from the o/w NE region on the phase diagrams, depending on the extent of NE domain. The spontaneous emulsification method was used to prepare various formulations containing 0.4 wt% of the drug. The nanosized character of NEs was evaluated, the cumulative drug release from of the selected formulations was determined for a period of 6 h, using a dialysis sac technique and the release efficiency for NEs was calculated. The therapeutic efficacy of the selected BZD-loaded NEs to lower the IOP was assessed by the calculation of various pharmacodynamic parameters (ie., E_{max}, T_{max} AUC_{0-6h}). In all cases, the average size of the droplets was found to be less than 40 nm. *In vitro* release studies indicated that the release efficiency in most of the NEs was higher as compared to the BZD ophthalmic suspension. Comparison of the pharmacodynamics parameters confirmed the equivalent efficacy of most BZD formulations.

Keywords: Nanoemulsion; Phase diagram, Brinzolamide, Ophthalmic delivery, Biological studies, Release efficiency.

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1. Introduction

Topically active carbonic anhydrase inhibitors (CAIs) have been developed for the treatment of glaucoma. Brinzolamide (BZD) is a topical CAI which exhibits selectivity, high affinity, and potent inhibitory activity for the carbonic anhydrase type II isozyme (CA-II), involved in aqueous humor

secretion. These characteristics have made BZD an effective drug in lowering the elevated intra-ocular pressure (IOP) associated with open-angle glaucoma or ocular hypertension. BZD is a poorly soluble powder and, therefore, has been developed and supplied as a sterile, aqueous ophthalmic suspension, formulated at physiological pH in an attempt to provide an ocular comfort profile. Its formulation has been optimized to provide a high degree of tolerability upon instillation, leading to a higher compliance rate by the patient. Preclinical pharmacologic evaluation studies have indicated that BZD acts specifically to inhibit CA without significant pharmacologic actions that could introduce undesired side effects. Although following topical ocular administration, BZD is absorbed into the systemic circulation; however, due to the therapeutic dose and low systemic absorption, its concentrations is very low and generally below assay quantitation limits and hence, the typical side effects associated with systemic administration occur at a lower incidence. The long tissue half-life in the eye favors a prolonged duration of IOP lowering [1].

Nanoemulsions (NEs) are lipid-based formulations with droplet size in the nanometric range and have attracted much attention due to their high potential in pharmaceutical applications. Although NEs and microemulsions are generally described in the literature indiscriminately, they are fundamentally different, particularly from thermodynamic stability point of view [2, 3]. Various definitions have been proposed in the literature regarding NEs. Generally accepted, NEs are transparent (or translucent), dispersions of water and oil stabilized by an interfacial film of combined surfactant and possibly co-surfactant molecules, and having the droplet size in the range of 50-200 nm (the upper limit depends upon the authors). NEs, also referred to as miniemulsions, are systems with ultralow interfacial tensions and unlike microemulsions (which are thermodynamically stable), they possess a relatively high kinetic stability [3-5] with no apparent droplet flocculation, coalescence, and creaming [2, 6, 7-9], so that they are sometimes described as "Approaching Thermodynamic Stability" [9]. Moreover, the large interfacial areas associated with the presence of nanosized droplets influencing the transport properties of the solubilized drug [10], high solubilization capacity, long term physical stability, ease of preparation and sterilization, improving solubility, bioavailability and mucosal permeability of poorly water soluble compounds [11-13], sustained release properties [14, 15] and ability to protect drugs from hydrolysis and enzymatic degradation in physiologic conditions [16-18] are among the advantages offered for nanoemulsions over unstable dispersions.

The aqueous suspension of BZD has a low bioavailability due to the need for solubilization of particles on the surface of the eye before the precorneal elimination processes occurs. On the other hand, the ophthalmic formulation of BZD is not a clear solution and one of the most frequently reported adverse events associated with the ophthalmic suspension was blurred vision and ocular discomfort following dosing. To the best of our knowledge, very few reports have been published investigating the improvement of BZD

bioavailability and therapeutic efficacy. Tuomela and his co-workers studied the application of nanocrystal-based drug delivery systems for ocular formulation development for poorly water soluble drugs. They formulated an ophthalmic, IOP reducing, nanocrystal suspensions from BZD, using various stabilizers, and investigated their IOP reducing effect *in vivo*. They showed that various BZD nanocrystal formulations had advantageous dissolution and absorption behavior and that the reduction achieved in experimentally elevated IOP was comparable to that obtained with a marketed product [19]. The main goal of the present investigation was to develop and characterize oil-in-water (o/w) NEs as novel ophthalmic delivery systems for BZD and evaluate their *in vivo* therapeutic efficacy. It was hypothesized that by loading BZD in NEs, its bioavailability would increase. As the preliminary step, the partial phase behavior of systems containing triacetin and Capryol™ 90 (as oils), various surfactants (namely, Cremophor RH 40, Brij® 35, Labrasol® and tyloxapol), Transcutol® P (as co-surfactant) and water at different surfactant/co-surfactant weight ratios (R_{sm}) was mapped on the triangle phase diagrams. Based on the phase behavior studies, various BZD-loaded NEs were then prepared using spontaneous emulsification method and their particle size and the release profile of the drug were investigated.

2. Materials and Methods

2.1. Materials

Triacetin (glycerol triacetate), isopropyl myristate (IPM) and methanol were purchased from Merck Chemical Company (Germany). Brij® 35 and tyloxapol were provided from Sigma-Aldrich (USA). Labrasol®, Transcutol® P, Capryol™ 90 (propylene glycol monocaprylate, type II, NF), Labrafac™ PG (propylene glycol dicaprylate/dicaprate NF) and Labrafac™ Lipophile WL1349 (medium chain triglycerides NF) were obtained as gift samples by Gattefossé (France). Cremophor® RH40 and BZD were gifted by Osvah Pharmaceutical Company (Tehran, Iran) and Bachem (Switzerland), respectively. Purified water was prepared by Milli-Q Plus Water Purification System (Millipore, France).

Table 1. Constituents of blank nanoemulsions.

Group	Oil	Surfactant	Co-surfactant
I	triacetin	Brij® 35	Transcutol® P
II	Capryol™ 90	Brij® 35	Transcutol® P
III	triacetin	Cremophor® RH40	Transcutol® P
IV	Capryol™ 90	Cremophor® RH40	Transcutol® P
V	triacetin	Labrasol®	Transcutol® P
VI	Capryol™ 90	Labrasol®	Transcutol® P
VII	triacetin	tyloxapol	Transcutol® P
VIII	Capryol™ 90	tyloxapol	Transcutol® P

2.2. Determination of Oil Solubility of BZD

Briefly, excess amounts of BZD were added to 1 mL of the various oil, namely triacetin, isopropyl myristate (IPM), Labrafac™ Lipophile WL1349, Labrafac™ PG, and Capryol™ 90). The mixtures were stirred for 72 h at room temperature to reach the equilibrium. After achieving equilibrium and to remove the excess amounts of the drug, samples were centrifuged at 12000 rpm for 10 min. The supernatant was filtered through a 0.2 µm membrane filter, diluted with a suitable solvent and the solubility of BZD in the oils was measured by UV spectrophotometer at 254nm.

2.3. Construction of Partial Pseudo-Ternary Phase Diagrams

Following the determination of BZD solubility in various selected oils, pseudo-ternary phase diagrams of quaternary systems composed of oils, surfactants, co-surfactant, and water at different surfactant/co-surfactant weight ratios (R_{sm}) were constructed in an attempt to identify the oil-in-water (o/w) nanoemulsion domains (Table 1). Appropriate amounts of the oil (triacetin and Capryol™ 90), surfactants (Cremophor RH 40, Brij® 35, Labrasol® and tyloxapol) and Transcutol® P (as co-surfactant) were weighed into screw-capped vials and the samples were mixed at room temperature until a clear solution was obtained. Phase diagrams were constructed by titrating these samples with aliquots of triple distilled water and stirring for a sufficiently long time to attain equilibrium. The course of each titration was monitored both visually for clarity, and through cross-polaroids in order to determine the boundaries of any birefringent liquid crystalline phase. The top apex of the phase diagrams represented a fixed surfactant/co-surfactant weight ratio (R_{sm} of 1;1, 1:2 or 2:1), whereas the other two apices represented oil and water. All mixtures produced optically transparent or translucent, non-birefringent solutions at low oil domains termed o/w NEs.

2.4. Preparation of BZD-loaded Nanoemulsions

Pseudo-ternary phase diagrams were constructed to obtain the components and their concentration ranges which can result in the formation of NEs. Following the identification of o/w NE domains on the phase diagrams, those systems indicated a relatively extended o/w NE region which allowed choosing an oil content for completely solubilizing the drug (depending upon the drug solubility in oil) with no effect on the NE area of the phase diagram were selected. BZD-loaded NEs were prepared by spontaneous emulsification technique according to the following briefly described procedure. The oil phase was separately prepared by mixing the appropriate amounts of an oil (5 wt%) and surfactant mixture (20 wt%). To the resultant mixture, a specific amount of BZD (0.4 wt%) was then added with constant stirring using mechanical stirrer, until a clear solution was obtained. At the final stage, the required amount of water (74.6 wt%) was added in a dropwise fashion with gentle agitation for at least 60 min at 1000 rpm in order to obtain a transparent/translucent NE (total weight of 1 g).

Among 24 possible formulations, 17 BZD-loaded nanoemulsions were prepared (Table 3).

2.5. Particle Size Analysis

The particle size and PDI of all BZD-loaded NEs were determined at room temperature by photon correlation spectroscopy, using a Malvern Zetasizer (Nano-ZS; Malvern Instruments, Worcestershire, UK)., equipped with a Nano ZS® Software for data acquisition and analysis. The analysis was performed three times to determine the mean values.

2.6. In vitro Release Studies

The release profile of BZD from developed NEs was evaluated using US Pharmacopeia dissolution apparatus type II in 250 mL of phosphate buffer solution pH 7.4 (as the release medium). Dialysis bags (MWCO 12 KD, Iran) were soaked in distilled water for 24 h and kept under refrigeration until use. One mL of each NE sample was packed in dialysis bag. The bag was then sealed with clips at both ends and placed in the release medium. The assembly was stirred at a speed of 50 rpm for 6 hours at 34 ± 0.2°C. Aliquots of 4 mL were withdrawn from the medium at predetermined time intervals for 6 h and equivalent volume of the fresh medium was added to maintain the volume of the medium at 100 mL and ensure the sink condition. The amount of released drug was measured spectrophotometrically at 254nm. This experiment was performed three times for each formulation and the percentage of the release efficiency (RE) was calculated using the following formula [20]:

$$RE = \frac{\int_0^t y \times dt}{y100 \times t} \times 100$$

where y indicates the percentage of the drug released at time t. Statistical analyses of the release data were carried out and a 0.05 level of probability was taken as the level of significance.

2.7. Biological Studies

A single-dose, cross-over design study was carried out for the assessment of therapeutic efficacy of selected BZD-loaded NEs. Five New Zealand albino rabbits weighting 1.5-2 kg with normal IOP were used based on the approved protocol of Institutional Animal Care and Use Committee of Shahid Beheshti University of Medical Sciences. A single 50 µL dose of each selected drug containing NE and the commercial product (Azopt®, suspension) was instilled topically in right eyes, whereas left eyes served as the control. The IOP was measured with a rebound tonometer (Icare, Finland) for 6 h. Measurements were done for five times at each interval and the average values were used to calculate the pharmacodynamic parameters, including maximum decrease in percentage of IOP (E_{max}), time required to achieve maximum reduction in IOP (T_{max}) and the area under the curve of decrease in percentage of IOP versus time (AUC_{0-6h}).

3. Results and Discussion

3.1. Screening of Oils

To formulate an efficient nanoemulsion and exploit their advantages, the components in the formulation should be pharmaceutically acceptable.

The most important criterion for the screening of components for nanoemulsion is, however, the solubility of poorly soluble drug in oils, surfactants and cosurfactants. The oil represents one of the most important excipients in the NE formulation because it can greatly influences the ability of nanoemulsion to maintain the drug in solubilized form or facilitate self-emulsification process. NEs, as isotropic, monophasic systems, must have good solubilizing capacity to incorporate single dose of drug in minimum volume of formulation. Therefore, screening of the appropriate oil is the primary requirement of NE development. In this investigation, various oils were employed for determination of solubility of BZD. The solubility of BZD in various vehicles is presented in Table 2. Amongst the various oily phases that were screened, triacetin and Capryol™ 90 provided the highest solubility of BZD. These oils are small and medium chain triglycerides with desirable polarity to solubilize poorly water soluble drugs such as BZD, so were chosen for further investigations [21].

3.2. Construction of Pseudo-Ternary Phase Diagrams

The partial pseudo-ternary phase diagrams of systems containing triacetin with four different surfactants and co-surfactants at various R_{sm} were constructed. Figures 1-8 show the phase diagrams of 4-component systems composed of triacetin or Capryol™ 90 (as oils) in the presence of 4 different surfactants and Transcutol® P (as co-surfactant) and water. As can be seen, in all phase diagrams, a clear, isotropic, transparent/translucent domain in the water-rich part of the diagram, designated as o/w, can be labeled, the extent of which depended upon the nature of the surfactant, oil and R_{sm}. It should be noted that because of the difficulties in accurately determining the boundaries between the NE domains and surfactant-rich area, regions with the extension up to 50 wt% of the surfactant mixture were considered as NEs.

Although there were some differences between the phase diagrams obtained in this study with various surfactants, oils and R_{sm}, it is, however, beneficial to mention the similarities. In general, the following generalizations can be made about the systems examined:

Table 2. Solubility of brinzolamide in different oils at 25°C (mean ± SD, n=3).

Oil	Solubility (mg/mL)
Triacetin	14.81 ± 0.61
Capryol™ 90	8.03 ± 0.23
Labrafac™ PG	0.96 ± 0.03
Labrafac™ lipophile	0.68 ± 0.10
Isopropyl myristate	0.38 ± 0.03

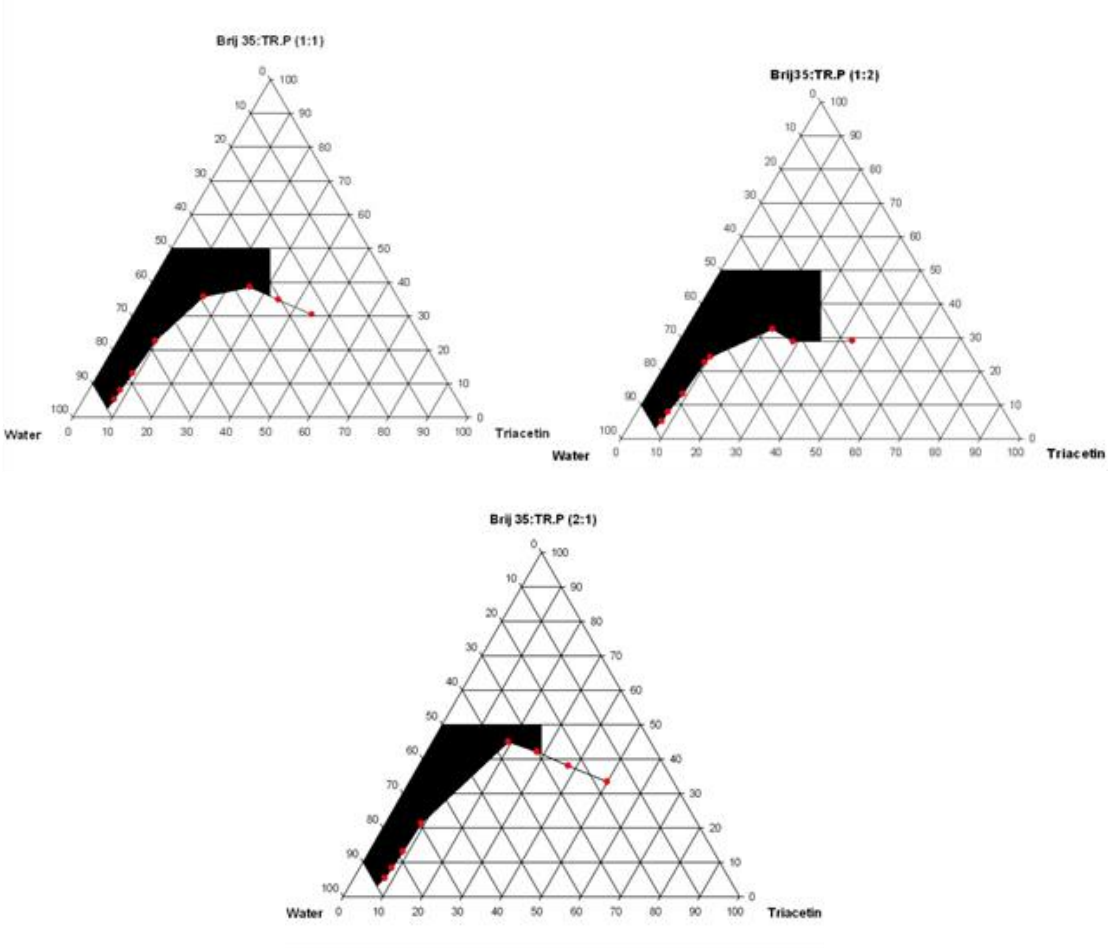

Figure 1. Partial pseudo-ternary phase diagrams of systems composed of triacetin, Brij 35 and Transcutol® P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

a) In all systems examined, irrespective of R_{sm}, the o/w domain was observed.

b) In the presence of any given surfactant mixture, the change in R_{sm} did not have a significant influence on the extent of NE regions.

c) In all systems investigated, no liquid crystal area was observed.

d) Oil solubilizing capacities increased with increasing surfactant/co-surfactant content, irrespective of the R_{sm}.

e) In Capryol™ 90-based systems, the extent of o/w domain is the least in the presence of Labrasol® and the greatest in the presence of Brij® 35. Nearly the same trend was observed when Capryol™ 90 was replaced by triacetin.

f) Regardless of the type of oil studied, the extent of o/w domain in the presence surfactant followed the order of Brij® 35> Cremophor® RH40, tyloxapol > Labrasol®.

The properties of NEs are governed by their compositions and therefore the components must be precisely selected in order to achieve a delivery carrier with desired characteristics. As mentioned earlier, in addition to pharmaceutical acceptability which is the most important criterion for the selection of the components, the solubility of the

drug in the oil and other excipients is also of considerable importance. Another important component of NE systems is the surfactant that stabilizes the interfacial area between oil and water and therefore plays a remarkable role on NE stability. These molecules are adsorbed rapidly at water/oil interface, reduce the interfacial tension to a very small value required for the formation of NE droplets and provide a flexible film around the droplets with an appropriate curvature at the interface. Additionally, it is also important to determine the proper surfactant concentration and use as low concentration as possible. It has been reported that nonionic surfactants are relatively less toxic compared to the ionic surfactants and therefore are preferred for drug delivery [21-24].

In this research, selection of the formulations was based on the criterion of using a minimum concentration of surfactant mixture (i.e., maximum 20 wt %), because of the safety concerns for ocular delivery and preparation of low viscose NEs to reduce irritation. Co-surfactants are also used along with surfactants for the formation of NEs. It has been suggested that the existence of these molecules may allow greater penetration of oil in the hydrophobic region of surfactant molecules, further lower the interfacial tension and increase the flexibility of the interface to take up the different curvatures required to form NEs [21, 25, 26].

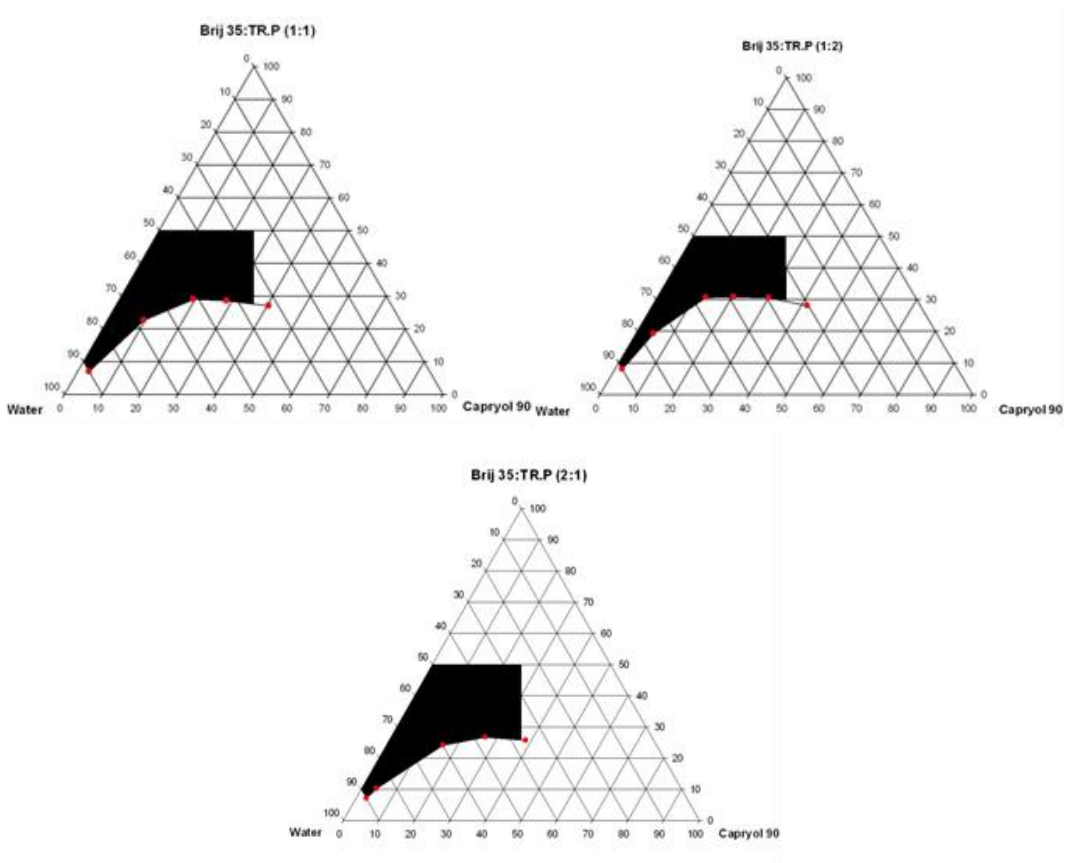

Figure 2. Partial pseudo-ternary phase diagrams of systems composed of Capryol™ 90, Brij® 35 and Transcutol® P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

Surfactants and co-surfactants are blended in the extent and position of NE regions on the phase diagrams. In this research, three different ratios of 1:1, 2:1 and 1:2 were chosen to evaluate the effect of decreasing concentration of surfactant with respect to co-surfactant and the effect of decreasing concentration of co-surfactant with respect to various weight ratios (R_{sm}), since it greatly affects surfactant.

3.3. Particle Size Analysis

The droplet size in NEs must be in a nanometer range. In this study, the particle size of the selected BZD-loaded NEs was measured using photoncorrelation spectroscopy to confirm the formation of nanoparticles. In addition, PDI was also determined to provide information about the deviation from the average size. Table 4 depicts the droplet size and PDI values of the selected formulations. It was observed that in all cases, the average size of the droplets is less than 40 nm.

As indicated in Table 4, except for the formulation NE1 (containing triacetin, Brij® 35, Transcutol® P at the R_{sm} of 1:1) and NE4 (containing Capryol™ 90, Brij® 35, Transcutol® P at the R_{sm} of 1:1), other systems had desired PDI values of less than 0.4, suggesting uniformity of the droplet size in the formulations prepared. It was also observed that the particle size in the systems prepared with triacetin and tyloxapol (formulations NE19 and NE20) was the least (9.82 and 8.97, respectively).

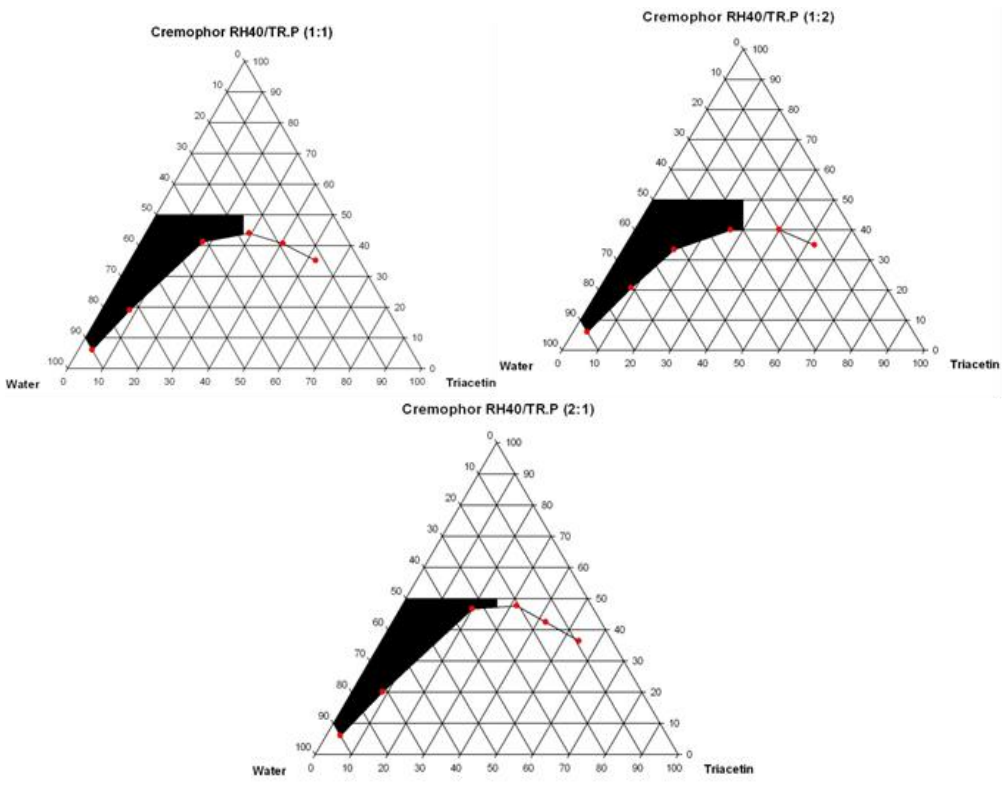

Figure 3. Partial pseudo-ternary phase diagrams of systems composed of triacetin, Cremophor® RH40 and Transcutol® P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

Table 3. Composition of BZD-loaded nanoemulsions, selected from the corresponding phase diagrams and prepared with 5 wt% oil, 20 wt% surfactant mixture, 0.4 wt% BZD and 74.6 wt% water at various R_{sm} and their release efficiency.

Group	Formulation	R_{sm}	Result*	Release efficiency (6 h)
I	NE1	1:1	+	72.61 ± 1.17
	NE2	2:1	+	66.31 ± 2.22
	NE3	1:2	+	71.60 ± 1.44
II	NE4	1:1	+	66.60 ± 4.48
	NE5	2:1	+	62.87 ± 4.79
	NE6	1:2	–	-
III	NE7	1:1	+	74.06 ± 1.40
	NE8	2:1	+	73.43 ± 1.46
	NE9	1:2	+	78.96 ± 1.27
IV	NE10	1:1	+	68.41 ± 3.20
	NE11	2:1	+	67.32 ± 2.48
	NE12	1:2	+	77.58 ± 2.97
V	NE13	1:1	–	-
	NE14	2:1	+	71.48 ± 1.84
	NE15	1:2	–	-
VI	NE16	1:1	–	-
	NE17	2:1	–	-
	NE18	1:2	–	-
VII	NE19	1:1	+	71.62 ± 1.87
	NE20	2:1	+	61.80 ± 4.36
	NE21	1:2	+	72.62 ± 1.16
VIII	NE22	1:1	+	60.41 ± 5.98
	NE23	2:1	+	49.68 ± 2.95
	NE24	1:2	–	-
		Suspension		71.05 ± 4.83

– ; not prepared, due to the turbidity or a limited nanoemulsion domain on the phase diagrams.

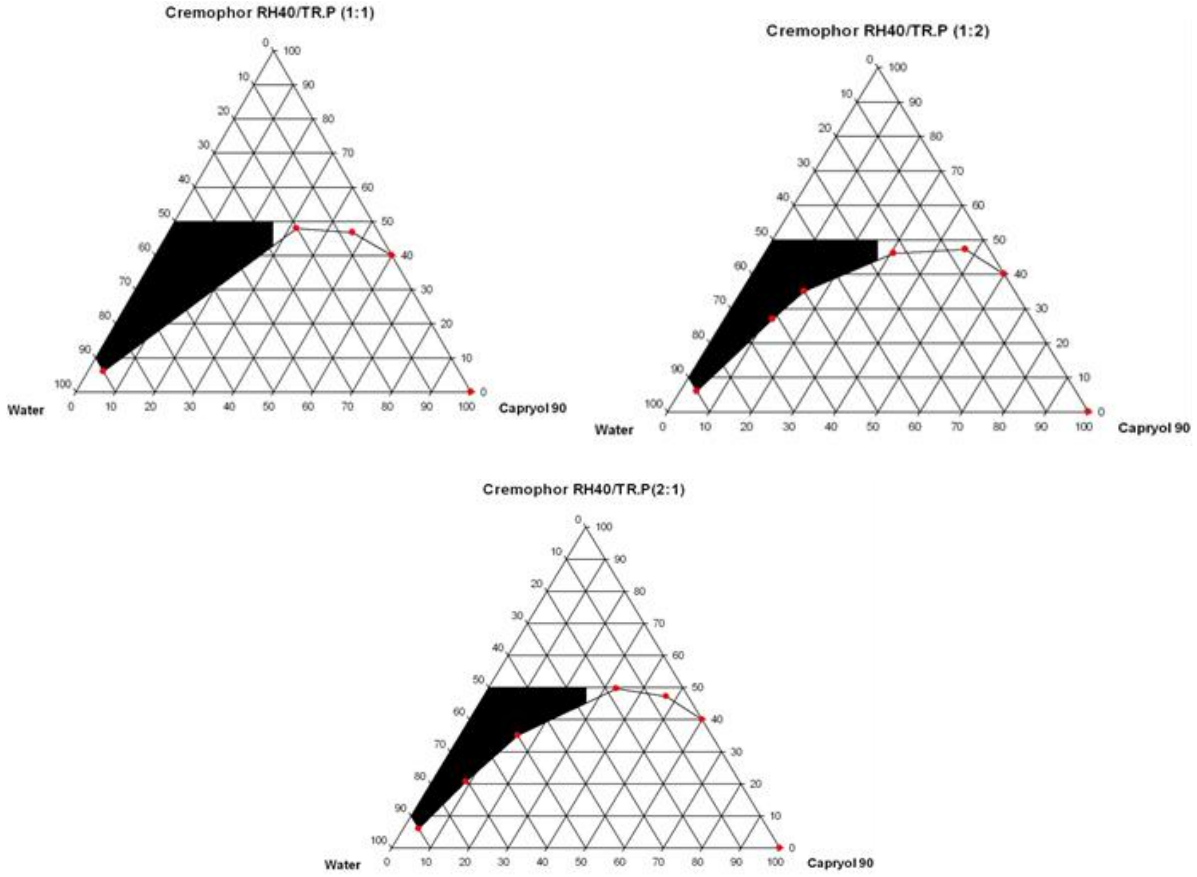

Figure 4. Partial pseudo-ternary phase diagrams of systems composed of Capryol™ 90 Cremophor® RH40 and Transcutol® P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

Table 4. Particle size and PDI value of the prepared BZD-loaded nanoemulsions.

Formulation	Z-Average (nm)	PDI
NE1	20.91	0.641
NE2	21.99	0.350
NE3	27.49	0.365
NE4	17.04	0.625
NE5	17.16	0.387
NE7	18.68	0.214
NE8	18.21	0.331
NE9	21.41	0.233
NE10	27.58	0.295
NE11	24.10	0.370
NE12	35.59	0.195
NE14	24.00	0.199
NE19	9.82	0.307
NE20	8.97	0.342
NE21	10.94	0.306
NE22	18.92	0.241
NE23	16.82	0.257

3.4. In vitro Release Studies

Most of the drug molecules are highly hydrophobic, therefore, the use of NEs as lipid-based vehicles has become progressively popular, because these systems are capable of solubilizing a lipophilic drug and providing a sustained release pattern of the solubilized drug. In this investigation, the potential of NE as a drug carrier for BZD was evaluated to ensure whether the drug could be released from formulations in a desired manner. Dissolution studies were performed to compare the release of BZD from 17 different NE formulations and the marketed suspension (Azopt®) and the terminal time point of BZD-release test was selected as 6 h. The release rate profiles are depicted in Figures 9 and 10 and the corresponding release efficiency data obtained after 6 h are presented in Table 3. As illustrated from the release plots, in most cases, 60% or more of the drug were released from the vehicle after 2 h, followed by a relatively constant slow BZD release within 6 h. The release rate was also found to change depending upon the ingredients added to NEs. In general, the following generalizations could be made from the systems investigated:

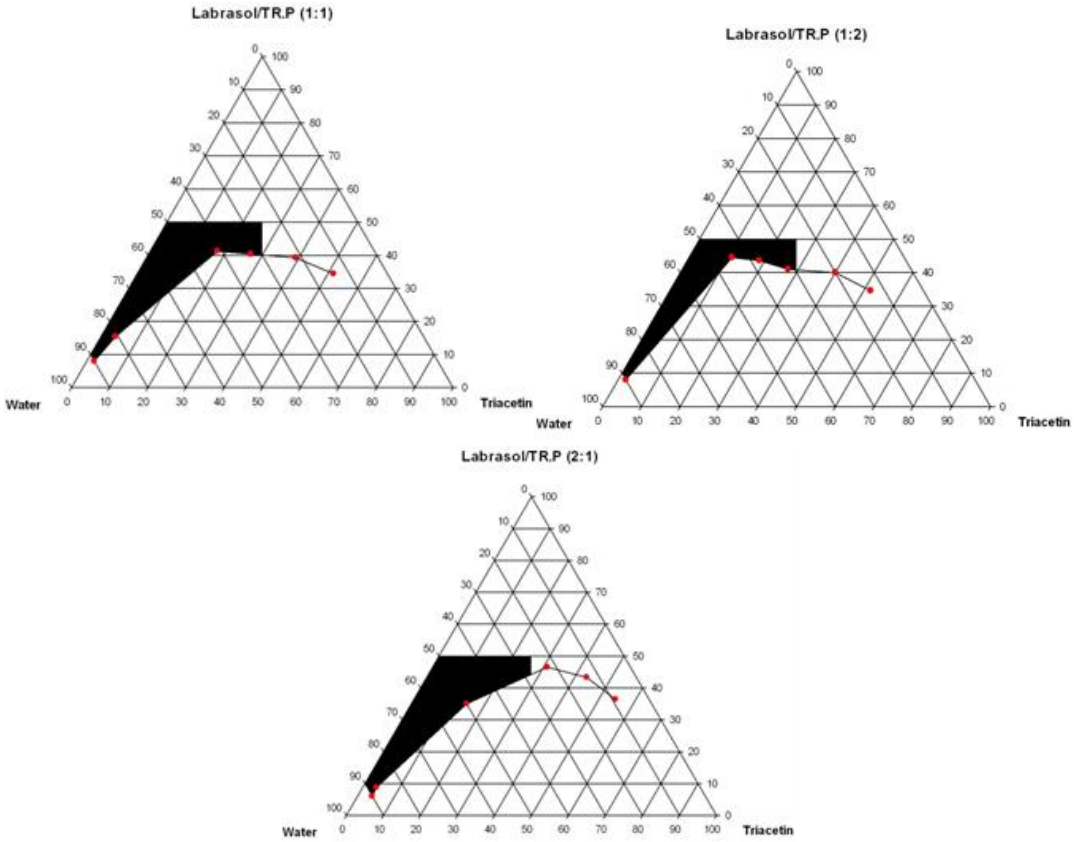

Figure 5. Partial pseudo-ternary phase diagrams of systems composed of triacetin, Labrasol® and Transcutol® P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

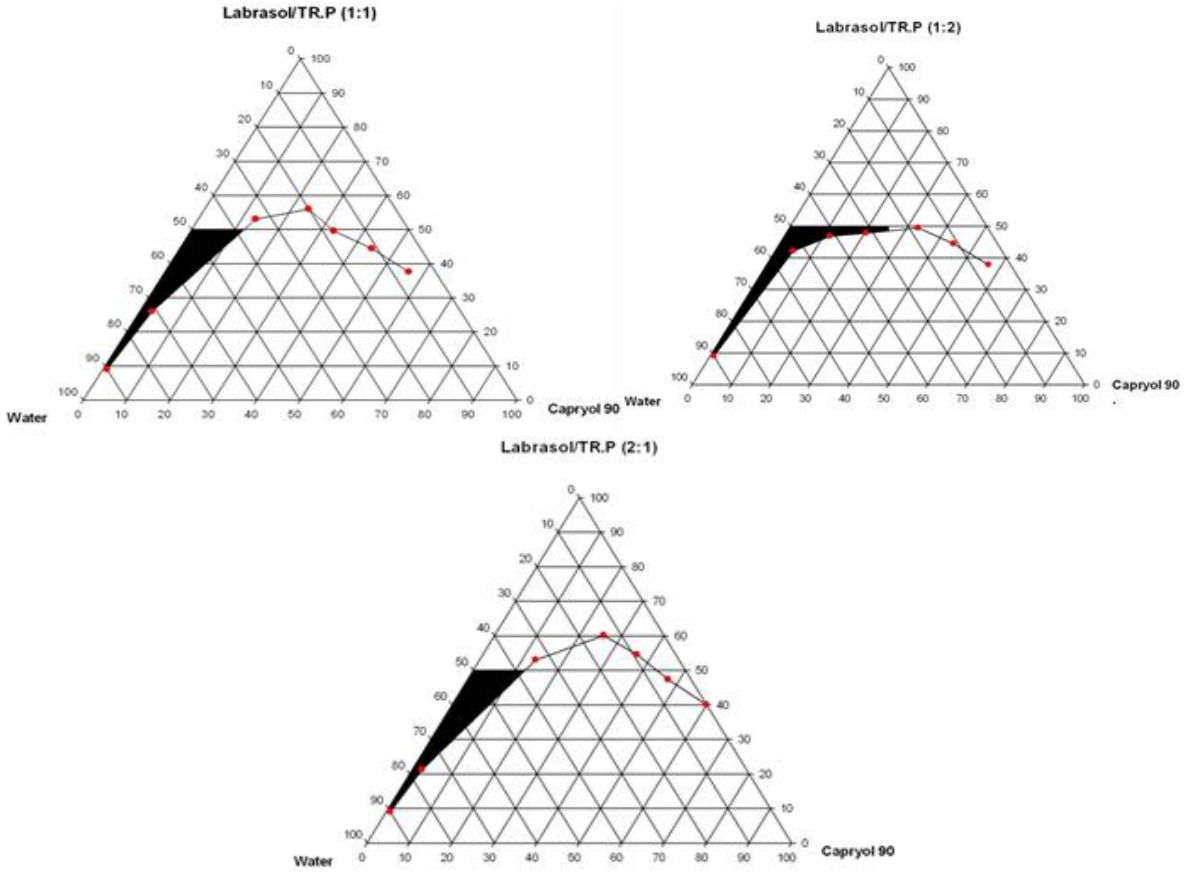

Figure 6. Partial pseudo-ternary phase diagrams of systems composed of Capryol™ 90 Labrasol® and Transcutol® P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

Triacetin-based NEs

a) As can be seen from the release plots, in all triacetin-based NEs, (except NE2 and NE20), more than 60% of the drug were released from the vehicle after 2 h. None of the formulations exhibited complete drug release after 6 h.

b) The highest release efficiency was obtained in case of formulation NE9 (78.96 ± 1.27), whereas the lowest release efficiency was observed with formulation NE20 (61.80 ± 4.36).

c) It was seen that for all NE formulations (except NE2 and NE20), the release efficiency was higher than that for the commercial suspension (71.05 ± 4.83). However, the decrease and increase in the release efficiency was significant in case of NE20 and NE9, respectively ($p < 0.05$).

d) Formulations prepared at R_{sm} of 2:1, showed more slow release profile compared to the other R_{sm} studied.

e) It seems that the release efficiency decreased in the presence of tyloxapol and increased in the presence of Cremophor® RH40.

Capryol™ 90-based NEs

a) As depicted from the release plots, more than 60% of the drugs were released from the vehicle after 2 h, except for NE5, NE22 and NE23. None of the formulations exhibited complete drug release after 6 h.

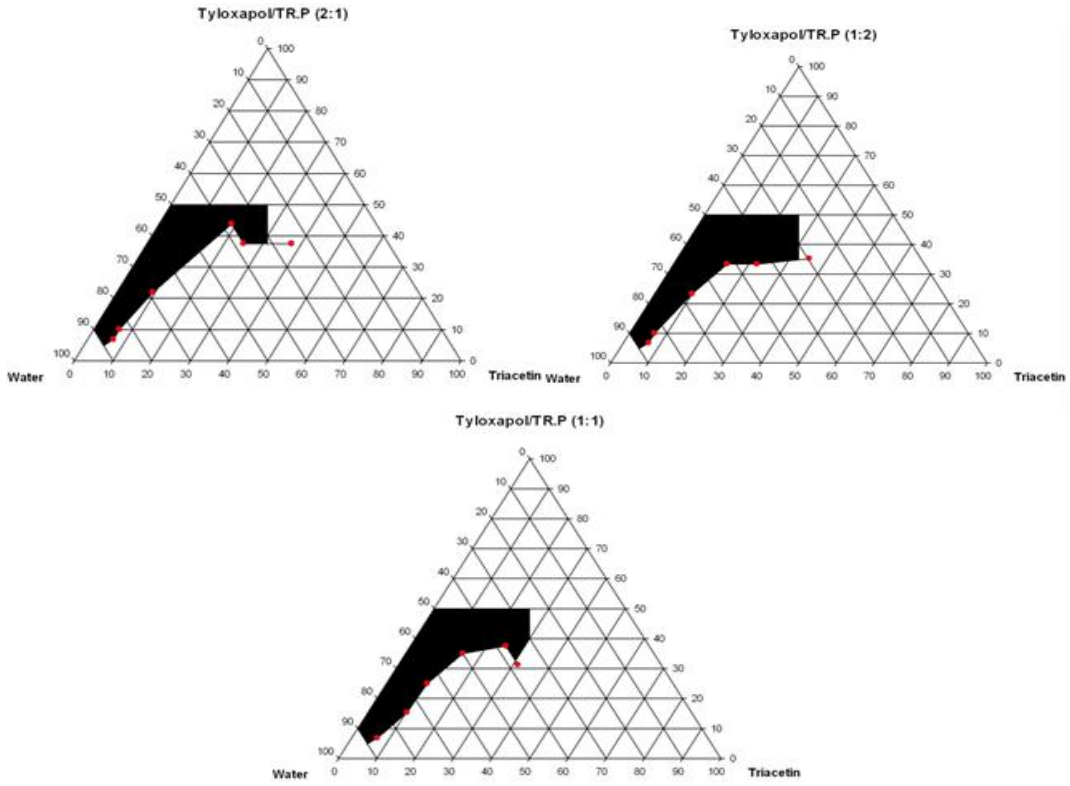

Figure 7. Partial pseudo-ternary phase diagrams of systems composed of triacetin, tyloxapol and Transcutol® P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

b) The highest release efficiency was obtained in case of formulation NE12 (77.58 ± 2.97), whereas the lowest release efficiency was observed with formulation NE23 (49.68 ± 2.95).

c) Compared to the commercial suspension, formulation NE12 was the only formulation that exhibited significantly higher release efficiency ($p < 0.05$).

d) Formulations prepared at R_{sm} of 2:1, showed more slow release profile compared to the other R_{sm} studied.

Several key factors influence the drug delivery potential of NEs, including droplet size and polydispersity, viscosity and drug solubility in oil. An enormous interfacial area due to the presence of nanosized droplets would permit faster rate of drug release from NEs. Although small differences were observed, however, all of the formulations investigated in this study had droplets in the nano range (less than 50 nm) with a PDI values generally less than 0.4 (0.199 in case of NE14). Lipophilic drugs, like BZD, are preferably solubilized in the oil phase of o/w NEs. As mentioned earlier, to develop an efficient o/w NE formulation for these drugs, drug loading in the system is a very crucial factor. NEs with the capability of maintaining the drug in the solubilized form provide a reservoir for the drug release. It is noticeable that all of the formulations investigated were prepared with 0.4 wt% of BZD and the results of the *in vitro* release studies indicated that the release efficiency in most of these formulations was higher as compared to BZD ophthalmic formulation which is marketed as 1% suspension.

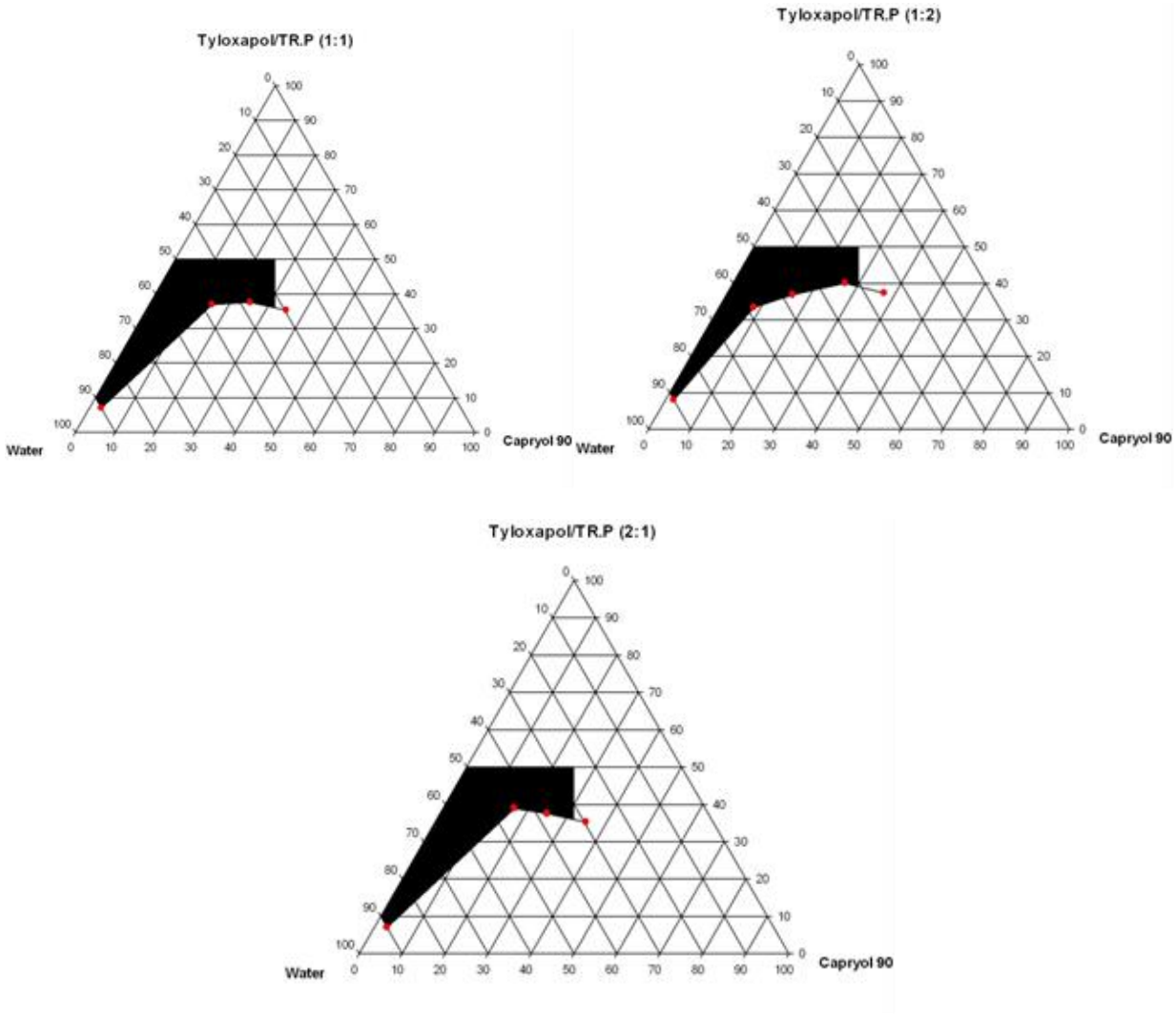

Figure 8. Partial pseudo-ternary phase diagrams of systems composed of Capryol^{TM} , 90 tyloxapol and Transcutol$^{®}$ P at different R_{sm} (black area of phase diagrams indicates the o/w nanoemulsion domain).

3.5. Biological Studies

Table 5 shows pharmacodynamic parameters after instillation of 7 BZD-loaded NEs into normotensive rabbit eyes in comparison with the marketed product (Azopt$^{®}$). The highest value for E_{max} was observed after instillation of NE5 (30.41%). Only two NEs (NE8 and NE20) exhibited lower values compared to Azopt$^{®}$. Maximum decrease in IOP occurred 1.0 to 2.2 h after applying BZD NEs, of which the least T_{max} was obtained for NE14. It was observed that the $AUC_{0\text{-}6h}$ values for most BZD NEs were similar or even higher than that forthe suspension, with the exception of NE8 and NE20, suggesting that the bioavailability of NEs is comparable to the market product. It should be noted that although the concentration of BZD in NEs was lower than that in the suspension (0.4% versus 1%), however, most of BZD formulations showed equivalent efficacy. This might be due to the penetration enhancing character of NEs by removing the mucus layer and disrupting tight junctions of corneal tissue [27].

Table 5. Pharmacodynamic parameters after topical administration of the selected BZD-loaded nanoemulsion formulations and the commercially available BZD suspension (Mean ±SD; n =3)**.**

Sample	E_{max} (%)	T_{max} (h)	AUC_{0-6h}
Suspension	25.09 ± 3.69	1.8 ± 0.45	97.00 ± 7.92
NE2	27.21 ± 4.52	2.0 ± 0.71	102.47 ± 28.45
NE5	30.41 ± 3.60	1.2 ± 0.61	112.22 ± 18.53
NE8	22.85 ± 2.54	1.6 ± 0.89	83.20 ± 8.44
NE10	28.37 ± 7.31	1.4 ± 0.55	96.87 ± 16.55
NE14	28.09 ± 7.18	1.0 ± 0.61	102.99 ± 21.80
NE20	24.22 ± 3.35	2.0 ± 0.71	90.22 ± 13.93
NE23	29.02 ± 5.67	2.2 ± 0.55	108.95 ± 15.01

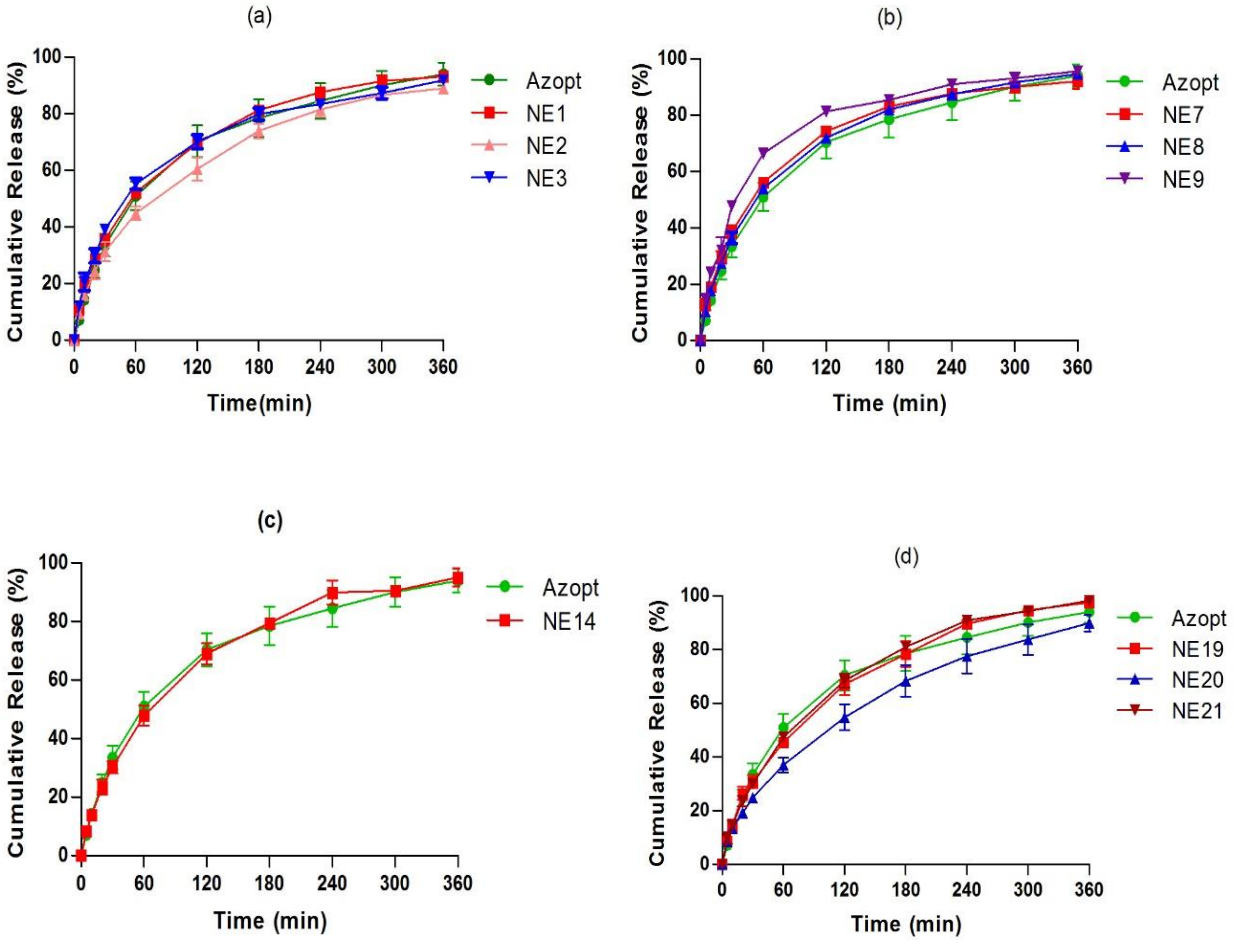

Figure 9. Comparison of the *in vitro* release profiles of BZD from the commercially available ophthalmic suspension (Azopt®) and triacetin-based NEs prepared with various surfactants, a) Brij® 35 b) Cremophor® RH40, c) Labrasol® and d) tyloxapol (mean ± SD, n=3).

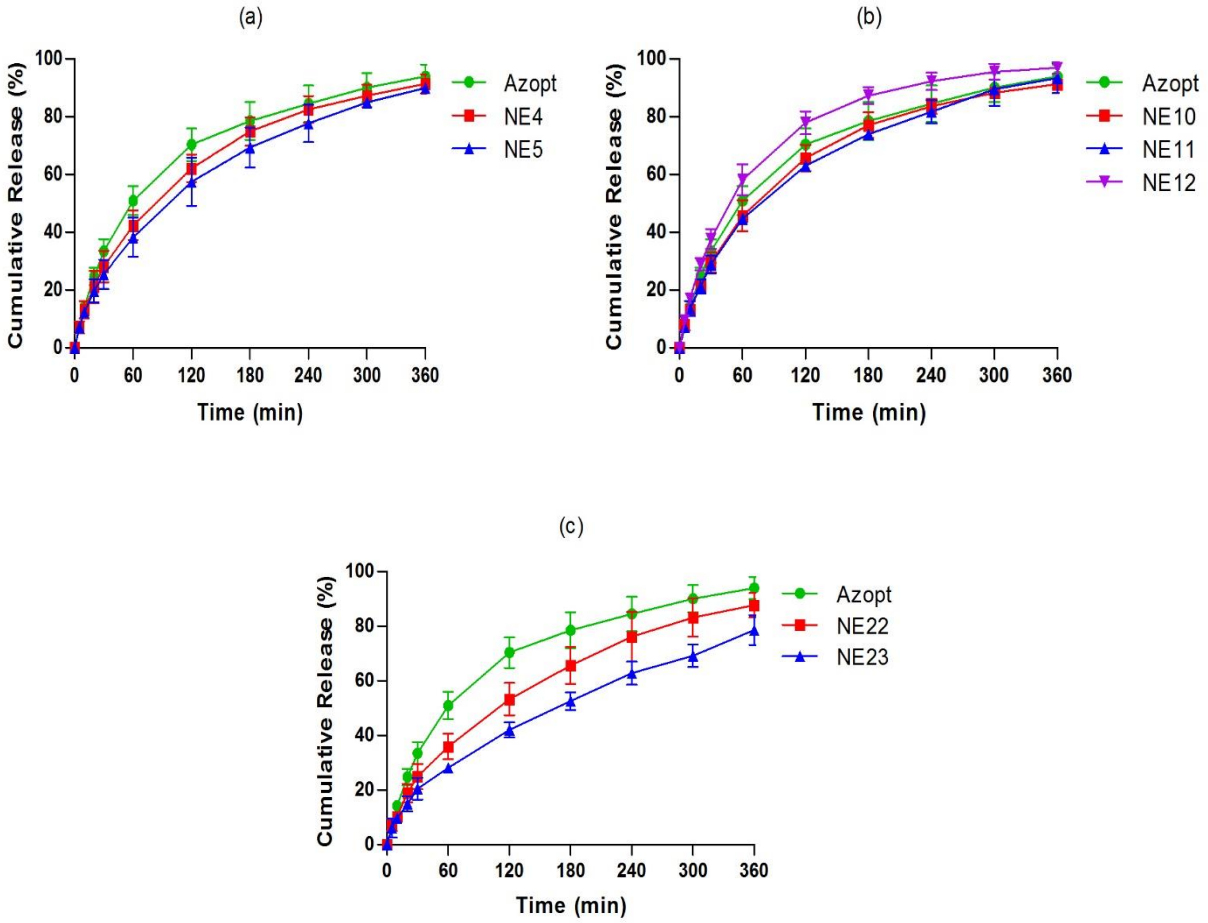

Figure 10. Comparison of the *in vitro* release profiles of BZD from the commercially available ophthalmic suspension (Azopt®) and Capryol™ 90-based NEs prepared with various surfactants, a) Brij® 35, b) Cremophor® RH40, c) Labrasol® and d) tyloxapol (mean ± SD, n=3).

4. Conclusion

Nanoemulsions have been shown to be promising carriers for drug delivery through various routes. Proper selection of components is critical for the development of an efficient NE, particularly for drugs with poor water solubility. Oil-in-water NEs were successfully developed by spontaneous emulsification method with triacetin and Capryol™ 90 with the use of various surfactants and employed for the incorporation of BZD into the oil phase. Our results revealed that the developed formulations could advantageously be employed to provide the same bioavailability as the commercially available suspension, even with lower drug content, and therefore, they may offer considerable potential as vehicles for ophthalmic delivery of BZD.

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