Journal of Applied Pharmaceutical Science Vol. 5 (Suppl 2), pp. 067-075, 2015
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2015.58.S11
ISSN 2231-3354

Natural and synthetic oil phase transition microemulsions for ocular delivery of tropicamide: efficacy and safety

Heba I El Agamy*, Gamal M El Maghraby

Department of Pharmaceutical Technology, College of Pharmacy, University of Tanta, Tanta, Egypt.

ARTICLE INFO

Article history:
Received on: 04/05/2015
Revised on: 07/06/2015
Accepted on: 18/06/2015
Available online: 04/09/2015

Key words:
Tropicamide, Microemulsion, Ocular irritation, Olive oil

ABSTRACT

The objective of this work was to investigate the efficacy and safety of natural oils (castor oil and olive oil) based phase transition microemulsion systems for ocular delivery with reference to ethyl oleate systems. Tropicamide was used as model drug and was incorporated in the formulation at a concentration of 0.5% w/w. The phase transition systems comprised the oil, surfactant and water with the phase behavior depending on the concentration of water. The tested systems included microemulsion (ME), liquid crystalline system (LC) and coarse emulsion (EM). The efficacy of these systems was evaluated by monitoring the mydriatic response in comparison to drug solution containing polyvinylpyrrolidone (PVP). Ocular irritation was monitored by visual inspection and tear flow estimation. Drug release depended on the formulation type and viscosity. Thus LC systems produced the slowest release rates followed by the ME with the EM producing the largest release rate. The mydriatic response versus time plots showed biphasic effect with two maxima (MR_{max}) which verified the systemic absorption of the drug. Both ethyl oleate and olive oil based systems were more effective than the control with respect to the area under the mydriatic response profile. However, castor oil based systems were comparable to the control. With respect to ocular irritation castor oil based system were the least irritant followed by olive oil systems with ethyl oleate systems being the most irritant. The study thus introduced olive oil based phase transition systems for ocular drug delivery with lower irritation compared to the synthetic oil systems.

INTRODUCTION

Optimized topical treatment of ocular diseases provides many advantages over systemic administration of drugs. The main advantage is reduction of systemic side effects. Unfortunately, ocular drug delivery requires careful consideration of the problems encountered after ocular application of drugs. These problems include the rapid tear turnover, transconjunctival absorption and loss of drug through nasolacrimal drainage. These factors result in short contact time after topical application of fluid formulation.

The short contact time together with the poor corneal permeability results in very low ocular availability of drugs with not more than 5% of the applied dose being able to penetrate the cornea (Meseguer *et al.,* 1994; Lang *et al.,* 1995). Therefore, a number of strategies were attempted to improve the extraordinary obstructed ocular drug delivery. This involved manipulating the corneal permeation and prolonging the precorneal retention of the formulations. These included formulation of semisolid products including bioadhesive hydrogels (Durrani *et al.,* 1995), development of in situ gel forming systems (Miller and Donovan, 1982), employing collagen shields or medicated contact lenses (Gurny *et al.,* 1985; Unterman *et al.,* 1988; Kaufman *et al.,* 1994), optimization of colloidal carriers such as nanoparticles, liposomes and niosomes (Fitzgrrald *et al.,* 1987; Calvo *et al.,* 1997; Vyas *et al.,* 1997; Pignatello *et al.,* 2002; Aggarwal *et al.,* 2005), or employing micellar systems (Pepic *et al.,* 2004). Microemulsions provide another attractive alternative for improved ocular drug availability.

They have the advantage of being thermodynamically stable with high ability to incorporate large quantities of drugs (Gasco *et al.,* 1989; Habe *et al.,* 1997; Vandamme, 2002). In addition, microemulsions have the tendency to undergo phase transition upon mixing with aqueous environment with some systems changing to liquid crystalline system upon dilution (Alany *et al.,* 2001). This behavior is similar to that of in situ gelling systems which is expected to prolong the residence time of the formulation after ocular application.

* *Corresponding Author*
Email: hebaelagamy1985@yahoo.com

Phase transition microemulsions based on ethyl oleate have been successfully employed as ocular delivery system and were reported to enhance the corneal permeability as well (Chan *et al.*, 2007).

However, this system was shown to affect the precorneal tear film providing some irritation (Chan *et al.*, 2008). This effect is expected taking into consideration the use of synthetic oil. Accordingly, the objective of this study was to investigate the efficiency and safety of vegetable oil based microemulsions for ocular drug delivery. Olive and castor oil based systems were tested with reference to the ethyl oleate formulation. Tropicamide was selected as a model drug for this study.

Tropicamide is antimuscarinic drug which is employed as mydriatic during funduscopic examination. Its mechanism of action depends on blocking the M4 muscarinic receptors of the eye so, controlling the pupil size and the lens shape. It is a poorly water soluble weakly basic drug which dissolves in acidic pH resulting in a solution which can be irritant to the eye (Dibalgi *et al.*, 2013). Therefore microemulsion phase transition systems can overcome this problem enhancing its efficacy and safety.

MATERIALS AND METHODS

Materials

Tropicamide was gift from Alexandria Company for Pharmaceutical Industries, Alexandria, Egypt. Castor oil, olive oil, sorbitan mono laurate (Span 20), polyoxy-ethylene 20 sorbitan mono-oleate (Tween 80), ethanol and potassium dihydrogen phosphate (pharmaceutical grade) were obtained from El Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. Ethyl oleate, polyvinylpyrrolidone (PVP) and the cellulose tubing were obtained from sigma Aldrich, St Louis, MO, USA.

Construction of pseudoternary phase diagram

Castor oil, olive oil and ethyl oleate oil were used separately as the oil phase with the goal of investigating the efficacy and safety of vegetable oil microemulsions relative to ethyl oleate based microemulsion. The published phase diagram for ethyl oleate based phase transition system which utilized a mixture of Span 20 and Tween 80 (2:3, weight ratio) as surfactant system was used in this study (Alany *et al.*, 2001). For castor oil and olive oil systems Tween 80 was used as the surfactant. The pseudo-ternary phase diagrams were constructed using water titration method at ambient temperature.

The oil and surfactant system were mixed at weight ratios of 0.5:9.5, 1:9, 1.5:8.5, 2:8, 2.5:7.5, 3:7, 3.5:6.5, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1. The prepared mixtures were titrated with water. The systems were visually characterized after equilibration to determine the phase change with increasing water content. Transparent fluid systems were characterized as microemulsion. Viscous systems showing oil strokes were considered LC with turbid liquid dispersions being identified as EM.

Preparation of ocular formulations

Table 1 presents the composition of the tested ocular formulations. The microemulsion formulations were prepared by mixing the oil with the required amount of surfactants followed by addition of water. The drug (0.5% w/v) was then dissolved in the prepared formulations with the aid of magnetic stirrer. The control employed aqueous drug solution (0.5% w/v) which was dissolved in phosphate buffer (pH 5) containing 0.25% w/v polyvinylpyrrolidone (PVP). To enhance drug dissolution the drug was co-ground with PVP before addition of the buffer with the aid of magnetic stirring until complete solubility.

Table 1: The composition of the investigated formulations.

Formulation	Composition (weight ratios)
Ethyl oleate formulations	
ME	Water, span 20,Tween 80, cardomol EO (10:22:33:35)
LC	Water,span20,Tween80,cardomol EO(25:17.6:26.4:30)
EM	Water span 20, ,Tween 80 , cardomol EO (85:4:6:5)
Castor oil formulations	
ME	Water , Tween 80, castor oil (10:70:20)
LC	Water, Tween 80, castor oil (27:60:13)
EM	Water, Tween 80, castor oil (70:25:5)
Olive oil formulations	
ME	Water, Tween 80, olive oil (15:65:20)
LC	Water, Tween 80, olive oil (21:65:14)
EM	Water, Tween 80, olive oil (70:25:5)
Control	0.25% PVP in di-hydrogen phosphate buffer

Characterization of the selected microemulsion formulation

The flow properties and viscosity of the tested formulation were determined using a DV III rotating Brookfield viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA). The electrical conductivity of the tested formulations was recorded by electrical conductivity meter (Hanna-HI 8733). Sodium chloride (0.1% W/V) was incorporated to provide measurable conductivity.

In vitro drug release

The developed ocular formulations were subjected to *In vitro* drug release study by using vertical glass Franz diffusion cells. These cells have a diffusional area of 2.27 cm^2 and the receptor compartment was 14 ml. The artificial membrane (Cellulose tubing, Sigma diagnostics, St. Louis, MO, USA) hydrated overnight distilled water for an overnight to ensure complete swelling. This provided fixed pore diameter throughout the experiments. The membrane was clamped between the donor and receptor compartments. To maintain sink conditions 20% (v/v) ethanol in water was used as receptor. This receptor was selected on the base of drug solubility in such fluid which was found to 32 mg/ml. This solubility was considered acceptable to maintain sink conditions taking into consideration the amount of drug in the donor (10mg in 2ml of formulation) and the volume of receptor compartment (14ml). The diffusion cells were incubated into a thermostatically controlled water bath with its temperature being adjusted to maintain the temperature of the membrane surface at 37 $\pm$ 1^{o}C to mimic *In vivo* conditions. The tested formulations

(2ml) were loaded into the donor compartment before occluding the donor compartments with aluminum foil. Receptor samples were taken periodically and replaced immediately with an equal volume of fresh receptor. The samples were analyzed for the drug content by UV spectrophotometric determination at 264 nm after suitable dilution with the receptor fluid if required. This required construction of a calibration curve which was linear in the range of (25 µg/ml to 110 µg/ml) with an equation of (y= 0.007x + 0.0563). The cumulative amount of drug released was calculated as a function of time and the release rate was determined in triplicate.

***In vivo* evaluation**

The mydriatic response obtained after application of the tested formulations to the rabbit eye was taken as a measure for the *In vivo* performance of the ocular formulation after topical application. The studies employed six New Zealand Albino rabbits weighing 2-3 kg. The study protocol was approved by the College of Pharmacy, University of Tanta, Ethical Committee.

The previously published protocol was followed (Chan *et al.*, 2007). The rabbits were fed a normal diet, exposed to alternating 12 hours light and dark cycles and restrained by wrapping with a towel during the experiments. All experiments were performed in the same room under standard lighting conditions. The same rabbits were used to test all formulations with 1 week wash out period between each formulation. The pupil diameter was measured from digital camera (Samsung 8MPixel digital camera, Vitenam) with a flexible ruler of 0.5 mm increments being held under the right eye to serve as a calibration scale (Fig. 1).

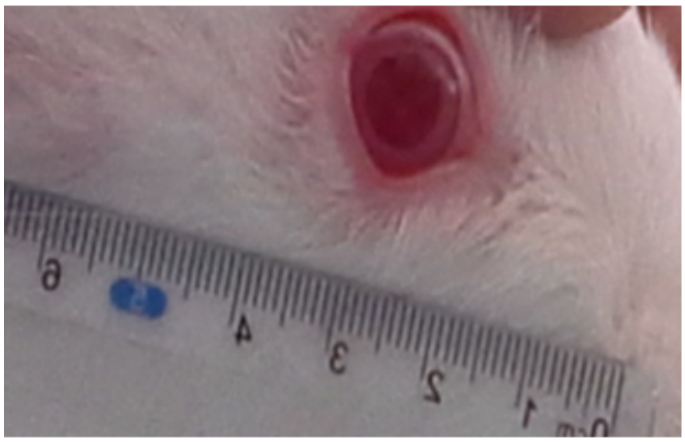

Fig. 1: A digital camera image showing the mydriatic response being monitored with a ruler serving as a calibration scale.

At zero time (before application) the pupil diameter was recorded four times in each rabbit and the average basal diameter was calculated. The test system (40 µl) was then instilled into the lower fornix of the conjunctival sac of the right eye with the left eye serving as a control. The mydriatic response was monitored and recorded using the digital camera at a 10 minute intervals for 300 minutes. The acquired images were used to determine the pupil diameter with reference to the calibration scale. The change of the pupil diameter versus time plots provided the mydriatic response profile. These profiles were used to calculate area under the curve (AUC_{0_300}), time required to achieve peak mydriatic response (T_{max}) the maximum mydriatic response (MR_{max}) and mydriatic response recorded 60 min, 120 min and 180 min post instillation (MR_{60}), (MR_{120}) and (MR180).

Ocular irritation studies

It was important to monitor the effect of composition of the ME systems on the ocular irritation. This was evaluated by visual inspection of the eye for redness. In addition the rate of tear flow was monitored through phenol red thread test**.**

Tear volume measurements (phenol red thread test)

The purpose of this test was to evaluate the ocular tolerance of the tested formulations. This was achieved by inserting a phenol red impregnated cotton thread into the inferior fornix of the conjunctival sac. The pH sensitive thread changes from yellow to red upon coming into contact with tears (Fig. 2). The red part of the thread was measured after 15 seconds. Tear deficiency is indicated when recording less than 10mm length for the wet red thread in 15 seconds (Chan *et al.*, 2008). To establish the base line tear volume, the thread was placed into the inferior fornix of the conjunctival sac of the left eye of the rabbit for 15 seconds, before measuring the length of the tear wetted portion (Craig *et al.*, 1997; Chan *et al.*, 2008). The tested formulation (40 □l) was instilled into the lower fornix of the conjunctival sac of the same eye, and a second phenol red thread test was done five minutes post instillation to evaluate the tear volume changes. The difference between the measured wetted length after and before instillation was then calculated. The more the difference in the wetted part, the more irritant is the formula.

Fig. 2: The phenol red thread test. Note the dark and bright discoloration of the thread indicating wet and dry portions respectively.

Statistical analysis

The Student T-test was used for assessing the significance of differences between formulations.

RESULTS AND DISCUSSION

Pseudo-ternary phase diagram

The phase diagrams of different oils with the selected surfactant system were constructed to determine the phase behavior of the system after mixing with increasing concentration

of water. These phase diagrams were used to select the composition of the formulations for ocular application and to predict the phase change after ocular application and mixing with the tears. With respect to the ethyl oleate based system the previously published phase diagram (Alany *et al.,* 2001; Chan *et al.,* 2007) was utilized with three different formulations (ME, LC and EM) being selected from this phase diagram according to the composition presented in Table 1. With respect to the olive oil and castor oil, Tween 80 was selected as the surfactant and the constructed phase diagrams of these systems are shown in Fig. 3. The olive oil based phase diagram showed five distinct zones depending on the composition of the ternary system.

These zones included w/o microemulsion zone which transforms to liquid crystalline system upon dilution with water. The later forms stable gel phase after further dilution with water before forming o/w coarse emulsion upon further dilution. In addition, an area of phase separation was detected at high concentration of oil (Fig. 3). The area occupied by each zone was measured with the ME zone occupying 23% of the total area of the phase diagram, the LC zone occupying 5% and the EM zone occupying 37%. Phase diagrams utilizing olive oil are available in literature with similar and dissimilar surfactant systems. For example, authors utilized both Tween 80 and span20 as surfactants with propylene glycol (PG) being employed as a cosurfactant (Salimi *et al.,* 2014). In this system the ME zone occupied 40%. This may be attributed to the use of PG as a co-surfactant which increases the area of ME. In another investigation the phase diagram of olive oil based system was constructed using cremophor EL and Tween 80 as surfactant and polyethylene glycol (PEG) as cosurfactant (Sha *et al.,* 2012). In this system the ME zone was around 20%. However, these studies did not show any liquid crystalline phase transition which is desirable for ocular delivery. For castor oil phase diagram the ME zone occupied 16% of the total area of the phase diagram, the LC zone occupied 15%, and the EM zone occupied 42%. Other investigators utilized Tween 80 with ethanol as surfactant/cosurfactant system for castor oil (Nazar *et al.,* 2008). Such system is not suitable for ocular application due to possible irritant effect of ethanol.

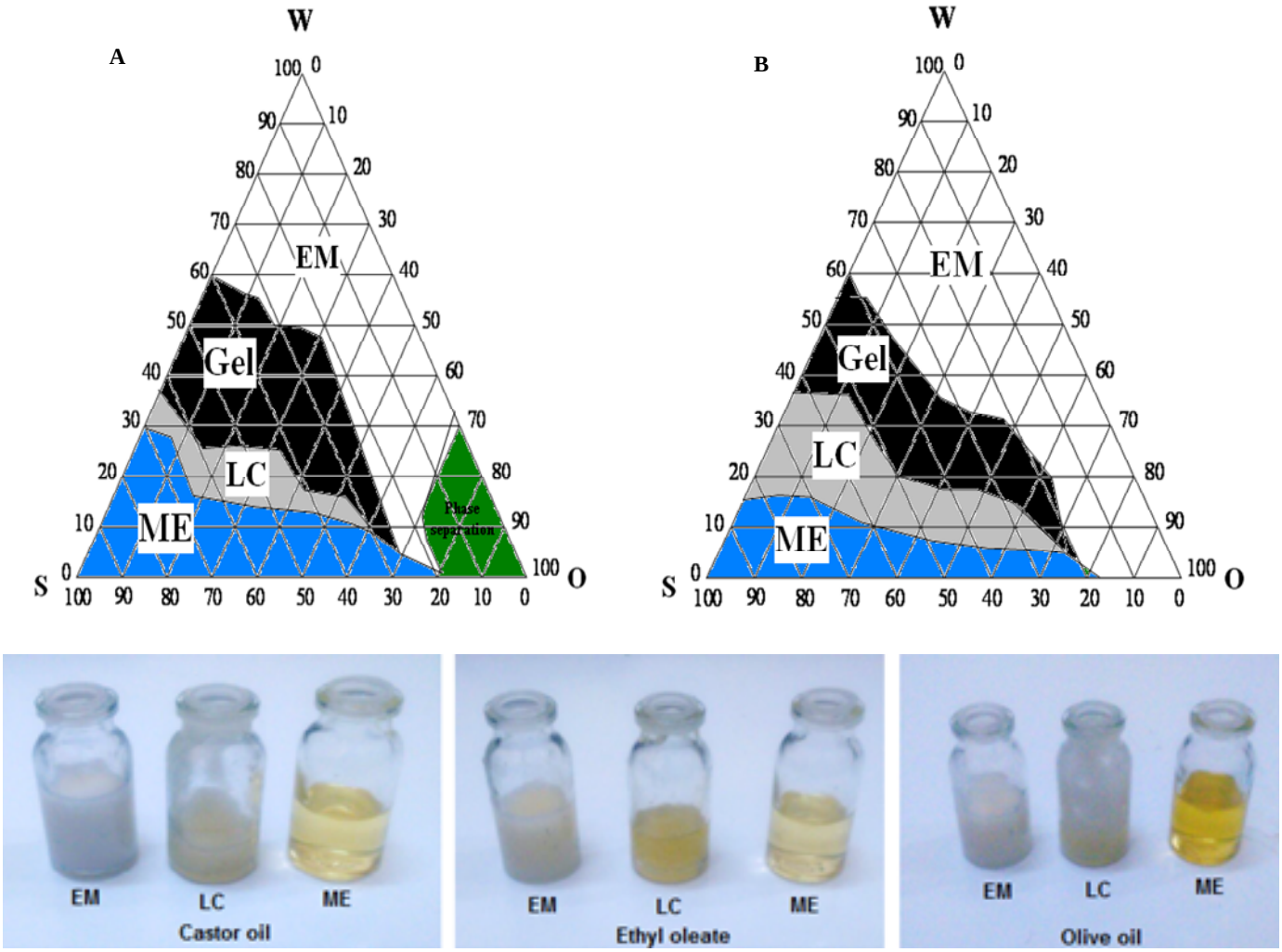

Fig. 3: Pseudoternary phase diagrams (a) olive oil and (b) castor oil and representative photographs for each phase.

Characterization of the selected formulations

The tested formulations included a range of systems of varying viscosity. These formulations included ME systems, LC systems and coarse emulsion formulations. All were selected from the constructed phase diagrams according to the composition presented in Table 1. The ME systems were selected to have a composition close to the LC border. This selection was done to provide a chance for thickening of the formulation after mixing with minute amounts of tears. The viscosity and flow behavior of ocular formulation is determining factor for the rate of drug release, contact time with eye and subsequently the onset and duration of action. Accordingly, the viscosity and flow behavior of different formulations were monitored with the goal of correlating their results with the recorded release pattern and *In vivo* performance of different formulations. Both ME and EM systems exhibited Newtonian flow pattern with the LC systems showing non-Newtonian, pseudoplastic flow behavior. The later exhibited a reduction in the viscosity upon increasing the shear rate indicating a shear thinning behavior. This property is desirable for ocular formulation so as not hinder the natural movement of the eye. Similar flow pattern was reported for phase transition systems (Chan *et al.,* 2007). The viscosity of all formulations was recorded at fixed stirring rate (100 rpm). The measured viscosity values are presented in Table 2. The viscosity increased as we move from the ME zone to the LC zone. This is expected due to structuring of the system upon gradual addition of water to the ME formulation. Further dilution of the LC systems to the coarse emulsion state resulted in significant reduction in the viscosity to reach values even smaller than that of the corresponding ME systems. The same pattern was recorded for all the tested systems irrespective to the composition. This behavior is similar to that recorded in literature for similar systems (Alany *et al.,* 2001; El Maghraby and Bosela, 2011; El Maghraby, 2012).

Table 2: The viscosity of the tested formulations. Results presented as mean ± SD, n=3

Formula name	Viscosity values (mpa s)
Castor ME	1376.7 ± 161.7
Castor LC	3923.3 ± 98.7
Castor EM	22.5 ± 2
Olive ME	1320 ± 55.6
Olive LC	9972.7 ± 45.6
Olive EM	10.8 ± 0.5
Ethyl oleate ME	167 ± 2.7
Ethyl oleate LC	1136 ± 7
Ethyl oleate EM	3.98 ± 0.1
PVP buffered solution	1.3 ± 0.1

For the electrical conductivity the measurements showed dependence of the electrical conductivity on the water content of the tested formulations. As the water content increases, the electrical conductivity increases. Therefore the ME formulations of the three tested oils ethyl oleate, olive oil and castor oil recorded the lowest electrical conductivity values of 0.17, 7.4, 2.4 □s, respectively. These values indicate that the tested ME systems are in the form of W/O microemulsion. Development of LC systems resulted in marginal increase in the conductivity before significant increase in electrical conductivity values in case of coarse emulsion formulation to record values of 488 to 680 μs. These results indicate that the coarse emulsion systems are in the form of O/W system. These findings are expected as it reflects the structural arrangement of the ternary system during phase transition in which the W/O system is transferred to lamellar liquid crystalline system before complete phase inversion into an O/W system. Similar findings were reported for phase transition systems (Alany *et al.,* 2001).

***In vitro* drug release**

Fig. 4 shows the *In vitro* release profiles of tropicamide from various ME, EM and LC formulations. The release data were fitted to various kinetic models to determine the kinetics of drug release. The model providing the best fit as indicated from the highest value for the correlation coefficient was taken as the kinetics for drug release and was used to calculate the rate of drug release. The results are presented in Table 3. The rate of drug diffusion from its aqueous solution in presence of 0.25% w/v PVP was taken as a control. This solution produced linear release as a function of time (Fig. 4). Taking into consideration the fact that the recorded diffusion of the drug from its solution is the maximum possible release, the linear profile will confirm the presence of sink conditions throughout the release studies with all formulations. Considering the drug release from different formulations, the recorded data were fitted better to the zero order (Fig. 4 and Table 3). With respect to the rate of drug release, there was a dependence on the type of the formulation and its viscosity. Thus liquid crystalline systems produced the slowest release rates followed by the microemulsion system with the coarse emulsion producing the largest release rate (Fig. 4 and Table 3). This trend was the same in the tested systems irrespective to its composition. Similar release pattern was reported for microemulsion systems of Quetiapine fumarate which has similar physicochemical properties compared to tropicamide (Parvanthi *et al.,* 2014). In contrast the freely water soluble drugs showed different release kinetics with the ME and LC fitting matrix diffusion pattern. This behavior was reported for pilocarpine hydrochloride and timolol maleate (Chan *et al.,* 2007; Hegde *et al.,* 2014). The recorded zero order release pattern of the drug from ME and LC systems can be due to the lipophilic nature of the drug with subsequent localization in the external oily phase.

Table 3: The correlation coefficient obtained after fitting the release data to different release models and the calculated release rate based on the best fit **.**

Formula	R^2			Release rate* ($\mu g\ cm^{-2} hr^{-1}$)
	Zero order	First order	Higuchi	
Ethyl oleate ME	0.999	0.922	0.982	88.7
Ethyl oleate LC	0.996	0.935	0.988	69.6
Ethyl oleate EM	0.977	0.921	0.975	279.5
Castor ME	0.986	0.914	0.971	71.1
Castor LC	0.997	0.90	0.974	70.9
Castor EM	0.985	0.895	0.963	283.2
Olive ME	0.986	0.916	0.97	67.5
Olive LC	0.988	0.918	0.975	63.7
Olive EM	0.986	0.87	0.954	229.3
PVP Solution	0.98	0.938	0.97	496

Note:*The release rate was calculated assuming zero order release kinetics

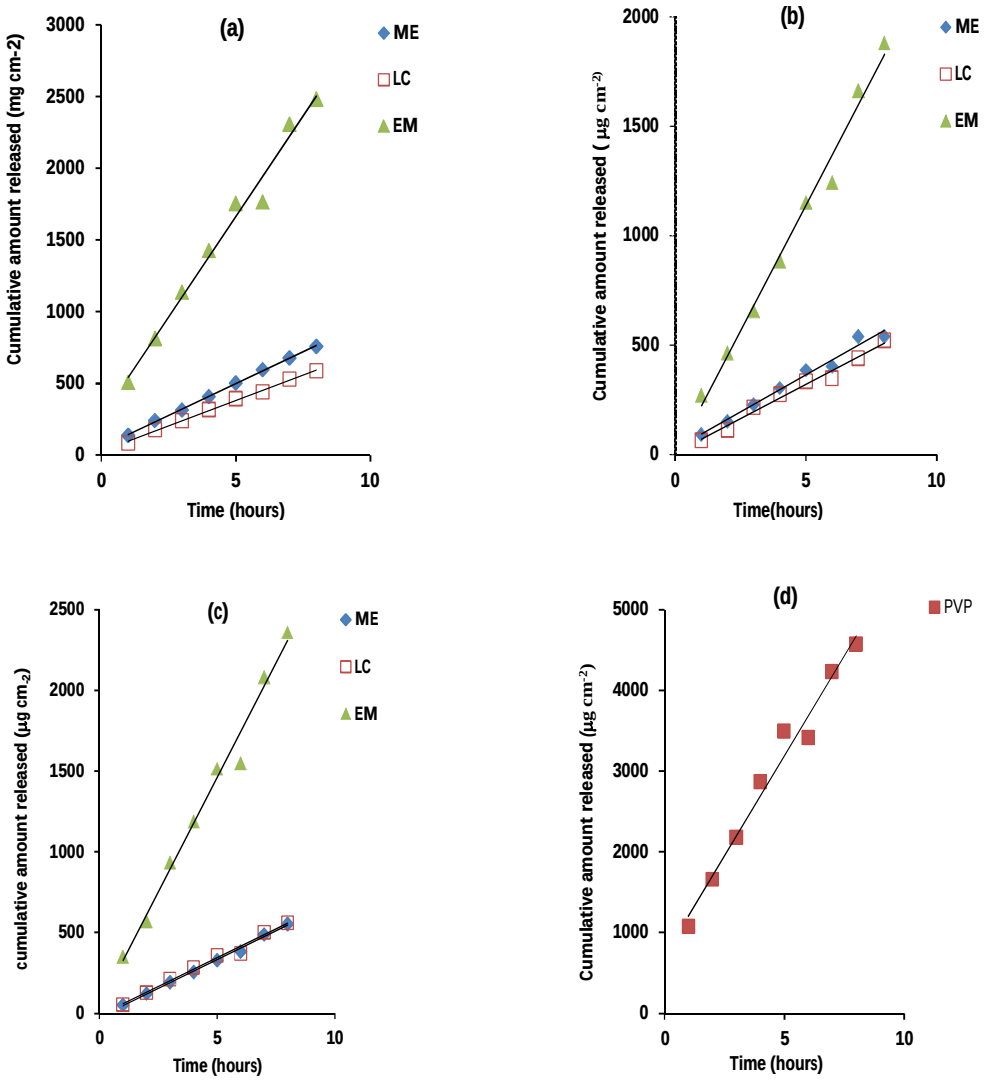

Fig. 4: *In vitro* drug release profiles from (a) ethyl oleate ME, LC and EM systems, (b) olive oil ME, LC and EM systems, (c) castor oil ME, LC and EM systems and (d) drug solution formulation. Formulation details are in Table 1.

In vivo evaluation

The mydriatic response obtained after ocular application of the drug in various formulations was recorded as a function of time. This was used to calculate the pharmacokinetic parameters which were used to compare the efficacy of different formulations. The results are shown in Fig. 5 with the calculated parameters being presented in Table 4. The mydriatic response versus time plots showed biphasic effect with two maxima (MR_{max1} and MR_{max2}, Fig. 5). The recorded MR_{max} values depended on the type and composition of the formulation. Thus within the ethyl oleate based system the emulsion formulation produced the highest MR_{max} value followed by the microemulsion system then the liquid crystalline system. The trend was shown in cases of olive oil and castor oil based systems (Fig. 5 and Table 4). Considering the previously published work on the ethyl oleate based phase transition system which utilized pilocarpine as a model drugs and revealed superiority of the ME with respect to the MR_{max} (Chan *et al.*, 2007) the recorded results here is contrary. The recorded trend for the MR_{max} correlated well with the release rate of the drug from the formulation suggesting that the drug release is the rate limiting step for at least the initial effect after ocular application. Another explanation for the superiority of the fluid system can depend on the previously published findings which highlighted the significant mydriatic response after lingual application of tropicamide (Schmidt *et al.*, 2006). This report reflected the existence of mydriatic effect after systemic absorption from the mouth and gastrointestinal tract. This means that nasolacrimal drainage and transconjunctival absorption can have a major effect on the recorded mydriatic response after ocular application of tropicamide. This may provide an explanation for the superiority of the less viscous systems. Accepting this explanation, we should expect higher MR_{max} values in case of drug solution which is the most fluid and has the drug in free form. However, this was not the case with the drug aqueous solution producing the smallest MR_{max}. This indicates that the recorded MR_{max} values are the sum of the local effect and the systemic effect and hence the inferiority of the

aqueous solution compared with the colloidal systems. Thus the superiority of ME system over drug solution depends on its contribution to the local effect in which the ME will provide greater chance for mixing with the pre-corneal tear film before subsequent transocular permeation (Chan *et al.*, 2007). Considering the effect of composition on the recorded MR_{max} values, the effect of oily component of each system was studied. Ethyl oleate based systems were superior followed by olive oil systems with those based on castor oil recording the smallest MR_{max} values (Table 4). This ranking can be explained on the base of the possible penetration enhancing effect of the oils. This means that pure ethyl oleate is better enhancer than olive oil which comprises oleic acid as the main oil which was better than castor oil which has recinoleic acid as the main component. The possible mydriatic effect of tropicamide after systemic absorption from the gastrointestinal tract as a consequence of nasolacrymal drainage made consideration of the duration of action of each factor a complex task. The superiority of ME system over tra ditional drug solution was reported when using ethyl oleate based ME for timolol maleate (Hegde *et al.*, 2014). Utilizing, isopropyl myristate based ME loaded with chitosan, the anti-inflamatory effect of dexamethasone drug in endotoxin-induced uveitis was enhanced significantly when compared with the market solution (Kesavan *et al.*, 2013). These studies reflect the efficacy of ME system irrespective to the oily component. With respect to the ocular delivery of tropicamide, alternative strategies have been employed. These included liposomes which were used as fluid or incorporated in a gel. Positively charged vesicles showed good potential for enhanced ocular delivery (Nagarsanker *et al.*, 1999). Dendrimers were also tried as another strategy and showed some success with tropicamide compared with phosphate buffer solution (Vandamme and Brobeck, 2014). In contrast, a vehicle containing an aqueous carboxymethyl kondagogu gum failed to provide significant enhancement in ocular delivery of tropicamide compared with the commercial tropicamide eye drops (Kumar and Ahuja, 2014).

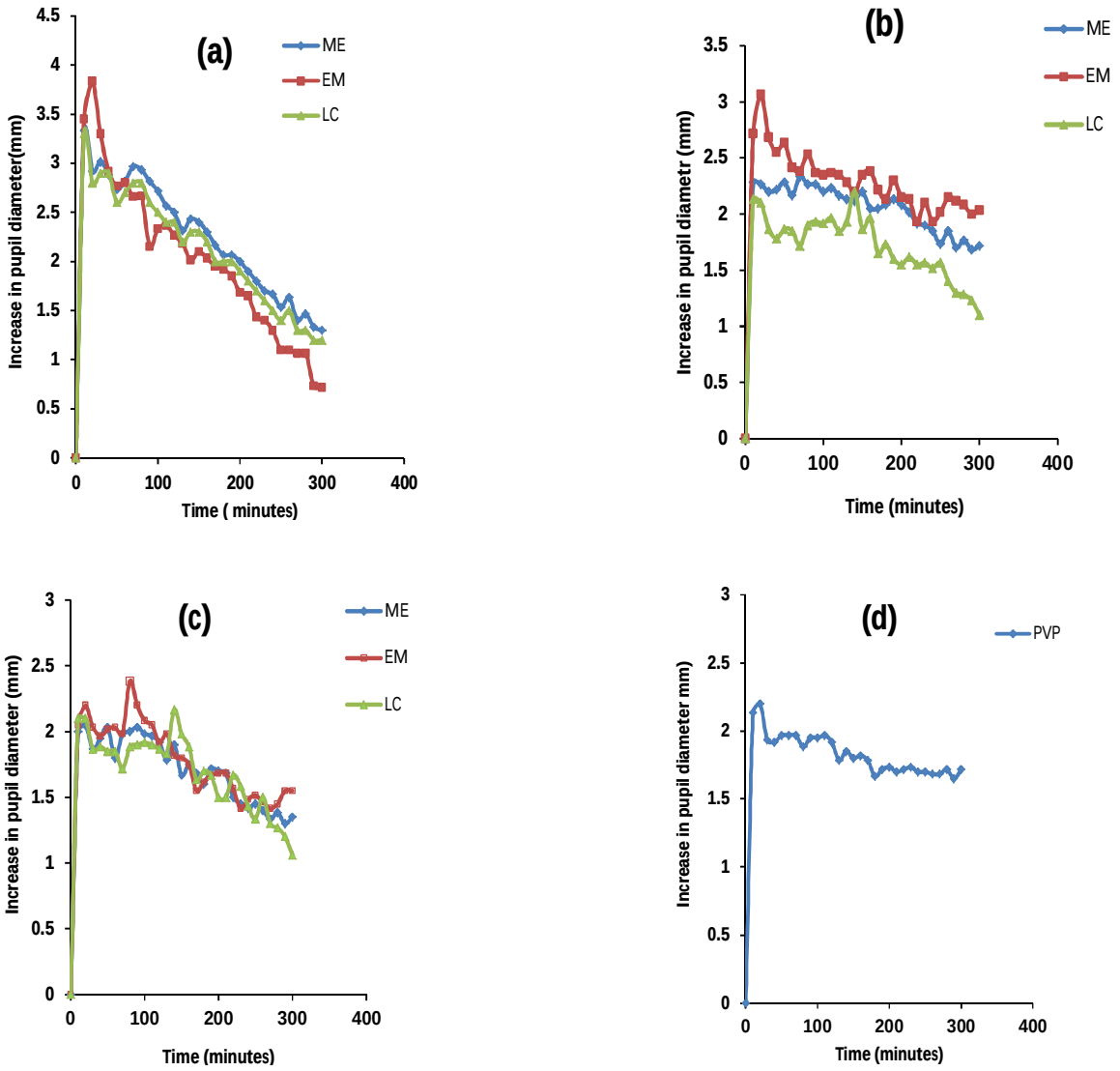

Fig. 5: Mydriatic response profiles obtained after ocular application of tropicamide (0.5% w/w) as (a) ethyl oleate phase transition systems,(b) olive oil based systems, (c) castor oil systems and (d) drug solution. Formulation details are in Table 1.

Table 4: Pharmacokinetic parameters calculated for tropicamide mydriatic response obtained after topical application of microemulsions, liquid crystalline systems, coarse emulsions and aqueous solution formulations.

Form	MR_{max1}	MR_{max2}	T_{max1}	T_{max2}	MR_{120}	MR_{180}	MR_{240}	MR_{300}	AUC
E ME	3.56 (0.77)*	3.5 (0.4)*	23.3 (8)	80 (11.5)	2.5 (0.46)	2.1 (0.35)	1.6 (0.37)	1.3 (0.5)	755 (98)
E LC	3.3 (0.5)*	3.4 (0.29)*	25 (5.1)	98.3 (15.6)	2.8 (0.41)	2.2 (0.4)	1.7 (0.3)	1.4 (0.44)	726 (98)
EEM	3.98 (0.4)*	2.68 (.32)	18.3 (1.6)	60 (6.6)	2.3 (0.36)	1.9 (0.33)	1.3 (0.3)	0.7 (0.11)	612 (65)
OME	2.7 (0.1)*	2.44 (0.19)	28.3 (11.3)	126 (23.6)	2.16 (0.27)	2.06 (0.16)	1.85 (0.16)	1.7 (0.16)	612 (44)
O LC	2.2 (0.1)	2.3 (0.15)	20 (8.1)	111.7 (15.2)	1.85 (0.17)	1.7 (0.09)	1.5 (0.06)	1.1 (0.08)	641 (26)
OEM	3.1 (0.12)*	2.66 (0.14)*	16.7 (2.2)	82 (9)	2.3 (0.13)	2.1 (0.11)	1.9 (0.16)	2 (0.05)	677 (29)
CME	2.3 (0.19)	2.2 (0.17)	31.6 (13.5)	101.6 (10.9)	1.9 (0.19)	1.6 (0.1)	1.4 (0.13)	1.35 (0.15)	488 (29)
C LC	2.2 (0.11)	2.1 (0.15)	21.7 (7.9)	156.7 (15.3)	1.86 (0.16)	1.7 (0.14)	1.4 (0.1)	1.06 (0.08)	507 (9)
CEM	2.3(0.04)	2.35 (0.1)	36.7 (12.3)	111.7 (19.7)	1.9 (0.09)	1.6 (0.13)	1.48 (0.08)	1.55 (0.1)	533 (13)
control	2.2 (0.1)	2.2 (.13)	12 (1.7)	68 (4.7)	1.9 (0.14)	1.6 (0.06)	1.7 (0.09)	1.7 (0.1)	542 (34)

Notes: Values between brackets are S.E.M., n=6. * Significantly different from the drug solution. The control was solution of the drug in water containing 0.25% w/v PVP. E is ethyl oleate, O is olive oil and C, castor oil.

Ocular irritation studies

The success of ocular drug delivery system is usually evaluated from the recorded pharmacological response while maintaining ocular safety. Accordingly, it was important to monitor the effect of composition of the ME systems on the ocular irritation. This was evaluated by visual inspection of the eye for redness. In addition the rate of tear flow was monitored through phenol red thread test. With respect to the visual appearance all of the tested formulations resulted in slight redness of the test eye relative to the control one. This started to reduce gradually throughout the day with the eyes returning back to normal after 24 hours. The results of the tear volume measurement were calculated as the difference in the length of the wetted portion of the thread measured after and before instillation of the formulations. The results are presented in Table 5. The results revealed higher tear flow rate in case of the synthetic oil (ethyl oleate) based systems compared with the corresponding natural oil based system. This finding indicates better safety profile for the natural oil containing formulations even if the composition of these oils contains fatty acids similar to the precursor of ethyl oleate. With respect to the effect of formulation, LC based systems showed the largest effect on the lacrymation with the ME based systems producing the smallest tear flow rate. This pattern was evident irrespective to the composition of the phase transition system. Similar pattern was reported for ethyl oleate based system (Chan *et al.*, 2008).

Table 5: The difference in the wetted length of the phenol red thread calculated from post-instillation minus pre-instillation length. Results are presented as mean ± SD, n=6

Formulation	Average difference (mm)
Ethyl oleate ME	8 ± 2.1
Ethyl oleate LC	14.2 ± 4.3
Ethyl oleate EM	11.2 ± 2.6
Castor ME	6.6 ± 1.5
Castor LC	9.3 ± 1.6
Castor EM	7.6 ± 2.0
Olive ME	5.2 ± 1.1
Olive LC	8.8 ± 4.1
Olive EM	6.5 ± 1.7
SOL	8.5 ± 3.1

CONCLUSION

The efficacy and safety of phase transition microemulsion systems depend on the composition of the system. Those based on natural oil may retain the safety and efficacy as ocular drug delivery formulation.

REFERENCES

Aggarwal D, Kaur IP. Improved pharmacodynamics of timolol maleate from mucoadhesive niosomal ophthalmic drug delivery system. Int J Pharm. 2005;290:155-159.

Alany RG, Tucker IG, Davies NM, Rades T. Characterizing colloidal structures of pseudoternary phase diagrams formed by oil/water/amphiphile systems. Drug Dev Ind Pharm. 2001;27(1):31-38.

Calvo P, Vila-Jato JL, Alonso MJ. Evaluation of cataionic polymer coated nanocapsules as ocular drug carriers. Int J Pharm. 1997; 153,41-50.

Chan J, El Maghraby GM, Criag JP, Alany RG. Effect of water-in-oil microemulsions and lamellar liquid crystalline systems on the precorneal tear film of albino New Zealand rabbits. Clin Ophthalmol. 2008;2(1):129-137.

Chan J, El Maghraby GM, Criag JP, Alany RG. Phase transition water-in-oil microemulsions as ocular drug delivery systems: *In vitro* and *In vivo* evaluation. Int J Pharm. 2007;328:65-71.

Craig J, Blades K. Preocular tear film assessment. Optician. 1997;219:20-27.

Dilbagi N, Kaur H, Ahuja M, Kumar S. Evaluation of tropicamide-loaded tamarind seed xyloglucan nanoaggregates for ophthalmic delivery. Carbohydr Polym. 2013;94:286-291.

Durrani AM, Farr SJ, Kellaway IW. Influence of molecular weight and formulation pH on the precorneal clearance rate of hyaluronic acid in rabbit eye. Int J Pharm.1995;118:243-250.

El Maghraby GM, Bosela AA. Investigation of self-microemulsifying and microemulsion systems for protection of prednisolone from gamma radiation. Pharm Dev Technol. 2011; 16(3): 237-42.

El Maghraby GM. Occlusive and non-occlusive application of transdermal delivery of progesterone: mechanistic studies. Sci Pharm. 2012;80(3):765-78.

Fitzgrrald P, Hadgraft J, Kreuter J, Wilson CG. A gamma scintigraphic evaluation of microparticulate ophthalmic delivery systems: liposomes and nanoparticles. Int J Pharm. 1987;40:81-84.

Gasco MR, Gallarate M, Trotta M. Microemulsions as topical delivery vehicles: ocular administration of timolol. J Pharm Biochem Anal. 1989;7(4):433-439.

Gurny R, Boye T, Ibrahim H. Ocular therapy with nanoparticulate systems for controlled drug delivery. J Control Rel. 1985;2:353-361.

Habe A, Keipert S. Development and characterization of microemulsions for ocular application. Eur J Pharm Biopharm. 1997;43:179-183.

Hegde RR, Bhattacharya SS, Verma A, Ghosh A. Physicochemical and pharmacological investigation of water/oil microemulsion of non-selective Beta blocker for treatment of glaucoma. Curr Eye Res. 2014;39(2):155-163.

Kaufman HE, Stuinemann TL, Lehman E, et al. Collagen based drug delivery and artificial tears. J Ocul Pharmacol. 1994;10(1):17-27.

Kesavan K, Kant S, Singh PS, Pandit JK. Mucoadhesive chitosan-coated cationic microemulsion of dexamethasone for ocular delivery: *In vitro* and *In vivo* evaluation. CURR EYE RES. 2013;38(3):342-352.

Kumar A, Ahuja M. Aqueous carboxymethyl gum Kondagogu as vehicle for ocular delivery. J Pharm Investig. 2014;44(4):237-242.

Lang GC. Ocular drug delivery conventional ocular formulations. Adv Drug Deliv Rev. 1995; 16:39-43.

Meseguer G, Gurny R, Buri P. *In vivo* evaluation of dosage forms : application of gamma scintigraphy to non-enteral routes of administration. J Drug target.1994; 2:269-288.

Miller SC, Donovan MD. Effect of poloxamer 407 gel on the miotic activity of pilocarpine nitrate in rabbits. Int J Pharm. 1982;12:147-152.

Nagarsenker MS, Londhe VY, Nadkarni GD. Preparation and evaluation of liposomal formulations of tropicamide for ocular delivery. Int J Pharm. 1999;190(1):63-71.

Nazar MF, Khan AM, Shah SS. Microemulsion system with improved loading of piroxicam: a study of microstructure. AAPS PharmSciTech. 2008;10(4):1286-1294.

Parvanthi M, Prathyusha A, Kiran RSV, Reddy JR. Preparation and evaluation of quetiapine fumarate microemulsions: A novel delivery system. Asian J Pharm Clin Res. 2014;7(4):208-213.

Pepic I, Jalsenjak N, Jalsenjak I. Micellar solutions of triblock copolymer surfactants with pilocarpine. Int J Pharm. 2004;272:57-64.

Pignatello R, Bucolo C, Ferrara P, et al. Eudragit RS100® nanosuspensions for the ophthalmic controlled delivery of ibuprofen. Eur J Pharm Sci. 2002;16:53-61.

Salimi A, Motaharitabar E, Goudarzi M, Rezaie A, Kalantari H. Toxicity evaluation of microemulsion (nanosize) of sour cherry kernel extract for the oral bioavailability enhancement. Jundishapur J Nat Pharm Product. 2014;9(1):16-23.

Schmidt KS, Hacker DV, Kass PH, Barkhoodarian AL. Effects of systemic administration of 0.5% tropicamide on intraocular pressure, pupillary diameter, blood pressure, and heart rate in normal cats. Vet Ophthalmol. 2006;9(2):137-139.

Sha X, Wu J, Chen Y, Fang X. Self Microemulsifying drug delivery system for improved oral Bioavailability of Probucol : Preparation and evaluation. Int J Nanomedicine. 2012; 7:705-12.

Unterman SR, Rootman DS, Hill JM, et al. Collagen shield drug delivery: therapeutic concentrations of tobramycin in the rabbit cornea and aqueous humor. J Cataract Refract Surg. 1988; 14:500-504.

Vandamme TF, Brobeck L. Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine and tropicamide. J Control Rel. 2005;102:23-38.

Vandamme TF. Microemulsions as ocular drug delivery system: recent developments and future challenges. Prog Retin Eye Res. 2002;21:15-34.

Vyas SP, Mysore N, Jaidey V, Venhastesan N. Niosomes based controlled ocular delivery of timolol maleate. Pharmazie. 1998; 53: 466-469.

How to cite this article:

El-Agamy H.I. and El-Maghraby G.M. Natural and synthetic oil phase transition microemulsions for ocular delivery of tropicamide: efficacy and safety. J App Pharm Sci, 2015; 5 (Suppl 2): 067-075.