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Research Article

NANOEMULSION COMPONENTS SCREENING OF QUETIAPINE FUMARATE: EFFECT OF SURFACTANT AND CO SURFACTANT

SNEH PRIYA[1]*, MARINA KOLAND[1], SUCHETHA KUMARI N[2]

[1]Department of Pharmaceutics, NGSM Institute of Pharmaceutical Sciences, Nitte University, Mangalore, Karnataka, India. [2]Department of Bio-Chemistry, K S Hegde Medical Academy, Nitte University, Mangalore, Karnataka, India. Email: snehpriya123@gmail.com

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ABSTRACT

Objective: Correct selection of oils, surfactants, and co surfactants along with their optimum concentration is essential to get stable and clinically acceptable nanoemulsions. The aim of the present study was, to provide an efficient screening approach for the excipients selection for the optimum nanoemulsion formulation development.

Methods and Results: The solubility of quetiapine (QTP) fumarate in the oils (Capryol 90, isopropyl myristate, castor oil, and olive oil), surfactants (labrasol, cremophor EL, Brij L23, Tween 20, and Tween 80), and co surfactants (ethanol, isopropyl alcohol, and propylene glycol) was taken as the criterion for selection. The maximum solubility of QTP was found to 35.54±0.91, 40.4±0.83, and 32.07±0.92 in Isopropyl myristate, Tween 20, and propylene glycol, respectively. The effect of Tween 20/propylene glycol mass ratio on the nanoemulsion formation was also studied by varying the ratio from 3:1 to 1:0 for the further optimization of the system. The highest nanoemulsion region was obtained at Tween 20/propylene glycol in the mass ratio of 3:1. Formulations were selected from the phase diagram at which concentration of oil was constant with increasing (30, 35, 40, 45% wt/wt) concentration of surfactant/co surfactant mass ratio (Smix) and subjected to thermodynamic stability tests. The optimized formulations were characterized for particle size, viscosity, pH and refractive index measurements. All the selected formulations were found to be stable, and the droplet size was found to be <100 nm.

Conclusion: The formulations were thermodynamically stable and can be effectively used for the drug delivery applications.

Keywords: Nanoemulsions, Pseudoternary phase diagram, Oil, Surfactant, Co surfactant.

INTRODUCTION

Nanoemulsions are isotropic, thermodynamically stable transparent or translucent systems of oil, water, and surfactants with a droplet size usually in the range of 10-200 nm. In many cases co surfactant or cosolvent is also used in addition to the surfactant [1,2]. Because of long-term stability, ease of preparation, and high solubilization of lipophilic drug molecules make nanoemulsions as a promising drug delivery tool [3,4]. Recently, it draws much interest in the area of nanoemulsions for nasal delivery for brain targeting [5,6]. They are also being investigated keenly for potential applications in ocular [7,8], pulmonary [9], transdermal 10], vaginal [11], and parenteral drug delivery [12].

Quetiapine (QTP) fumarate was selected as a model drug for this study (log p=2.8). It is a short-acting atypical antipsychotic approved for the treatment of schizophrenia, bipolar disorder, and along with an antidepressant to treat major depressive disorder. It also has an antagonistic effect on the histamine H1 receptor [13]. These systems often require high surfactant concentration, and this may lead to toxicity and irritancy problems. Therefore, cautious selection of surfactants along with their optimum concentration is required. The influence of the surfactant/co surfactant mass ratio (Smix) on the nanoemulsion formation region also formed an important aspect of the study. Optimum selection would aid in better formulation with desirable attributes. Therefore by considering all the objective of this study was to provide an efficient screening approach for the proper selection of oils, surfactants, and co surfactants for the nanoemulsion formulation development.

METHODS

Materials

Quetiapine (QTP) fumarate was received as generous gift from Orchid Pharmaceuticals Pvt. Ltd., (Chennai, India). Propylene glycol monocaprylate (Capryol 90), caprylocaproyl macrogol-8-glyceride (Labrasol) (Gattefosse, Gennevilliers, France), and Brij L23 were gift samples from Colorcon Asia (Mumbai, India). Polyoxy-35-castor oil (Cremophor EL), Tween 20 (Polyoxyethylene sorbitan monolaurate), and Tween 80 (sorbitate monooleate) were procured from Sigma-Aldrich Chemicals Pvt. Ltd., USA. Isopropyl myristate, castor oil, olive oil, high-performance liquid chromatography (HPLC) grade water methanol and acetonitrile were purchased from E-Merck (Mumbai, India). Ethanol, isopropyl alcohol, and propylene glycol were procured from S.D Fine Chemicals (Mumbai, India). All other chemicals and solvents were of analytical grade.

Nanoemulsion components screening and selection

Screening of oil, surfactant and co surfactant based on solubility of QTP

The solubility of QTP in various oils, surfactants, and co surfactants were determined by adding an excess amount of drug in 2 ml of the oils (Capryol 90, isopropyl myristate, castor oil, olive oil), surfactants (Labrasol, Cremophor EL, Brij L23, Tween 20, and Tween 80) and co surfactants (ethanol, isopropyl alcohol, and propylene glycol) separately in 5 ml capacity stopper vials, and mixed using a vortex mixer. The mixture vials were then kept at 25±1.0°C in shaker for 72 hrs to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3,000 rpm for 15 minutes. The concentration of drugs in the supernatant was analyzed by HPLC method after proper dilution [10].

HPLC analysis of drug

Determination of QTP was performed by a validated HPLC method developed in our laboratory [14]. A Shimadzu-model HPLC equipped with quaternary LC-10A VP pump, variable wavelength programmable ultraviolet (UV)/visible detector, SPD-10AVP column oven (Shimadzu),

SCL 10AVP system controller (Shimadzu), Rheodyne injector fitted with a 20-µl loop was used, and the data were recorded and evaluated using Class-VP 5.032 software. Chromatographic separation was achieved on a reversed-phase C-18 column, LiChrospher®100 (250×4.6 mm inner diameter, 5 µm) using a mobile phase consisting of phosphate buffer (pH 3), acetonitrile and methanol (ratio 50:40:10) at a flow rate of 0.5 ml/minute with UV detection at 254 nm. The mobile phase was filtered through 0.45 µm membrane filter and degassed by sonication before use.

Effect of Smix on nanoemulsion formation

Surfactant was blended with co surfactant in the weight ratios of 1:0, 1:1, 2:1, 3:1, and 4:1. These Smix were chosen in increasing concentration of surfactant with respect to co surfactant for detailed study of the phase diagrams. Aqueous titration method was used for the construction of the pseudoternary phase diagrams, which involves stepwise addition of water to each weight ratio of oil and surfactants, and then mixing the components with the help of vortex mixer at 25°C. The nanoemulsion phase was identified as the region in the phase diagram where clear, easily flowable, and transparent formulations were obtained based on the visual observation. Nine different combinations in different weight ratios of oil and Smix, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1, were taken. One axis of the pseudo- three-component phase diagram represented the aqueous phase, the other represented the oil phase, and the third represented the Smix. Pseudoternary phase diagrams were plotted by using CHEMIX School Version 3.60 and nanoemulsion region was selected from there [4,10].

Preparation of nanoemulsions

Nanoemulsion was prepared by ultrasonication method by using probe sonicater (Table 1). In this method, the droplet size of conventional emulsion is reduced with the help of sonication mechanism. Only small batches of nanoemulsion can be prepared by this method. In this method, the oil phase and the aqueous phase were first prepared. Oil phase contain drug, oil and Smix, and aqueous phase contain water. Oil phase were prepared by dissolving drug in oil and Smax mixture by Probe sonication at amplitude of 50, pulse 5 seconds on and 5 seconds off for 3 minutes. The oil phase was added dropwise to hot aqueous phase (50°C) with continuous stirring at 250 rpm. This conventional emulsion was then probe sonicated at amplitude of 50, pulse 5 seconds on and 3 seconds off for 10 minutes to get nanoemulsion [15].

Characterization of nanoemulsions

Thermodynamic stability studies

To conquer the problem of metastable formulation, thermodynamic stability tests were performed. Some representative formulations were taken from the o/w nanoemulsion region of the phase diagram constructed at Smix 3:1, since it showed the maximum nanoemulsions area and were subjected to the thermodynamic stability tests such as heating-cooling cycle, freeze-thaw cycle, and centrifugation. The selected formulations were centrifuged at 3500 rpm for 30 minutes. Those formulations that did not show any phase separations were taken for the heating and cooling cycle. Six cycles between refrigerator temperatures 4°C and 45°C storage at each temperature of not <48 h were conducted. The formulations that were stable at these temperatures were subjected to the freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulations between 0°C and +25°C [4,10].

Emulsification efficiency

Initial evaluation of emulsifying properties was carried out by visual assessment. The time taken for the nanoemulsions to emulsify in the water and get miscible in it was considered as the emulsifying time of that nanoemulsions formulation. Take 1 ml of nanoemulsions formulation and pour it into water, and the time taken for emulsification was noted and kept for 24 hrs to categorize for its clarity and stability. Experiments were performed in three replicates for each sample. After 24 hrs, emulsification efficiency of the resultant nanoemulsions was categorized by visual assessment (Table 2) [16].

pH measurements

The apparent pH of the nanoemulsion formulations was measured by a pH meter (systronics, India) in triplicate at 25°C.

Viscosity

The viscosity of the nanoemulsions was determined by Brookfield R/S plus rheometer (Brookfield Engineering, Middleboro, MA) using a C50-1 spindle in triplicate at 25°C [4].

Refractive index

The refractive index of the system was measured by an Abbe refractometer (Bausch and Lomb Optical Company, Rochester, NY) by placing one drop of the formulation on the slide in triplicate at 25°C.

Droplets size and size distribution

The droplet size, polydispersibility index (PDI) and zeta potential of SLNs was determined using zetasizer by dynamic light scattering (Nano ZS, Malvern Instruments, UK). Six replicates were measured, and values were measured as mean±standard deviation. The zeta potential of a droplet is the overall charge that the particle acquires in a particular medium. Knowledge of the zeta potential of nanoemulsions helps to assess the stability of the formulation during storage [4].

Fluorescence optical photomicroscopy

Morphology and structure of the nanoemulsion were studied using Olympus BX53 fluorescence microscope. A drop of nanoemulsions was placed on a clean glass slide and viewed by fluorescence microscope under ×20 magnification.

RESULTS AND DISCUSSION

The important criterion for selection of materials for the nanoemulsion formulation development is that the components are pharmaceutically acceptable, non-sensitizing and nonirritant to the skin and mucosa.

Solubility study

The solubility of drug in oil, surfactant and co surfactants is the most important criterion for the screening. The solubility of QTP in different oils/surfactants/co surfactants was determined (Table 3). The solubility of QTP was found to be the highest in isopropyl myristate as compared to other oils. Same way, Tween 20 (surfactant) and propylene glycol (co surfactant) were showed maximum solubility of drug as compared to other surfactant and co surfactant respectively. Hence isopropyl myristate, Tween 20, and propylene glycol were selected

Table 1: Composition of QTP nanoemulsions

Formulation code	Drug (mg)	Oil (% w/w)	Smix (2:1) % w/w)	Water (% w/w)
QNE1	10	5	30	65
QNE2	10	5	35	60
QNE3	10	5	40	55
QNE4	10	5	45	50

QTP: Quetiapine

Table 2: Visual assessment of emulsification efficiency

Dispersability and appearance	Self-emulsification time (minute)	Grade
Compositions spreads rapidly in water forming clear and transparent nanoemulsion	<1	+++ (very good)
Compositions droplets spread in water to form turbid emulsion	3-5	+ (good)
Compositions do not spread in water and form coalescence of oil droplets	Not emulsified	- (poor)

Table 3: Solubility studies of QTP in various components

Ingredients	Solubility in mg/ml*±SD
Oils	
Capryol 90	29.58±0.84
Isopropyl myristate	35.54±0.91
Castor oil	19.3±0.45
Olive oil	13.81±0.79
Surfactants	
Tween 80	20.15±0.69
Tween 20	40.4±0.83
Labrasol	30.43±1.02
Cremophor EL	33.56±0.78
Brij L23	26.24±0.57
Co surfactant	
Ethanol	25.4±0.57
Isoproply alcohol	20.2±0.86
Propylene glycol	32.07±0.92
Polyethylene glycol 400	16.88±1.14

*: ???, QTP: Quetiapine

as a component of nanoemulsion as oil, surfactant and co surfactant respectively.

The solubility of the drug in the oil phase is important for the nanoemulsion to maintain the drug in solubilized form. The proper selection of surfactants is very necessary because large amounts of surfactants may cause gastrointestinal and skin irritation when administered orally and topically, therefore, it is important to determine the surfactant concentration properly and use the minimum concentration in the formulation. Nonionic surfactants are relatively less toxic than their ionic counterparts and typically have lower critical micelles concentration [4,17].

Surfactant and co surfactant having HLB value >10 are considered to prefer for making o/w nanoemulsion, the right blend of low and high HLB surfactants leads to the formation of a stable nanoemulsion upon dilution with water [18]. In this study, Tween 20 is selected as a surfactant having the HLB value 16. Fluid interfacial film and brief negative interfacial tension is rarely achieved by the use of a single surfactant usually required the addition of a co surfactant. The presence of co surfactant decreases the bending stress of the interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form a nanoemulsion over a wide range of compositions [19]. Therefore, propylene glycol with the HLB value of 3.4 was selected as a co surfactant selected for the study.

Effect of surfactant and co surfactant mass ratio on nanoemulsion region in pseudoternary phase diagram

The existence of nanoemulsion formation zone can be illustrated with the help of the pseudoternary phase diagram. Phase diagrams were constructed using isopropyl myristate as phase oil and Tween 20 and propylene glycol as the surfactant and co surfactant, respectively. The shaded region of phase diagrams showed nanoemulsion region. In Fig. 1, low-nanoemulsion area was observed when Tween 20 was used alone without co surfactant, i.e., at the Smix ratio 1:0 (Fig. 1a). Probably, when the co surfactant is absent or present at lower concentrations, the surfactant is not able to sufficiently reduce the o/w interfacial tension. The maximum concentration of oil that could be solubilized, as can be seen in the phase diagram 1a., was 21% wt/wt at 54% wt/wt of Smix. When co surfactant was added with surfactant in equal amounts, a higher nanoemulsion region was observed compared with previous one, perhaps because of the further reduction of the interfacial tension and increased fluidity of the interface at Smix 1:1 (Fig. 1b). On further increasing the surfactant concentration, i.e. at Smix 2:1 (Fig. 1c), the nanoemulsion region increased in size as compared to the region in Smix 1:0 and Smix 1:1. The maximum concentration of oil that could be solubilized was 31% wt/wt at 45% wt/wt of Smix in phase diagram 1c. When the surfactant concentration is further increased in the Smix ratio of 3:1, an increased in the nanoemulsion region was observed when compared with Smix 2:1 in Fig. 1d, and its maximum concentration of oil that could be solubilized was 35% wt/wt at 40% wt/wt of Smix. In Fig. 1 it can be said that, when surfactant concentration was increased in comparison to co surfactant, the nanoemulsion region increased up to the 3:1 Smix ratio, but in the 4:1 ratio, it was decreased, indicating that the optimum emulsification has been achieved. Hence from Fig. 1, 3:1 Smix ratio was selected for further preparation of nanoemulsions.

The usual preference is to select formulations with the lowest surfactant concentration for oral administration. However, for transdermal delivery, where enhanced skin permeation is the aim, it is not purposeful to select the lowest surfactant concentration. The surfactant concentration should be chosen so that it gives the maximum flux, which is an important criterion. This is usually not obtained with formulations that contain the highest amount of surfactant since high surfactant concentration decreases the thermodynamic activity of the drug in the vehicle, and the affinity of the drug to the vehicle becomes greater. Therefore, formulations should be optimized judiciously. As it could be seen from the phase diagrams, the surfactant or Smix that is able to increase the dispersion entropy, reduce the interfacial tension and increase the interfacial area, and thus, a lower the free energy of the nanoemulsion system to a very low value with the minimum concentration, and that is thermodynamically stable, is a prospective candidate for efficient drug delivery.

Characterization of the selected nanoemulsions

Thermodynamic stability tests

All formulations were found to be stable because no phase separation, turbidity, creaming, or cracking was observed. Thermodynamic stability confers long shelf life to the nanoemulsion as compared to ordinary emulsions. It differentiates them from emulsions that have kinetic stability and will eventually phase-separate [17].

Emulsification efficiency

All formulation showed very good emulsification efficiency and self-emulsification time was found to be <1 minute. Emulsion efficiency depends on concentration of oil and Smix. As the concentration of Smix will increase self-emulsification time will decrease and as the oil content will increase, self-emulsification time will increases simultaneously.

Viscosity, pH, and refractive index

The viscosity of nanoemulsions is a function of the surfactant, water and oil components and their concentrations. It is also depend on types of nanoemulsion. Increasing the water content lowers the viscosity of in both o/w and w/o type of nanoemulsions, while decreasing the amount of surfactant and co surfactant increases interfacial tension between water and oil resulting in increased viscosity of w/o type of nanoemulsions whereas decrease in o/w type of emulsion [20]. Viscosity tends to increase with the oil content. Nanoemulsions formulation results proved that as concentration of Smix increased, viscosity of formulation were increased. Viscosity is also very important for stability as well as efficient release of drug from nanoemulsions. In general, formulation that possess lower viscosity, expected to exhibit faster release of active ingredients. The pH values of all the formulations were found in the range of 6-7. The result of that they will avoid the nasal mucosal irritation. The mean value of the refractive index for all the formulations was relatively similar. However, a slight increase in the refractive index was seen from formulations QNE1-QNE4 (Table 3). This might be attributed to a decrease in the water content, as water has a comparatively lower refractive index (the refractive index of water is 1.334) [4].

Droplets size, PDI and zeta potential

The droplet size of nanoemulsions was deceased as the concentration of Smix increased (Table 4). Nevertheless, the droplet size of all

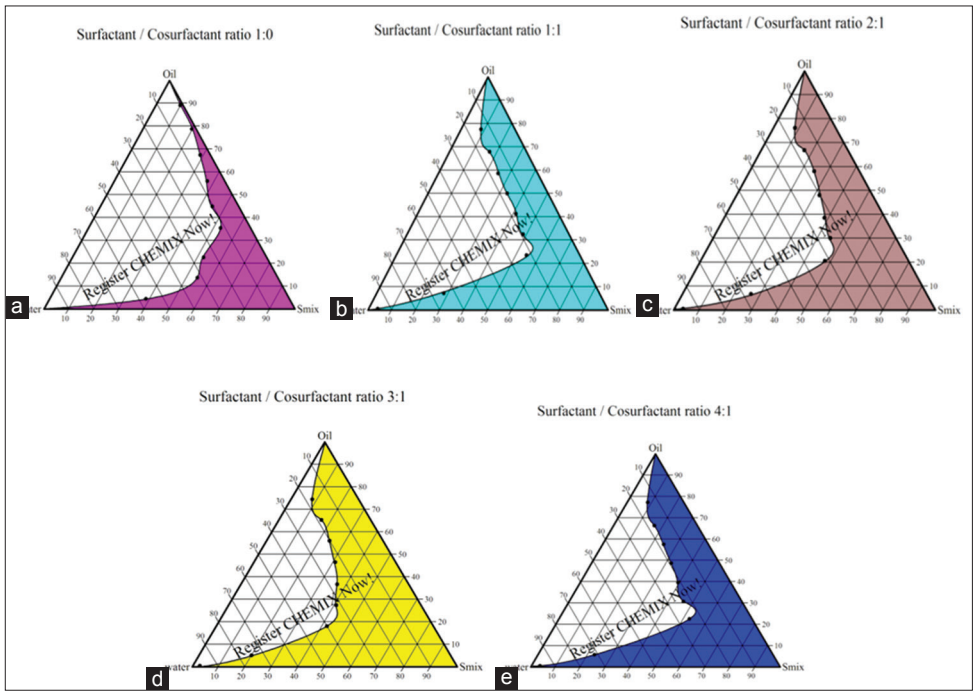

Fig. 1: Pseudoternary phase diagrams indicating o/w nanoemulsion region of isopropyl myristate (oil), water, Tween 20 (surfactant), and propylene glycol (co surfactant) at different Smix ratios. (a) surfactant/cosurfactant ratio 1:0, (b) surfactant/cosurfactant ratio 1:1, (c) surfactant/cosurfactant ratio 2:1, (d) surfactant/cosurfactant ratio 3:1, (e) Surfactant/cosurfactant ratio 4:1

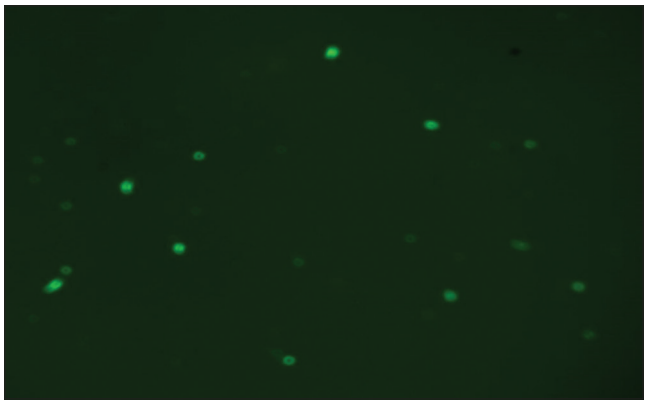

Fig. 2: Fluorescence optical micrographs

the formulations was in the nano range, i.e., below 100 nm. The PDI values of all nanoemulsions were <0.22. The low PDI values indicated that droplet size was uniform within each formulation. The zeta potential of all nanoemulsions formulation ranged from −30.56±2.04 to −39.18±1.05. The preceding results suggested an improvement in the stability of the nanoemulsions because at larger zeta potentials colloidal nanodispersions are more likely to be stable as the charged droplets within them more strongly repel one another, thus overcoming the natural tendency to aggregate.

From Table 4, it was found that formulation QNE2 has sufficient viscosity (>30 cps) which is adequate for retention of formulation in nasal cavity for sufficient absorption through nasal mucosa, and from Table 5, it was found that the droplets size of QNE2 was small enough which should be ideal for delivery into central nervous system as reported in study carried out in this area. Hence, QNE2 was selected as optimized formulation for further investigations.

Shape and morphology

The shapes of droplets were found to be spherical and uniform in size. The droplets in the nanoemulsion appear bright which represent oil phase, and the surroundings are dark; a "positive" image was seen using fluorescence optical photomicroscopy (Fig. 2).

Table 4: Viscosity, emulsifying time, pH and refractive index of QTP nanoemulsions

Formulation code	pH±SD	Viscosity (cps)±SD	Refractive index±SD	Emulsifying time (seconds)±SD
QNE1	6.71±0.41	30.6±0.13	1.339±0.11	16.1±0.021
QNE2	6.08±0.29	34.5±0.24	1.345±0.22	18.3±0.042
QNE3	6.92±0.35	68.8±0.35	1.352±0.09	20.8±0.034
QNE4	6.24±0.51	90.6±0.26	1.359±0.21	23.5±0.053

QTP: Quetiapine, SD: Standard deviation

Table 5: Droplets size, PDI and zeta potential of QTP loaded nanoemulsions

Formulation code	Mean globule size (nm)	PDI	Zeta potential (mV)
QNE1	60.27±1.25	0.213	−31.15±1.05
QNE2	58.19±2.01	0.189	−39.18±2.04
QNE3	50.76±0.51	0.189	−35.31±2.13
QNE4	47.67±1.06	0.209	−30.56±1.55

PDI: Polydispersibility index, QTP: Quetiapine

CONCLUSION

For proficient nanoemulsion formulation, an appropriate selection of components is essential. The study perceptibly demonstrated the impact of the surfactant/co surfactant weight ratio in the formulation of nanoemulsion systems. It is possible to achieve desirable properties by appropriately varying the level of oil, surfactants, and secondary surfactants.

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