ISSN: 2279 - 0594

Journal of Biomedical and Pharmaceutical Research

Available Online at www.jbpr.in

CODEN: - JBPRAU (Source: - American Chemical Society)

Volume 4, Issue 6: November-December: 2015, 21-34

Research Article

# Formulation of Amisulpride loaded Nanoemulsion Drug Delivery System for the Treatment of Schizophrenia

**Nirvesh Chaudhri*, Girish C. Soni, S. K. Prajapati**

Department of Pharmaceutics, Institute of Pharmacy, Bundelkhand University Jhansi-284002, Uttar Pradesh India

Received 20 Nov. 2015; Accepted 14 Dec. 2015

**ABSTRACT**

The treatment of schizophrenia has advanced because the therapeutic efficacy, tolerability, and safety profiles of atypical antipsychotics. Amisulpride is an atypical antipsychotic drug, effective for positive and negative symptoms of schizophrenia with unique receptor pharmacology. As could be predicted from the pharmacologic profile of a pure D2/D3 receptor blocker. Nanoemulsion formulation containing Amisulpride was developed by ultra sonication method. Formulations were prepared using oil (oleic acid and IPM), two surfactants ( Labrasol, and Tween 20 "Smix") and co-surfactant (PEG 400). Optimized formulation, containing 10% oil, 2:1 as Smix, co-surfactant ratio 1 and 2 as ratio of Labrasol and Tween-20 in Smix was prepared. Amisulpride is practically insoluble in water and suffers from irregular and low bioavailability (48%). The current study is aimed at developing and optimizing a Nanoemulsion formulation of amisulpride in order to improve oral absorption of amisulpride through GIT. It exhibited faster and more complete dissolution of amisulpride than marketed tablet regardless of the type and pH of the dissolution medium. Also, it showed a significant improvement of the bioavailability of amisulpride in rats. Optimized Nanoemulsion showed significant ($p<0.001$) increase in vivo bioavailability.

**Keywords:** Amisulpride, Nanoemulsion formulation, phase diagram, pharmacokinetic, Schizophrenia.

## INTRODUCTION

Schizophrenia is a severe brain disorder in which people interpret reality abnormally. Schizophrenia may result in some combination of hallucinations, delusions, and extremely disordered thinking and behavior. The word "schizophrenia" does mean "split mind," but it refers to a disruption of the usual balance of emotions and thinking. Schizophrenia is a chronic condition, requiring lifelong treatment. The large majority of people with schizophrenia show substantial improvement when treated with antipsychotic drugs [1].

Amisulpride, is a benzamide derivative selectively block cerebral dopamine D2 and D3 receptors. When administered at an oral daily dose of 50 mg, it improves the dopaminergic neurotransmission with a D2 dopaminergic receptors pre-synaptic inhibition and it is used in the treatment of Schizophrenia. It suffers from low bioavailability (48%) with high inter-individual variability. It is metabolized in liver only to a minor degree. Since, the bioavailability doesn't exceed the 90% limit of high permeability according to Biopharmaceutics Classification System; therefore, amisulpride could be classified as a drug with low permeability [2].

This low and irregular bioavailability could be attributed to several factors. Amisulpride is practically insoluble in water and is a weekly basic drug (pKa = 9.37). It shows pH dependent solubility because it has one ionisable amino group which can be charged at acidic pH values, making the molecule more soluble. Thus, basic drugs might dissolve completely in the stomach and latter precipitate in the intestine because of the rapid pH increase and extensive dilution of excipients. Accordingly, in order to improve the oral absorption of basic drugs having poor solubility, it is tremendously essential to increase the solubility of basic drugs and to prevent its precipitation in neutral media. This could be due to low solubility and being a substrate for P-glycoprotein efflux [3].

Nanoemulsion is a class of emulsions that have been used as a mean of enhancing oral bioavailability of poorly absorbed drug. Nanoemulsion drug delivery system has gained

*Corresponding author: Nirvesh Chaudhri | E-mail: neersingh5551@gmail.com

more attention due to enhanced oral drug delivery system has gained more attention due to enhanced oral bio-availability enabling reduction in dose, more consistent temporal profiles of drug absorption, selective targeting of drug toward specific absorption window in GIT. The aim of this study is to develop and optimize Amisulpride loaded Nanoemulsion formulation containing bio-enhancers and P-glycoprotein inhibitors components, for the improvement of dissolution and oral absorption of amisulpride [4, 5].

## MATERIALS AND MATHODS

Amisulpride was a gift from Talent india Pvt. Ltd (Ahmadabad, India). Oleoyl macrogol-6 glycerides (Labrafil M 1944) and Labrasol were gifts from Gattefosse Asia Pvt. Ltd. (Mumbai, India). Polyoxyethylene glycol sorbitan monooleate (Tween 20), Polyoxyethylene sorbitan monolaurate (Tween 80) and Isopropyl myristate were obtained from Himedia laboratories Pvt. Ltd. (Mumbai, India). Oleic acid was purchased from Qualikems fine chemical Pvt. Ltd. (Vadodara, India). Polyethylene glycol (PEG 400) and polypropylene glycol (PG) were purchased from Central drug house (New Delhi, India). All other chemicals used were of analytical reagent grade.

### Solubility of Amisulpride

Before starting the phase diagram one must have to select the oil, surfactant and co-surfactant in which the drug shows maximum solubility, to be in the desired solubility range, which is essential for the formulation of nanoemulsion drug delivery system. The solubility of Amisulpride in various oils, surfactants and cosurfactants was determined by dissolving an excess amount of drug in 2 ml of each of the selected oils, surfactants, and cosurfactants in 5-ml capacity Stoppard vials separately. A combination of oils was also used for determination of solubility. An excess amount of drug was added to each 5-ml-capacity stoppard vial and mixed using a vortex mixer. The mixture vials were then kept at 37±1.0$^0$C in an isothermal shaker for 72 hours to get to equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3000 rpm for 15 minutes. The supernatant was taken and filtered through a 0.45-µm membrane filter. The concentration of Amisulpride was determined in each oil, surfactant, cosurfactant, and combination of oils by UV spectrophotometer at their respective λmax at 226nm [6, 7].

### Pseudo-ternary phase diagram

Pseudoternary phase diagrams were constructed using aqueous titration to determine regions where nanoemulsions formed. The surfactant (Labrasol+Tween 20) and the co-surfactant (PEG 400) were selected for nanoemulsion formulation and mixed (as Smix) in different volume ratios (1:1, 1:2, 1:3, 2:1, 3:1, 4:1). For each phase diagram, the selected oil phase (Oleic acid+IPM) and a given Smix ratio were mixed in different volume ratios ranging from 1:9 to 9:1 in different glass vials. Different combinations of oil and Smix (1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1 and 9:1) were prepared to maximize the precision of the phase boundary delineation. Slow titration with aqueous phase was done for each weight ratio of oil and Smix under moderate stirring, and visual observation was used for transparent and easily flowable nanoemulsion [8]. The percentage of water, oil and surfactant /cosurfactant mix at which there was visual evidence of the formation of a nanoemulsion, were plotted on the ternary phase diagram with the axes representing the aqueous phase, the oil and the Smix.

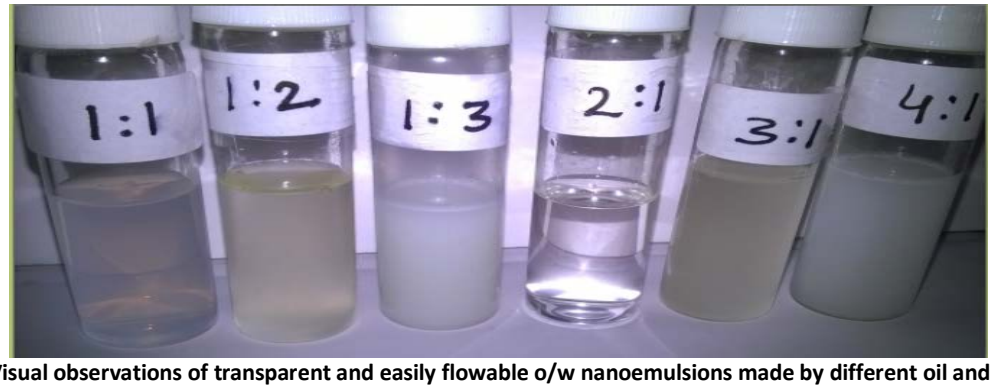


**Figure 1: Visual observations of transparent and easily flowable o/w nanoemulsions made by different oil and Smix ratio**

**Selection of nanoemulsion formulation**

The pseudoternary phase diagrams which shows maximum nonaemulsion region was taken for further studies. From the phase diagrams, a number of nanoemulsions formulations were taken with different ratio of oil, Smix and water.

**Preparation of nanoemulsion by ultra sonicator**

Drug loaded nanoemulsion formulations were prepared using an ultrasonication method. Separately, in the oil phase, consisting of 10 ml of oleic acid+IPM (1:1) the drug was added to the oil phase and stirred with the help of magnetic stirrer. The surfactant and cosurfactant mixture was prepared by Smix ratio (1:1, 1:2, 1:3, 2:1, etc.) Gradually, the Smix (2:1) was added to the oil phase under stirring conditions (Table 1). The oil droplet particle size in the course emulsion formed was further reduced by ultrasonication at 21% amplitude and 50% duty cycle using sonicator (Sonic – vibra cell Bandelin RK 100 H,Germany) ultrasound instrument for 10 minutes [9, 10].

**Table 1: Composition of nanoemulsion excipients**

| Sr. no. | Name of excipients | Amount of excipients used in nanoemulsion formulation | | | | | |
|---|---|---|---|---|---|---|---|
| | | NE1 | NE2 | NE3 | NE4 | NE5 | NE6 |
| 1. | Oleic acid+ IPM (1:1) | 10 | 10 | 10 | 10 | 15 | 15 |
| 2. | Labrasol+ Tween20 (2:1) | 33.3 | 30 | 32 | 20 | 34 | 31.7 |
| 3. | PEG 400 | 16.7 | 15 | 16 | 20 | 17 | 15.8 |
| 4. | Water | 40 | 45 | 42 | 50 | 34 | 37 |

**Characterization of nanoemulsion**

**Thermodynamic Stability Studies**

Selected formulations were subjected to different thermodynamic stability tests to assess their physical stability [11].

1. Heating–cooling cycle: Six cycles between refrigerator temperature (4°C) and 45°C with storage at each temperature of not less than 48 h were conducted, and the formulations were examined for stability at these temperatures.

*2.* Centrifugation test: Formulations were centrifuged at 3500 rpm for 30 min, and examined for phase separation.

*3.* Freeze–thaw cycle: The formulations were subjected to freeze–thaw cycles between−21°C and +25°C and observed for any phase separation.

**Droplet size and zeta potential measurement**

Droplet size and zeta potential of the nanoemulsion was determined by photon correlation spectroscopy that analyzes the fluctuations in light scattering due to Brownian motion of the particles using a Zetasizer (1000 HS, Malvern Instruments UK). The formulation (0.1 ml) was dispersed in 50 ml of DI water in a volumetric flask, mixed thoroughly with vigorous shaking and light scattering was monitored at 25 °C at 90̊ angle [12].

**Transmission Electron Microscopy**

The morphology and structure of the nanoemulsion were studied using transmission electron microscopy (TEM, H7500, Hitachi, Japan). TEM operating at 200 kV capable of point-to point resolution was used. A combination of bright-field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. To perform the TEM observations, the nanoemulsion formulation was diluted with DI water (1/100). A drop of the diluted nanoemulsion was directly deposited on the holey film grid and observed after drying.

**Viscosity, Refractive index, %Transmittance and pH**

Viscosities of nanoemulsion were measured using a Brookfield rotational rheometer (Brookfield, RVIII model, Stoughton, MA, USA) with cone and plate geometry. 1 ml of the formulation was used for viscosity determination. The refractive index of the system was measured by an Abbe refractometer (Rajat scientific work, Moradabad, India) by placing one drop of the formulation on

the slide in triplicate at 25°C and then compare it with water (R.I = 1.333). The percent transmittance of the nanoemulsion was measured using UV-Visible double beam spectrophotometer keeping distilled water as blank at 226nm. Measurement of pH of the samples was made by using the pH meter (cyberscan, eutech instrument) [13, 14].

**In vitro drug release studies**

The in vitro drug release of Amisulpride from the nanoemulsion formulation was determined by dialysis bag method. 0.1N HCl and pH 6.8 buffer were used as medium for in vitro release studies. 1ml of formulation was placed in the dialysis bag(single dose containing 50mg of Amisulpride), which was subjected to release study in 500 ml of dialyzing media (0.1N HCl and phosphate buffer pH 6.8) stirred at a speed of 100 rpm and temperature 37±0.5°C. Samples were withdrawn at predetermined time intervals. In order to maintain sink conditions, an equal volume of medium was replaced. The samples were analyzed by the UV-Visible spectrophotometer at 226nm to determine the concentration [15].

**In vivo studies**

Approval to carry out in vivo study was obtained from Bundelkhand University, Institutional Animal Ethics committee, CPCSEA, Institute of Pharmacy, (Registration no: 716/02/a/CPCSEA), approval (BU/ Pharma/ DAEC/ 15/ 02) and their guideline were followed for the studies. The nanoemulsion formulation (NE2), which showed the highest release profile of drug on in vitro studies, was taken for in vivo studies. The animals used for in vivo experiments were adult Wister rats (200-250 g). The animals were kept under standard laboratory conditions, temperature at 25±2°C and relative humidity (60±5%). The animals were housed in polypropylene cages, with free access to standard laboratory diet (Lipton feed, Mumbai, India), and water ad labitum.

**Induce Schizophrenic-like symptoms in Wister rats**

Schizophrenic-like symptoms have been induced in a rat model after the administration of MK- 801 as an NMDA antagonist but Memantine (NMDA antagonist) are used as an alternative drug [16]. Memantine acting on the glutamatergic system by blocking NMDA receptors and acts as an agonist at the dopamine D2 receptor [17, 18]. It has substituted for phencyclidine in rodent and primate drug discrimination studies [19]. These are show common *adverse drug reactions* include confusion, dizziness, drowsiness, headache, insomnia, and hallucinations [20].

**Procedure**

Three groups were made for the study, and eight rats were kept in each group. First; controlled group (Nanoemulsion formulation), second; Test group (Pure drug suspension), third; Standard group (Marketed Amisulpride tablet).

The formulations (nanoemulsion, marketed tablet and drug suspension) were given orally (5mg/ kg). The rats were anesthetized using chloroform and blood samples (0.5 ml) were withdrawn from retro-orbital vein of rat at 0.5, 1, 2, 4, 6, 8, 12 and 24 h, in centrifuged tubes and centrifuged at 5000 rpm for 10 min. The plasma was separated and stored at -20 °C until drug analysis was carried out using U.V spectroscopy [21].

**Pharmacokinetic and statistical analysis:**

The pharmacokinetic parameters were obtained by a non-compartmental analysis using computer software, Kinetica (version 5, Thermo Fischer Scientific). The maximum plasma concentration (Cmax, μg/ ml) and the time to reach Cmax (Tmax, h) were directly obtained from individual plasma concentration time curve. The area under the curve AUC0-24 (μg.h/ ml) was determined as the area under the plasma concentration-time curve [22].

The relative bioavailability F was calculated using the following equation:

$$F\ (\text{Relative bioavailability}) = \frac{(AUC)\ NE}{(AUC)\ \text{tablet}} \times 100$$

**Stability studies of optimized formulation**

Stability studies on optimized nanoemulsion were performed by keeping the sample at 4±0.5$^0$C, 25±0.5$^0$C and 40±0.5$^0$C. These studies were performed for the period of 3 months. The droplet size, viscosity, refractive index and electrical conductivity were determined at 0, 1, 2 and 3 months [23].

## RESULT AND DISCUSSION

### Solubility of Amisulpride

The solubility of amisulpride in various oils, surfactants and co-surfactants is presented in Table 2). The solubilising efficiency of the oily phase for the drug is the key determining factor for oil selection. Amongst the various tested oils, oleic acid+IPM had the largest solubilising capacity for amisulpride (56.94±9.23mg/ml), so it was chosen for nanoemulsion formulation [24]. The solubility of amisulpride in Labrasol (30.28±0.32 mg/ml) was higher than in Tween 20 (20.26±0.23 mg/ml). In addition, amisulpride exhibited the highest solubility in Labrasol+Tween 20 among the tested surfactants (42.23±2.73 mg/ml). Hence, a mixture of Labrasol and Tween 20 was chosen as surfactant mixture "Smix" for nanoemulsion formulation. Regarding co-surfactant selection, the solubility of the drug will be the perspective criteria particularly due to the substantially high dose of amisulpride. PEG 400 showed the maximum solubility of amisulpride (40.41±0.27 mg/ml) and so it was the co-surfactant of choice in the present study. Labrasol, Tween 20 and PEG 400 are known to have inhibitory effect on P-glycoprotein efflux that is responsible for the low bioavailability of many drugs [25].

**Table 2: Solubility of drug in different Oils, surfactant and cosurfactant**

| Sr. no. | Name of oils | Sulubility (mg/ml) | Name of surfactant and cosurfactant | Sulubility (mg/ml) |
|---|---|---|---|---|
| 1. | Castor oil | 16.96±4.29 | Gelucire | 28.28±0.32 |
| 2. | IPM | 10.65±2.05 | Tween 80 | 15.65±0.35 |
| 3. | Captex 355 | 4.43±0.32 | Tween 20 | 20.26±0.23 |
| 4. | Olive oil | 6.51±0.02 | Labrasol | 30.28±0.32 |
| 5. | Oleic acid | 48.25±0.021 | Span 20 | 16.22±0.25 |
| 6. | Capmul MCM | 29.82±4.22 | Labrasol+ Tween 20 | 42.23±2.73 |
| 7. | Caproyl 90 | 24.53±0.60 | PEG 300 | 35.80±1.02 |
| 8. | Captex 200 | 5.25±0.02 | PEG 400 | 40.41±0.27 |
| 9. | Oleic acid+ IPM | 56.94±9.23 | PG | 26.74±0.23 |
| 10. | Oleic acid+ Labrasol | 33.99±7.16 | Labrafil M1944 | 2.47±0.58 |

### Pseudo-ternary phase diagram

Pseudo ternary phase diagram were constructed to know the range of nanoemulsion. Pseudoternary phase diagram were constructed separately for each Smix ratio, so that O/W nanoemulsion region could be identified and optimized. In figure (2) the surfactant and cosurfactant were mixed in same ratio 1:1 where the concentration of oil 10% has been solubilized and the nanoemulsion area increased. It may be due to reduction of the interfacial tension, increasing the fluidity of the interface, thereby increasing the entropy of the system. With further increase in cosurfactant i.e. smix ratio 1:2, 1:3 (Figure 3, 4,), it was observed that nanoemulsion area has been decreased which states that high amount of cosurfactant mixture may not have the effect on interfacial tension.

When surfactant concentration was increased with respect to co-surfactant, Smix ratio 2:1 (Figure 5), it was seen that nanoemulsion area was increased compared to 1:1 and nearly 10% oil could be solubilised with the smix concentration of 45%. When the surfactant was further increased to smix ratio 3:1 and 4:1 (Figure 6, 7), nanoemulsion area was found to be decreased with same % oil being solubilised at same smix concentration. Hence,

using a constructed phase diagram, the optimum ratios (2:1) of the components are used for nanoemulsion formulation which would remain stable and prevent drug precipitation [26].

After each 5% addition of the aqueous phase to the oil: Smix mixture, visual observation was made and recorded in Table 3. Through visual observation the following categories were assigned:

1. Transparent and easily flowable: oil/water nanoemulsions (NE)
2. Transparent gel: nanoemulsion gel (NG)
3. Milky or cloudy: emulsion (E)
4. Milky gel: emulgel (EG)

**Table 3: Visual observation during aqueous phase titration for phase diagram constraction using smix ratio**

| OIL: SMIX(ml) | OBSERVATION MADE AFTER EACH ADDITION OF AQUEOUS PHASE (ml) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 |
| 1:1 | NE | NE | NE | NE | NE | NE | NE | NE | NE | E |
| 1:2 | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| 1:3 | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| 1:4 | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| 1:5 | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| 1:6 | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| 1:7 | NE | NE | NE | NE | NE | NE | NE | NE | NE | NE |
| 2:1 | NE | NE | NE | NE | NE | NE | NE | NG | NG | NG |
| 3:1 | NE | NE | NE | NG | NG | EG | EG | E | E | E |
| 4:1 | NE | NG | EG | E | E | E | E | E | E | E |
| 5:1 | EG | EG | E | E | E | E | E | E | E | E |
| 6:1 | EG | E | E | E | E | E | E | E | E | E |
| 7:1 | E | E | E | E | E | E | E | E | E | E |

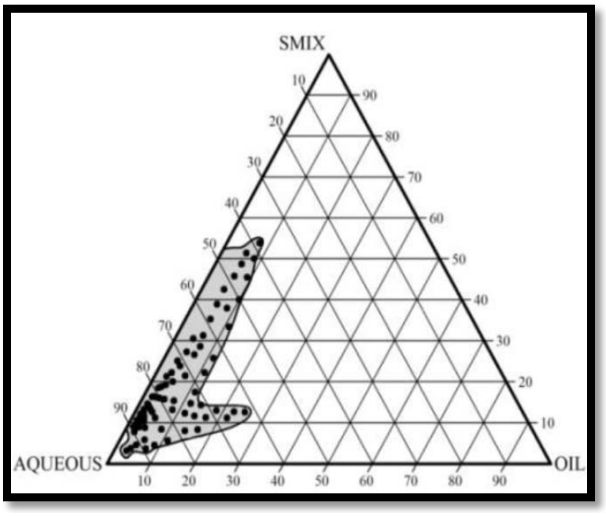


**Figure 2: nanoemulsion region at smax1:1**

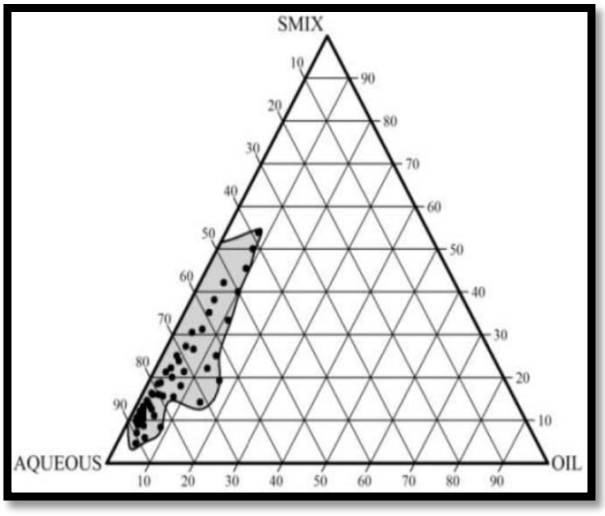


**Figure 3: nanoemulsion region at smax1:2**

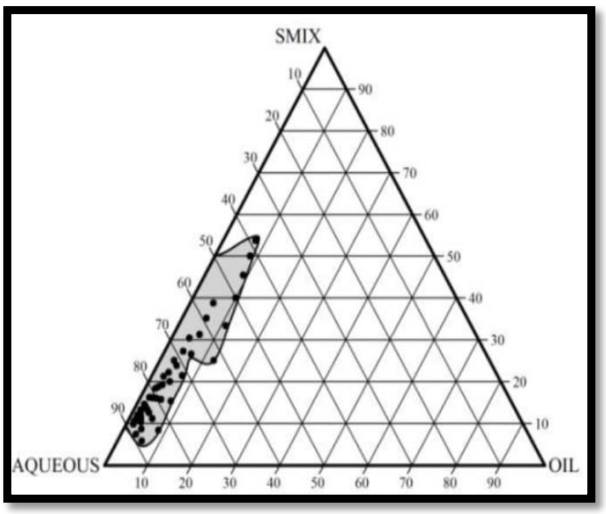


Figure 4: nanoemulsion region at smax1:3

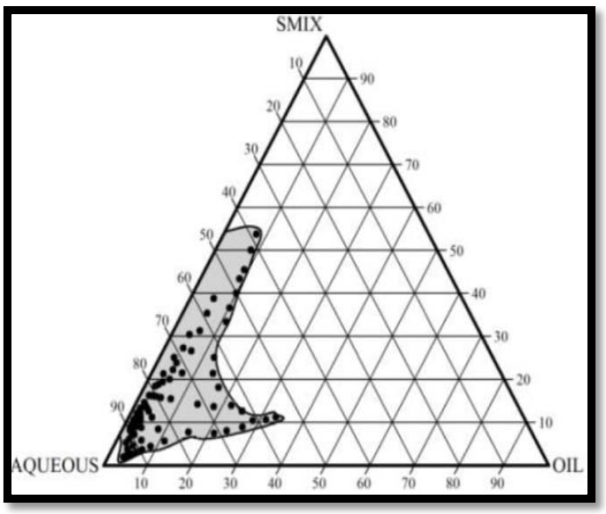


Figure 5: nanoemulsion region at smax 2:1

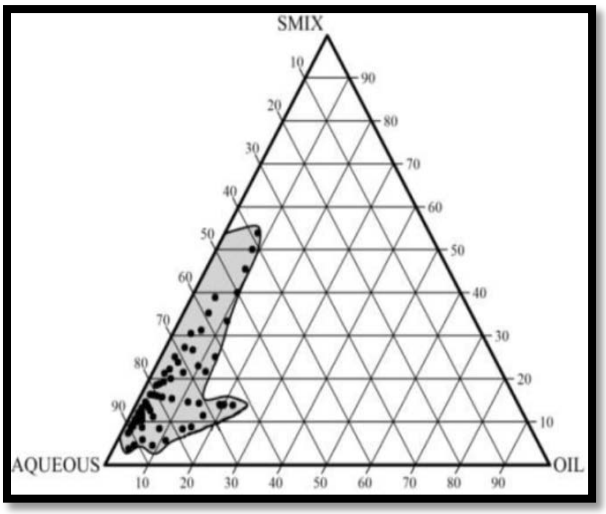


Figure 6: nanoemulsion region at smax3:1

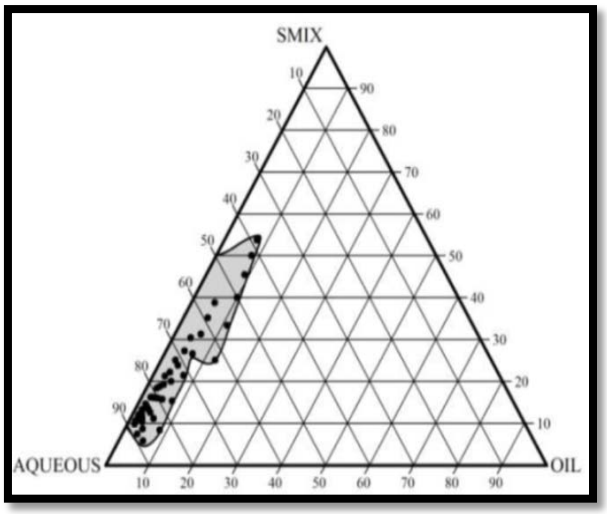


Figure 7: nanoemulsion region at smax4:1

## Characterization of nanoemulsion

### Thermodynamic stability study

It is the thermo stability that differentiates nano- or microemulsions from macroemulsions that are kinetically unstable that eventually lead to phase separation. Thus, the selected nanoemulsion formulation was subjected to different thermodynamic stability stress tests by using centrifugation cycle, heating cooling cycle and freeze thaw cycle. The formulation was stable during each stress cycle; hence it can be said as thermodynamically stable as shown in Table 4.

**Table 4: Thermodynamic stability test of different selected NE formulation**

| Sr. no. | Formulation code | Freezthraw cycle | | Centrifugation | Heat and cooling cycle | | Dispersibility tests | Inference |
|---|---|---|---|---|---|---|---|---|
| | | $4^0$C | $45^0$C | | $-21^0$C | $+25^0$C | | |
| 1 | NE 1 | √ | √ | √ | √ | √ | A | Pass |
| 2 | NE 2 | √ | √ | √ | √ | √ | A | Pass |
| 3 | NE 3 | √ | √ | √ | √ | √ | A | Pass |
| 4 | NE 4 | √ | √ | √ | √ | √ | A | Pass |
| 5 | NE 5 | √ | √ | √ | √ | √ | A | Pass |
| 6 | NE 6 | √ | √ | √ | × | √ | B | Pass |

**Transmission Electron Microscopy**

TEM images post dilution showed that spherical shape and the smooth surface morphology of the oil droplet were formed with sizes ranging from 50 to 100 nm as shown in Figure 7. The nanoemulsion droplets emerged as dark and the surroundings were found to be bright.

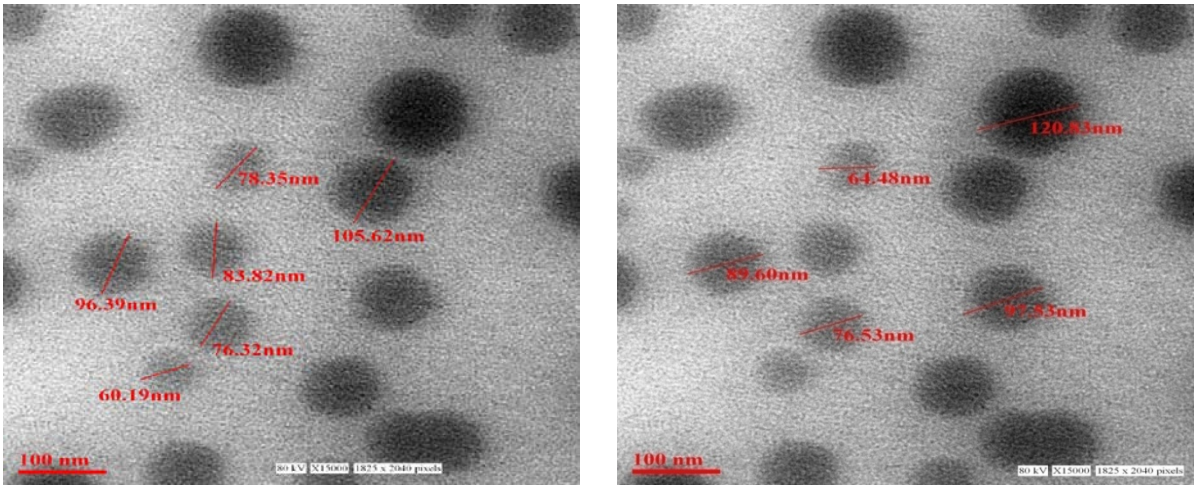


**Figure 8: Droplet sizes of NE formulation are determination by TEM**

**Droplet size, Zeta potential and polydispersity Determination**

The characteristics of nanoemulsions such as droplet size, Zeta potential and polydispersity value were given in Table 5. The parameters for physicochemical characters of the optimized formulations were as follows: 62.89-90.38nm for the average size of all nanoemulsion vehicles particle size. The droplet size of formulation NE2, containing 10% of oil was 62.89nm, which was lower as compared to other formulations (Figure 8). Polydispersity signifies the uniformity of droplet size within the formulation. The polydispersity value of the formulations was very low (<0.2) which indicated uniformity of droplet size within the formulation. The zeta potential values of NE2 formulations was found to be around -39 mV.

**Table 5: Droplet size, Zeta potential and polydispersity determination of NE Formulation**

| Sr. no. | Formulation code | Partical size(nm) | Polydispersity Index (PDI) | Zeta potential (mV) |
|---|---|---|---|---|
| 1. | NE 1 | 76.53 | 0.150 | -26.5 |
| 2. | NE 2 | 62.89 | 0.141 | -39.0 |
| 3. | NE 3 | 84.29 | 0.169 | -21.9 |
| 4. | NE 4 | 90.38 | 0.186 | -18.4 |
| 5. | NE 5 | 83.33 | 0.167 | -22.4 |
| 6. | NE 6 | 89.60 | 0.178 | -19.2 |

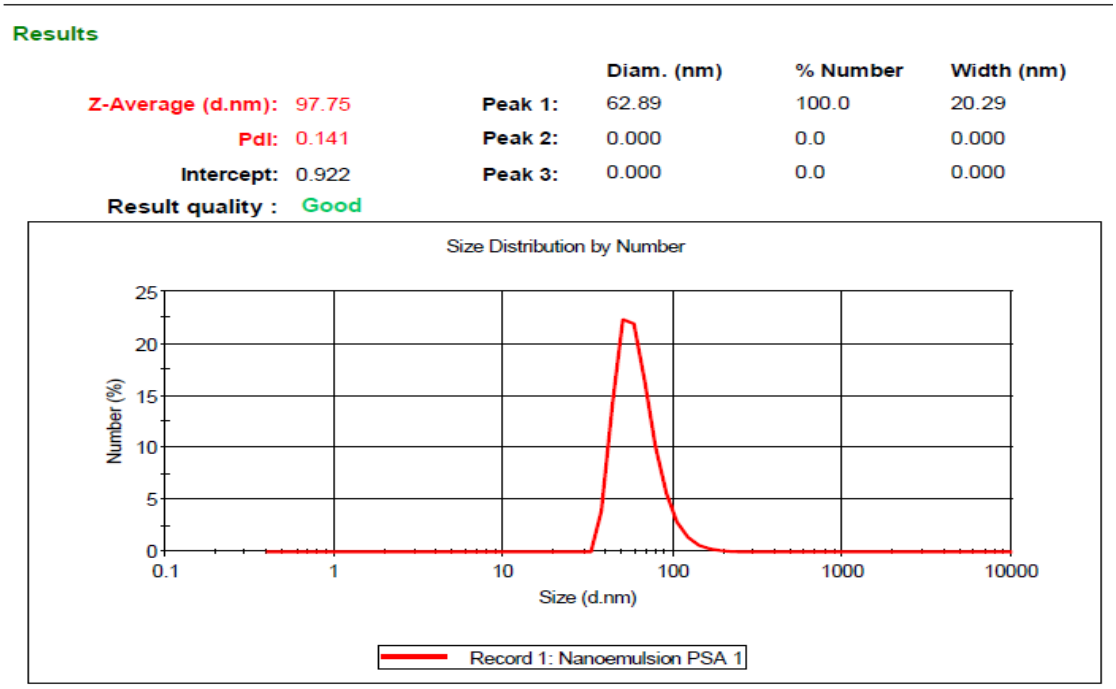


**Figure 9: Droplet size and polydispersity determination by zetasizer**

**Viscosity, Refractive index, %Transmittance and pH**

The characterization of the selected nanoemulsion was determined (Table 6). The viscosity of formulation NE2 (19.36 cp) was lower than that of other formulation. Lower viscosity is one of the characteristics of nanoemulsion formulations. The RI and % Transmittance values of all the nanoemulsion formulations were shown in same Table and the mean values of RI for NE-2 formulation were found to be 1.36 and % Transmittance was 99.40%. These values were close to the RI of water (1.33) because these nanoemulsions were of O/W type. The pH of nanoemulsion was found to be in the range of 4.8±0.2 to 5.7±0.4. The range is suitable for oral administration.

**Table 6: Viscosity, Refractive index, %Transmittance and pH determination of NE formulation**

| Sr. no. | Formulation code | Viscosity (cp) | Refractive index (RI) | % Transmitance | pH |
|---|---|---|---|---|---|
| 1. | NE 1 | 23.40 | 1.31 | 98.96 | 5.3±0.16 |
| 2. | NE 2 | 19.36 | 1.36 | 99.40 | 5.7±0.36 |
| 3. | NE 3 | 25.53 | 1.37 | 98.63 | 4.8±0.15 |
| 4. | NE 4 | 27.69 | 1.40 | 96.55 | 5.6±0.67 |
| 5. | NE 5 | 25.02 | 1.29 | 97.23 | 5.0±0.25 |
| 6. | NE 6 | 26.98 | 1.39 | 98.25 | 5.4±0.50 |

**In vitro drug release study**

Dissolution studies by dialysis bag method were performed to compare the release of drug from six different NE formulations (NE1−NE6) against marketed tablet and aqueous drug suspension having same quantity (50 mg) of AMS. The release of drug from all NEs was much faster and higher in 1.2 pH and phosphate buffer pH 6.8 than the marketed tablet and aqueous drug suspension. The optimised NE- 2 formulation showed highest 96.33±0.70% drug release in 1.2 pH in contrast, the tablet and aqueous drug suspension released 48.20±1.43 % and 40.28±2.63 of the drug in 24 h due to low aqueous solubility. The comparative drug release profile is depicted in Figure 9, 10. The cumulative amount of drug delivered from NEs after 24 h were calculated using PCP-Disso-V2.08 software, Pune, India. The optimized formulation NE- 2 having higher drug release (96.33%), optimum globule size (62.89nm), minimum polydispersity value (0.141), lower viscosity (19.36cp) was selected for the in vivo study.

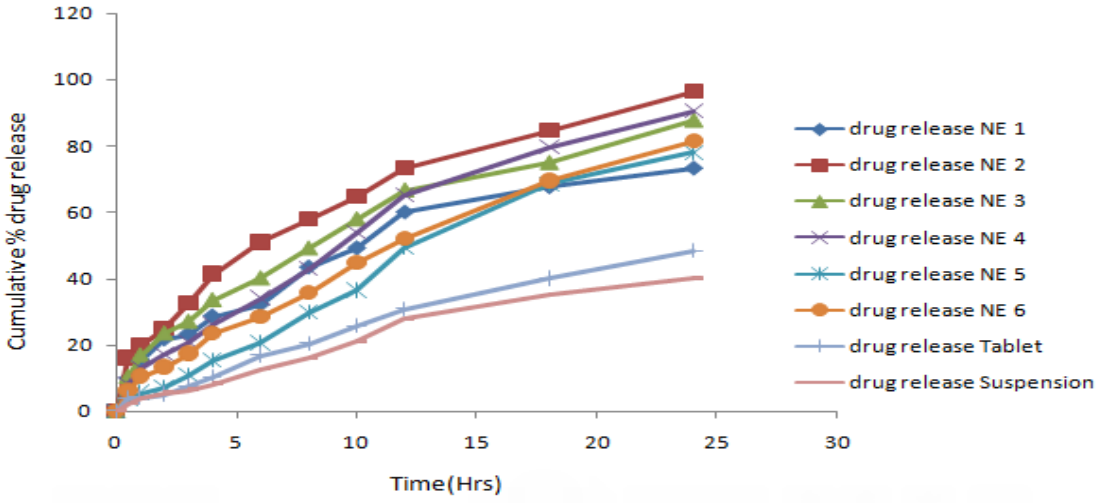


**Figure 10: In vitro release profile of amisulpride formulation in 1.2 pH buffer**

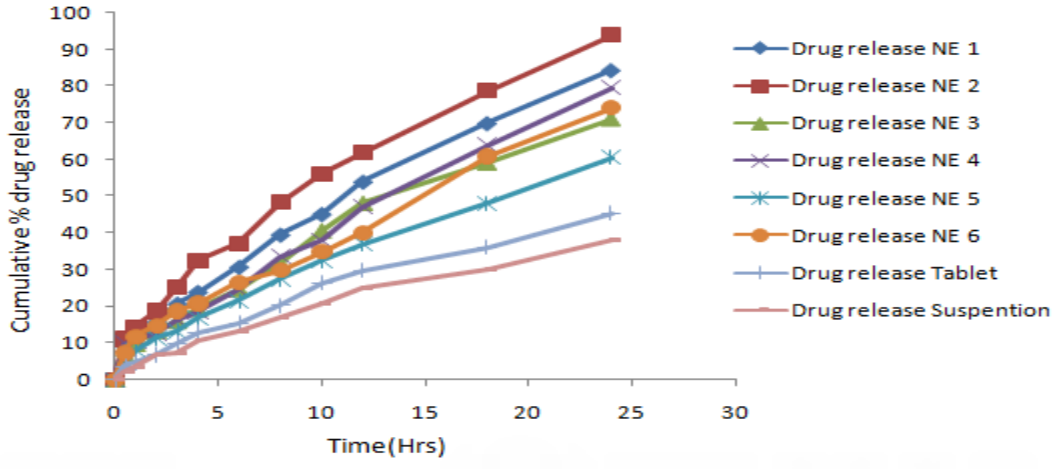


**Figure 11: In vitro release profile of amisulpride formulation in 6.8 pH buffer**

### In vivo studies

In vivo bioavailability study in rats, Figure 11 shows the mean amisulpride plasma concentration vs. time profiles obtained after single oral administrations of the optimized NE- 2 formulation, tablet and the aqueous suspension (50 mg/ml). The mean pharmacokinetic characteristics are summarized in Table 7 that showed Tmax and Cmax of NE2 were 4 h and 14.25±0.14 µg/ml, respectively, as compared to those of tablet which were 4h and 8.49±0.10 µg/ml, drug suspension 6h and 5.98±0.92 µg/ml respectively. Statistically the difference in Tmax of NE-2 was extremely significant ($p < 0.001$) when compared to Tmax of tablet and highly significant ($p < 0.01$) when compared to drug suspension. The difference in Cmax of NE-2 formulation was extremely significant ($p < 0.001$) when compared with tablet formulation, and drug suspension. It was also observed that $AUC_{0-t}$ and $AUC_{tot}$ of NE-2 formulation were 55.6±1.4 µg/ml and 57.5±5.3 µg h/ml, respectively. The difference in the values of MRT is not significantly different ($p > 0.05$).

Statistical analysis was carried out by ANOVA using Tukey's test.

Statistical significance is:

Nanoemulsion vs. Tablet: $p < 0.001$

Nanoemulsion vs. Suspension: $p < 0.01$

Higher drug concentration in blood indicates better systemic absorption of amisulpride from NE. The oral relative bioavailability of amisulpride from optimized NE- 2 formulation exhibited a 1.38-fold increase compared with the orally administrated of marketed tablet and 2.27-fold increase compared with the aqueous drug suspension.

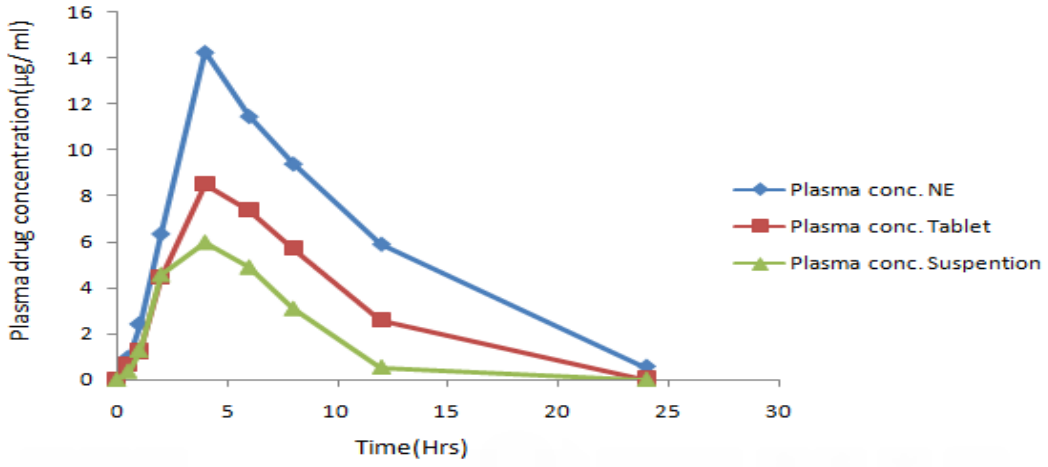


**Figure 12: Comparison of plasma concentration of drug formulation after oral administration as plain drug suspension, marketed formulation and optimized NE formulation.**

**Table 7: Important pharmacokinetic parameters of Amisulpride formulation**

| Sr. no. | pharmacokinetic parameters | Plain drug Suspension | Marketed tablet | NE formulation |
|---|---|---|---|---|
| 1. | Cmax (µg/ml) | 5.98±0.78 | 8.49±0.10 | 14.25±0.14 |
| 2. | Tmax (hrs) | 6.0 | 4.0 | 4.0 |
| 3. | $AUC_{0-n}$ (µg.h/ml) | 27.18±6.16 | 34.63±1.14 | 55.60±1.4 |
| 4. | $AUC_{tot}$ (µg.h/ml) | 29.28±2.90 | 38.20±5.96 | 57.50±5.3 |
| 5. | $T_{1/2}$ (hrs) | 5.63±1.76 | 5.89±1.09 | 7.63±0.41 |
| 6. | MRT (hrs) | 2.21±1.03 | 2.94±2.09 | 4.19±1.64 |

**Stability studies**

All nanoemulsion formulation was characterized for droplet size, viscosity, pH, and RI for the period of three months. It was found that the droplet size, viscosity and RI of NE-2 formulation were not significantly changed during 3 months of storage period at $4^0$C (Table 8 and Figure 12). These results indicated that the optimized formulation was stable as there were no significant changes in physical parameters [28].

**Table 8: Stability studies of optimized nanoemulsion (NE 2) were performed by keeping the sample at refrigerator temperature ($4^0$C), room temperature ($25^0$C) for the period of 3 months.**

| Time(months) | Temperature ($^0$C) | Partical size | RI± SD |
|---|---|---|---|
| 0 | 4.0±0.5 | 62.89 | 1.36 |
| 1 | 4.0±0.5 | 62.89 | 1.36 |
| 2 | 4.0±0.5 | 62.89 | 1.36 |
| 3 | 4.0±0.5 | 64.82 | 1.37 |
| 0 | 25.0±0.5 | 62.89 | 1.36 |
| 1 | 25.0±0.5 | 64.82 | 1.39 |
| 2 | 25.0±0.5 | 69.48 | 1.40 |
| 3 | 25.0±0.5 | 69.48 | 1.40 |

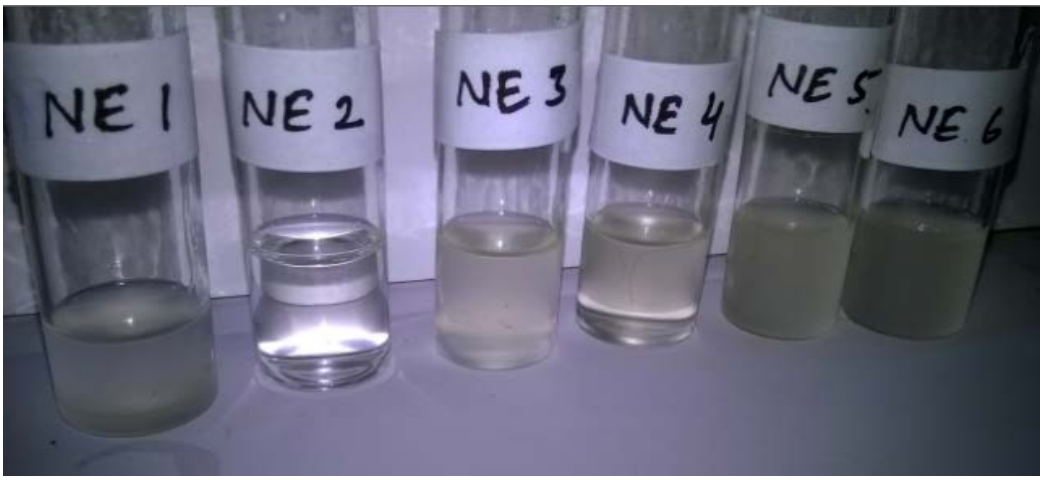


**Figure 13: Stability studies of optimized nanoemulsion at ($4^0$C) and ($25^0$C) for the period of 3 months.**

## CONCLUSION

Amisulpride was successfully formulated as Nanoemulsion formulation. It exhibited faster and more complete dissolution of amisulpride than marketed tablet regardless of the type and pH of the dissolution medium. Also, it showed a significant improvement of the bioavailability of amisulpride in rats. Optimized Nanoemulsion showed significant ($p<0.001$) increase in vivo bioavailability. The oral relative bioavailability of amisulpride from optimized Nanoemulsion exhibited a 1.38-fold increase compared with the orally administrated marketed tablet. This improved oral bioavailability of amisulpride formulation. The plan of this work consists of compatibility test, construction of the pseudo ternary phase diagram to know the range of nanoemulsion, selection of the formulation and incorporation of the drug, evaluation of the formulation, bioanalytical analysis and stability study.

### Conflict of Interests

The authors do not have any conflict of interests in the preparation and publication of this paper.

## ACKNOWLEDGEMENT

The authors are thankful to Talent india Pvt. Ltd. (Ahmadabad, India) for providing the gift sample of Amisulpride.

## REFERENCES

1. Schizophrenia spectrum and other psychotic disorders. In: Diagnostic and Statistical Manual of Mental Disorders DSM-5. 5th ed. Arlington, Va.: American Psychiatric Association; 2013.
2. Xiberas X, Martinot JL, Mallet L, Artiges E, Canal M, Loc'h C, Maziere B, Paillère-Martinot ML: In vivo extrastriatal and striatal D2 dopamine receptor blockade by amisulpride in schizophrenia. J Clin Psychopharmacol 2001;21:207–214.
3. Boyer P, Lecrubier Y, Puech A J, Dewailly J, Aubin F. Treatment of negative symptoms in schizophrenia with amisulpride. Br J Psychiatry. 1997;166: 68–72.
4. International 1. Sarker DK, Engineering of nanoemulsion for drug delivery, Current Drug Delivery, 2005;(2): 297-310.
5. Sole, I., Maestro A., Gonzalez C., Solans C. and Gutierrez J. M. Optimization of Nano-emulsion Preparation by Low-Energy Methods in an Ionic Surfactant System. Langmuir, 2006; vol. 22, 8326-8332.
6. Chhabra G, Chuttani K, Mishra AK, Pathak K. Design and development of nanoemulsion drug delivery system of amlodipine besilate for improvement of oral bioavailability. Drug Dev Ind pharm. 2011;37(8): 907-16.
7. Bouchemal K, Briançon S, Perrier E, Fessi H. Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation International Journal of Pharmaceutics. 2004; 280:241–251.
8. Sheikh S, Faiyaz S, Sushma T, Farhan J, Roop K, Khar MA. Development and bioavailability assessment of ramipril nanoemulsion formulation. European Journal of Pharmaceutics and Biopharmaceutics. 2007; 66: 227–243.
9. Kentish, S.; Wooster, T.; Ashok kumar, M.; Balachandran, S.; Mawson, R.; Simons, L. The use of ultrasonics for nanoemulsion preparation. Innovat. Food Sci. Emerg. Tech. 2008; 9, 170-175.
10. Jafari, S.; He, Y.; Bhandari, B. Nano-emulsion production by sonication and micro-fluidization- a comparison. Int. J. Food Prop. 2006; 9, 475-485.
11. Liew, J. C. L., Nguyen, Q. D. & Ngothai, Y. The stability of N-dodecane/brine nanoemulsions produced by phase inversion temperature method.Conference: Towards a Sustainable Astralasia, 2008; 1625-1634.
12. Craig DQM, Barker SA, Banning D, Booth SW. An investigation into the mechanisms of self-emulsification using particle size analysis and low frequency dielectric spectroscopy. Int J Pharm. 1995; 114: 103Y110.
13. Chiesa, M.; Garg, J.; Kang, Y.T.; Chen, G. Thermal conductivity and viscosity of water-in-oil nanoemulsions. Colloids Surf., A 2008; 326, 1-2, 67-72.
14. Cheong, J.N.; Tan, C.P. Palm-based functional lipid nanodispersions: preparation, character-ization and stability evaluation. Eur. J. Lipid Sci. Technol. 2010; 112, 557-564.
15. Oliveira, AF; Chunha, DA; Ladriere, L et al. "In vitro use of free fatty acids bound to albumin: A comparison of protocols". Bio Feedback. Bio Techniques (Letter to the Editor), 2015; 58: 228–33.

**16.** Liu Y, Tang Y, Pu W, Zhang X, Zhao J. Concentration of DA, Glu and GABA in brain tissues in schizophrenia developmental model rats induced by MK-801. Zhong Nan Da Xue Xue Bao Yi Ban 2011; 36: 712-9.

**17.** Monastero R, Camarda C, Pipia C, Camarda R,. Visual hallucinations and agitation in Alzheimer's disease due to memantine: report of three cases. J Neurol Neurosurg Psychiatry. 2007; 78:546

**18.** Memantine inhibits ATP-dependent K+ conductances in dopamine neurons of the rat substantia nigra pars compacta. J Pharmacol Exp Ther. 322(2):721-9.

**19.** Li Z, Boules M, Williams K, Peris J, Richelson E. The novel neurotensin analog NT69Lblocks phencyclidine (PCP)-induced increases in locomotor activity and PCP-induced increases in monoamine and amino acids levels in the medial prefrontal cortex. Brain Res 2010; 1311: 28-36.

**20.** Carr KD, Kim GY, Cabeza de Vaca S. Rewarding and locomotor-activating effects of direct dopamine receptor agonists are augmented by chronic food restriction in rats. Psychopharmacology 2001;154(4):420–8.

**21.** Hadi El Lakiss, Mihaela Ilie, Daniela Luiza Baconi, Derivative UV spectrophotometry used for the assay of diazepam from human blood plasma. FARMACIA, 2012; Vol. 60, 4.

**22.** Zhang, G., Terry, A.V., Jr, Bartlett, M.G.; Simultaneous estimation of five antipsychotic drugs in rat plasma by high performance liquid chromatography with ultraviolet detection; Journal of Chromatography B, 2007; 856: 20–28.

**23.** Tadros, T.; Izquierdo, P.; Esquena, J.; Solans, C. Formation and stability of nano-emulsions. Adv. Colloid Interface Sci. 2004; 108-109, 303-318.

**24.** Hamazaki K, Hamazaki T, Inadera H. Fatty acid composition in the postmortem amygdala of patients with schizophrenia, bipolar disorder, and major depressive disorder. J Psychiatr Res. 2012; 46:1024-8.

**25.** Yang S, Gursoy RN, Lambert G, Benita S. Enhanced oral absorption of paclitaxel in a novel self microemulsifying drug delivery system with or without concomitant use of P-glycoprotein inhibitors. Pharmaceutical Research, 2004; 21:261-270.

**26.** Aboofazeli R, Lawrence C B, Wicks S R, Lawrence M J; Investigations into the formation and characterisation of phospholipid microemulsions. III. Pseudo-ternary phase diagrams of systems containing water–lecithin–isopropyl myristate and either an alkanoic acid, amine, alkanediol, polyethylene glycol alkyl ether or alcohol as cosurfactant, Int. J. Pharm. 1994; 111, 63–72.

**27.** Sarfaraz Ahmad, Nitesh Kumar et al: Stability testing of Betamethasone Dipropionate Nanoemulsion (O/W) having salmon fish oil. Internationale Pharmaceutica Sciencia. 2014; 2(8):179-183.