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DESIGN AND IN-VITRO EVALUATION OF OLANZAPINE- LOADED SELF NANOEMULSIFYING DRUG DELIVERY SYSTEM

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ABSTRACT

Purpose: The purpose of this work was to prepare olanzapine-loaded self-nanoemulsifying drug delivery systems (SNEDDS) with enhanced self-emulsification properties and hence, a better chance for oral absorption. **Methods:** Various oils, surfactants and co-surfactants were investigated. Preliminary investigations were carried out for the selection of the proper ingredients of the self-emulsifying system depending on the drug solubility and the emulsification power. Ternary phase diagrams were then constructed for the identification of the adequate proportions of ingredients of the self-emulsifying systems. Self-emulsification time, effect of dilution (with different volumes at different pH values), mean globule size as well as polydispersity index values (PDI) were used to compare between the prepared formulas. Formulas with PDI values < 0.3 were selected to be loaded with different amounts of the drug and they were physically evaluated. **Results:** Two optimum systems loaded with 20 mg olanzapine were found to fulfill the criteria of SNEDDS. Both systems were composed of oil mixture (isopropylpalmitate:Capryol 90®(3:1 w/w)), surfactant mixture (Cremophor RH40®:Tween® 80 (1:1 w/w)) and a co-surfactant (Transcutol® HP) at different proportions. They had rapid self-emulsification time (< 15 sec) and were robust to dilution and pH change. Also, they had mean globule size < 20 nm and maintained their PDI <0.3.**Conclusion:** Adjusting the components of the ternary system and their proportions facilitates the preparation of olanzapine- loaded self-nanoemulsifying systems with satisfactory physical characteristics.

1. INTRODUCTION

Olanzapine is an antipsychotic drug which belongs to the thienobenzodiazepine class. The drug is effective in the treatment of positive and negative symptoms of schizophrenia [1]. After oral administration, olanzapine was found to be only 60% bioavailable. This poor bioavailability is attributed to the poor aqueous solubility of the drug (0.192 mg/mL) and its extensive metabolism in the liver producing inactive metabolites [2]. For these reasons, enhancing the drug solubility and protecting it from hepatic metabolism is a desirable approach aiming to improve its therapeutic performance.

For oral route of drug administration, incorporation of the drug in lipid- based delivery systems has attained increasing interest as a means of bypassing the drug passage in the hepatic portal vein and consequently its hepatic degradation. This is believed to be attained chiefly by targeting lymphatic transport via Peyer's patches along the gastrointestinal tract. Nano-emulsions are among the lipid- based drug delivery systems that have been currently investigated for their many advantages. Besides their relative stability and easy manufacturing techniques, nano- emulsions offer the drug a large interfacial area for partitioning between oil and water [3]. Thus, formulating lipophilic drugs in such delivery systems enhances their rate of dissolution and consequently increases their oral absorption [4].

Self- nanoemulsifying drug delivery systems (SNEDDSs) are isotropic mixtures of oils (natural or synthetic) and surfactants (solid or liquid) in addition to hydrophilic solvents, co-solvents and co-surfactants [5]. These mixtures form o/w emulsions by the addition of water with little or no energy input [6]. Therefore, when taken orally, SNEDDSs will directly form o/w emulsions with the gastrointestinal fluids. The globular sizes of the formed emulsions were found to be in the nanometric range ranging from 20-200 nm [5& 6].

Although attaining increasing interest in the field of pharmaceutical researches, SNEDDSs are rarely available in the market. The remarkable product containing self- emulsifying delivery system is Neoral®, which showed significant enhancement of cyclosporin A bioavailability as reported by Porter et al [7].

Accordingly, the aim of this work is to design olanzapine- loaded SNEDDS with optimized physicochemical characteristics. First, the adequate components of the self- emulsifying system as well as their optimum proportions were determined among different oils, surfactants and co-surfactants according to the drug solubility and the emulsification power. The optimum composition of the self- nanoemulsifying system was determined depending upon self-emulsification time, globule size and globule polydispersity index on dilution. The prepared

self- nanoemulsifying systems were loaded with different amounts of olanzapine and their physical characteristics (mean globule size and polydispersity index) were evaluated on dilution in order to select the best formula for olanzapine- loaded SNEDDS.

2. MATERIALS AND METHODS

2.1. Materials

Olanzapine was kindly donated by ApexPharma Company for Pharmaceuticals, Cairo, Egypt. Maisine™ 35-1, Peceol™, Capryol 90®, Labrasol®, Transcutol® HP, Lauroglycol™ FCC and Labrafil® M 2125 CS were gifted by Gattefosse Company, Lyon, France. Cremophor RH40® was purchased from BASF Company, Germany. Acconon MC8-2 was gently donated by Abitec Corporation, Janesville. Isopropylpalmitate (IPP) was obtained from Sigma Chemical Company, St Louis, USA. Tween® 80 (PEG Sorbitan monooleate) was bought from ADWIC, El-Nasr Company for Pharmaceuticals, Egypt. Potassium dihydrogen phosphate and disodium hydrogen phosphate were purchased from Riedel-de Haën, Sigma-Aldrich, GmbH, Germany. All other reagents were of analytical grade and used as received. All water used was deionized, distilled water.

2.2. Design of the SNEDDSs:

2.2.1. Study of the drug solubility in different oils, surfactants and co-surfactants

The equilibrium solubility of olanzapine in different oils was determined using the method described by Dixit and Nagarsenker [8]. Briefly, an excess amount of the drug was added to 3 mL of the investigated oil in a vial and shaken for 72 hours at 30±0.5ºC in a thermostatically controlled shaking water bath (Oscillating thermostatically controlled shaker, GallenKamp, England) to attain equilibrium. The contents of the vials were then centrifuged at 3000 rpm for 10 min using an ultracentrifuge (Model 8880, Centurion Scientific Ltd., W. Sussex, UK) to precipitate undissolved olanzapine. Aliquots from the supernatants were then withdrawn and filtered through a cellulose filter (Millipore® filter 0.22 µm). The ultraviolet absorbance of the filtrates was measured at 277 nm (using Shimadzu UV Spectrophotometer, 1601/ PC, Japan) after appropriate dilution with methanol and their olanzapine content was calculated. The investigated oils were Maisine™35-1, Peceol®, Isopropylpalmitate (IPP) and Capryol 90®, in addition to mixtures of IPP and Capryol 90® at ratios of 1:1, 2:1 and 3:1 w/w

The experiment was repeated to determine the drug solubility in the investigated surfactants and co-surfactants by replacing the investigated oils with the investigated surfactants (Acconon MC8-2, Cremophor RH40®, Labrasol® and Tween® 80) or co-surfactants (Transcutol® HP, Lauroglycol™ FCC and Labrafil® M 2125 CS).

Each experiment was carried out in triplicate and the results were represented as mean values ± standard deviations. Statistical analysis of data was performed using the software SPSS 19.0 (SPSS Inc., Chicago, IL, USA) applying one-way ANOVA test followed by post hoc multiple comparisons using least significant difference (LSD). The results were considered significantly different when p- values were ≤ 0.05.

2.2.2. Preliminary screening of the emulsification efficiency of different surfactants

Emulsification efficiency of the investigated surfactants was estimated according to the method described by Date and Nagarsenker [9]. Accordingly, 300 mg of the surfactant (Acconon MC8-2, Cremophor RH40®, Labrasol® and Tween® 80) were added to 300 mg of the oily phase (Maisine™35-1, Peceol®, IPP and Capryol 90®) and the mixtures were homogenized by heating at 50±0.5ºC for 2 min. From each mixture, 50 mg were diluted with distilled water up to 50 mL in a stoppered conical flask. The stoppered flasks were inverted several times and the number of flask inversions required to form a uniform emulsion (with no turbidity or phase separation) was counted. Furthermore, the formed emulsions were left on rack for 2 hours, then their transmittance was measured at 638.2 nm (by means of UV Spectrophotometer) using distilled water as blank. The percent transmission was calculated for each emulsion in triplicates and the average values ± SD were calculated.

2.2.3. Preliminary screening of the emulsification efficiency of different co-surfactants

The emulsification efficiency of the co-surfactants (Transcutol® HP, Lauroglycol™ FCC and Labrafil® M 2125 CS) was assessed by the same method described for the investigated surfactants with slight modifications. The tested oils were the 1:1, 2:1 and 3:1 mixtures of IPP and Capryol 90®, where 300 mg of the oily phase were mixed with 100 mg of the co-surfactant in the presence of 200 mg of Cremophor RH40®, Tween® 80 or their 1:1 w/w mixture.

2.3. Optimization of the composition of the designed SNEDDSs

2.3.1. Construction of ternary phase diagrams:

Three ternary mixtures of the chosen oil, surfactants and co- surfactant were prepared. The prepared mixtures contained a 3:1 mixture of IPP and Capruol90® as the oil phase, Transcutol® HP as the co- surfactant and Cremophor RH40®, Tween® 80 or their 1:1 mixture as the surfactant. Each ternary mixture was prepared with varying proportions of its components and the ternary phase diagram was constructed for each mixture according to the criteria described by Zhang et al [10]. For each point on the phase diagram, one gram of the corresponding ternary mixture was diluted to 10 mL with distilled water, magnetically stirred at 37°C and immediately observed visually for emulsion formation, phase separation and presence of any precipitate.

Only clear or slight bluish dispersions were considered in the nanoemulsion area of the diagram [10]. The diluted nanoemulsions were left for 24 hours for stability assessment [11].

2.3.2. Determination of self-emulsification time:

The selected self- emulsifying system (oil phase: 3:1 w/w mixture of IPP and Capryol 90®, surfactant: 1:1 mixture of Cremophor RH40® and Tween® 80 and co-surfactant: Transcutol® HP) was prepared with different proportions of its components. The detailed composition of the prepared SNEDDSs is given in table (1).

For each of the prepared self- emulsifying systems, the time for self- emulsification was determined according to the method described by Obitte et al [12].One gram of the prepared system was diluted with 200 mL of phosphate buffer (pH = 6.8) and gently agitated using a magnetic stirrer at 37°C. Then it was visually inspected and the time required for the disappearance of the preconcentrate and formation of clear mixture of nanosized globules was recorded.

Table (1): Percent w/w composition of the selected SNEDDS

Formula	Oil [a]	Surfactant [b]	Co-surfactant [c]
I	10	30	60
II	10	40	50
III	10	50	40
IV	10	60	30
V	10	70	20
VI	10	80	10
VII	20	40	40
VIII	20	50	30
IX	20	60	20
X	20	70	10

[a] Oil is IPP:Capryol 90®, 3:1 mixture.

[b] Surfactant is Cremophor RH40®:Tween® 80,1:1 mixture.

[c] Co-surfactant is Transcutol® HP.

2.3.3. Effect of dilution on emulsion characteristics

For prepared self-emulsifying system (composition is given in table (1)), one gram was diluted with different diluents. The diluents used differed in their volume and pH value. The prepared emulsions were either 10 times or 100 times diluted with phosphate buffer of pH 6.8 or pH 7.4.

The diluted systems were mixed using a magnetic stirrer at 37°C and stored at ambient temperature for 12 hours, then visually checked for any signs of phase separation. In addition, mean globule size and polydispersity index (PDI) of the resultant SNEDDS were determined using Zetasizer Nano ZS (Malvern Instruments, UK).

2.4. Preparation of olanzapine- loaded SNEDDS:

Olanzapine (5, 10, 15 and 20 mg) was added to one gm of the optimized self- nanoemulsion formula (Formulas IV and VIII), heated to 50±0.5ºC for 5 min and sonicated till the drug was totally dissolved.

2.5. Characterization of olanzapine- loaded SNEDDSs:

One gram of the olanzapine-loaded SNEDDS (formulas IV and VIII) was diluted to 10 mL with phosphate buffer (pH 6.8) and the mixture was stirred using a magnetic stirrer at 37°C. The prepared mixtures were stored in tightly closed glass vials for 1 week at room temperature and checked for the presence of any precipitate.

In addition the globule size and the PDI values were determined for each diluted formula after one- week storage at room temperature

2.6. Transmission Electron Microscopy (TEM)

The morphology of olanzapine-loaded SNEDDSs of formulas IV and VIII was viewed using a Transmission Electron Microscope, (JEM-1230, Jeol, Tokyo, Japan). Samples were prepared by the negative staining technique. The tested formulas were dispersed into distilled water and then copper grid coated with collodion film was put into the above dispersion for several times. After being stained by 2% phosphotungistic acid (PTA) solution and dried at room temperature, the samples were ready for the TEM investigation at 70 kV.

3. RESULTS

3.1. Design of the SNEDDSs:

3.1.1. Study of the drug solubility in different oils, surfactants and co-surfactants

The mean values of olanzapine saturation solubility in the investigated single oils are presented in figure (1- a). As shown in figure (1- a), the highest saturation solubility of olanzapine in single oil was found to be in Capryol 90® followed by Peceol™, Maisine™ 35-1 and IPP, in order. The differences in drug solubility in the investigated oils were found to be statistically significant where the calculated p- values were less than 0.05.

The values of olanzapine saturation solubility in the prepared mixtures of IPP and Capryol 90® are also presented in figure (1- a) and they were found to be intermediate between the values of the drug solubility in both individual fatty acids. However, no significant differences were

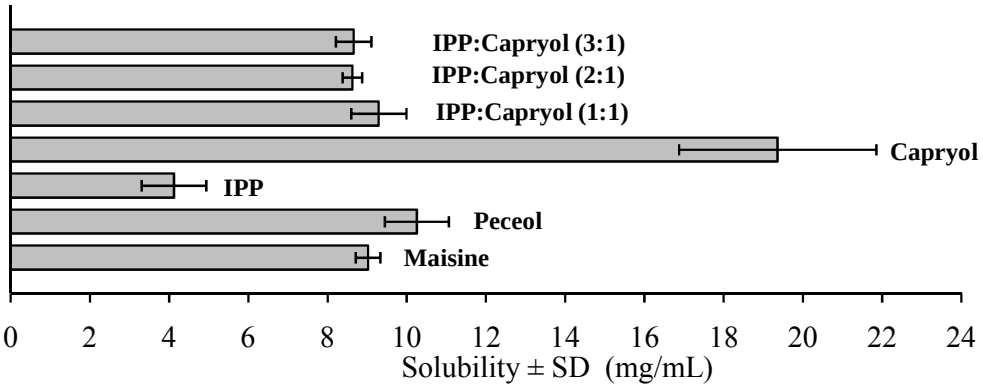
detected between the results of olanzapine solubility in the investigated oil mixtures at ratios 1:1, 2:1 and 3:1 w/w IPP: Capryol 90®, where all p- values exceeded 0.05.

Regarding the drug saturation solubility in the investigated surfactants, it is obvious from figure (1- b) that Labrasol® solubilized the drug more efficiently than Acconon MC8-2, Cremophor RH40® and Tween® 80. With respect to co-surfactants, olanzapine showed its highest solubility in Transcutol® HP followed by Lauroglycol™ FCC and finally, Labrafil® M 2125 CS (p- values <0.05). These results are graphically illustrated in figure (1-c).

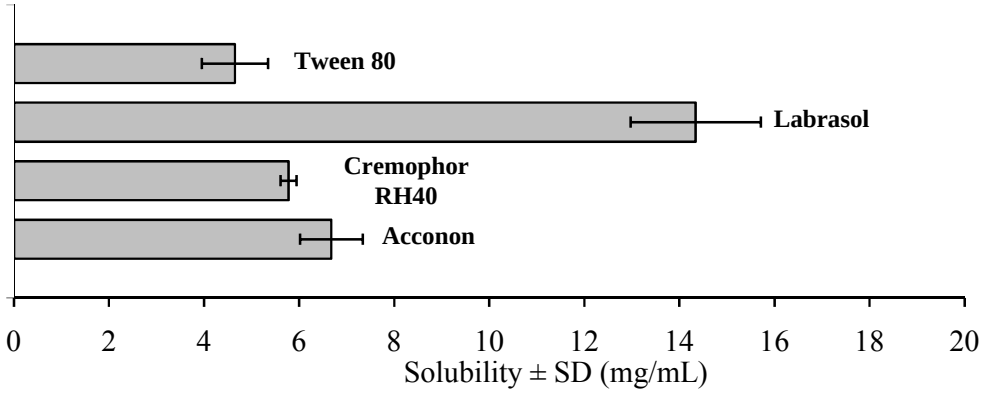
3.1.2. Preliminary screening of the emulsification efficiency of different surfactants

The results of the emulsification efficiency tests are given in table (2). As shown in table (2), for all the tested oils, the largest number of flask inversions and the least percent UV transmition were reported for Acconon MC8-2. On the other hand, relatively small numbers of flask inversions were needed for emulsion formation using Cremophor RH40® or Tween® 80 as emulsifying agents. Moreover, the percent UV transmition of the emulsions prepared using the afore mentioned emulsifiers with IPP or Capryol 90® as oils approached 100%(being 97.7 % and 100.5%, respectively for Cremophor RH40®,101.0 %, 100.1%,respectively for Tween® 80)

(a)



(b)



(c)

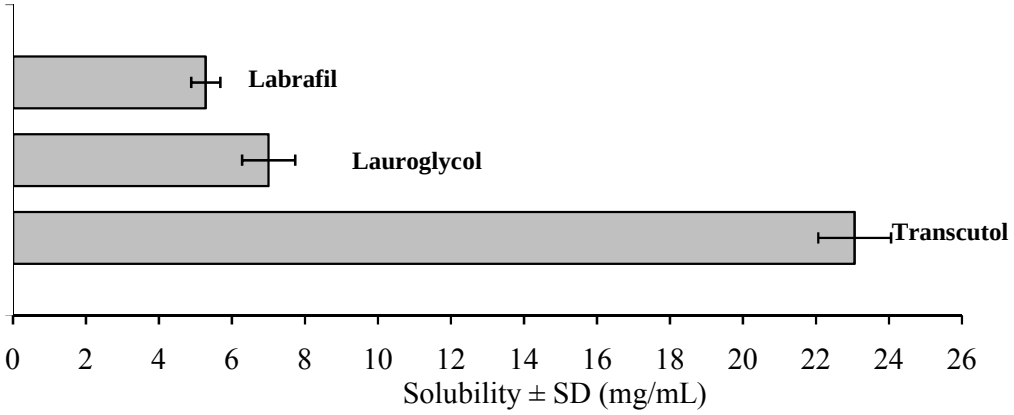


Fig.(1): Saturation solubility of olanzapine in different oils (a), surfactants (b) and co-surfactants (c) at 30ºC.

Table (2): Percent UV transmition for emulsions prepared by the investigated surfactants with the tested oils

Surfactant	Oil			
	Maisine™ 35-1	Peceol™	IPP	Capryol 90®
Acconon MC8-2	21.6±2.68 (>50)	14.7±1.55 (>50)	74.0±3.74 (11*)	49.0±3.53 (>50)
Cremophor RH40®	95.5±3.81 (1*)	96.6±3.67 (1*)	97.7±1.48 (1*)	100.5±0.42 (1*)
Labrasol®	83.3±2.40 (8*)	44.2±2.54 (>50)	88.2±3.61 (5*)	50.1±4.59 (>50)
Tween® 80	77.9±1.76 (11*)	60.0±3.74 (>50)	101.0±1.48 (1*)	100.1±0.92 (1*)

Results are mean values ± SD

In parenthesis, the number of flask inversions required for emulsion formation (*medians).

3.1.3. Preliminary screening of the emulsification efficiency of different co-surfactants

The investigated co-surfactants were tested with the selected oil mixtures of IPP:Capryol 90® (1:1, 2:1 and 3:1 w/w) using Cremophor RH40®, Tween® 80 and their mixture (1:1 w/w) as surfactants. The number of flask inversions required for emulsion formation as well as the

percent UV transmittance results of the prepared emulsions was estimated. Statistical analysis of data revealed that all the investigated co-surfactants possessed the same emulsifying power under the stated experimental conditions (data not shown).

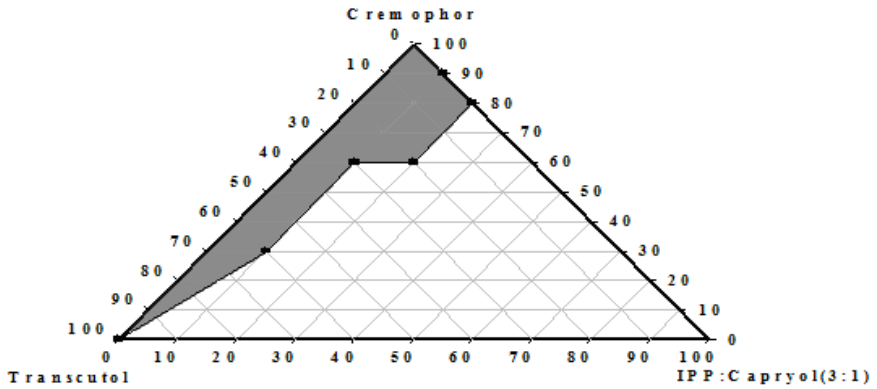
3.2. Optimization of the composition of the designed SNEDDSs

3.2.1. Construction of ternary phase diagrams:

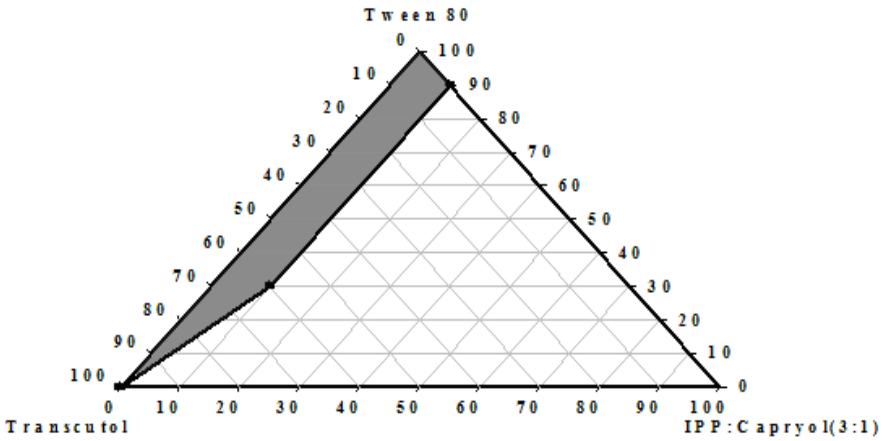
The ternary phase diagrams constructed for the prepared emulsion systems are illustrated by figure (2). Results represented in figure (2) show that among the investigated emulsion systems, those prepared using Cremophor RH40® as the surfactant produced ternary phase diagrams with wider nanoemulsion regions in comparison to those prepared using Tween® 80 surfactant. Moreover, emulsion systems containing 1:1 mixture of both surfactants produced the widest nanoemulsion region.

All the systems which gave clear nanoemulsions after dilution showed no phase separation after a period of 24 hours standing on shelf.

(a)



(b)



(c)

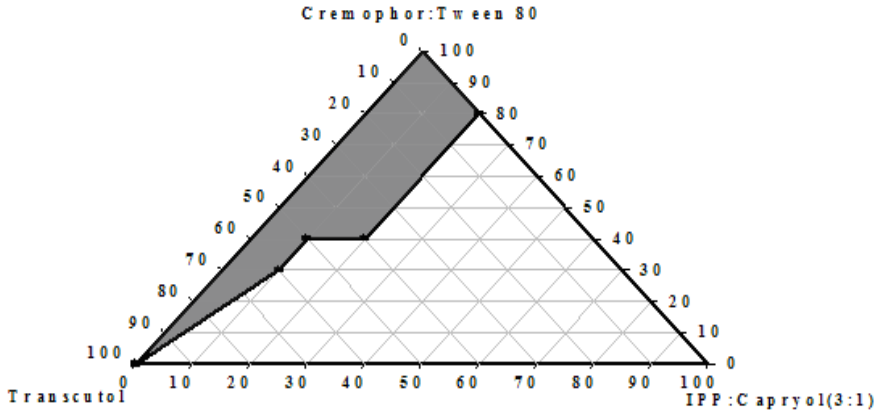


Fig.(2): Ternary phase diagram of self- emulsifying systems containing 3:1 IPP:Capryol 90® oil phase, different surfactants and Transcutol® HP co-surfactant.

The shaded areas represent the nanoemulsion regions

3.2.2. Determination of self-emulsification time:

The recorded self- emulsification times for the ten tested formulas are represented in table (3). From the results presented in table (3), it was evident that all the tested formulas were self-emulsified within 11.01±0.04 to 26.02±0.40 sec.

Table (3): Self-emulsification time of the selected SNEDDS

Formula	Self-emulsification time ±S.D. (sec)
I	13.10 ±0.10
II	12.21 ±0.13
III	11.01 ±0.04
IV	11.32 ±0.09
V	16.13 ±0.15
VI	15.33 ±0.14
VII	17.32 ±0.06
VIII	12.31 ±0.28
IX	19.30 ±0.26
X	26.02 ±0.40

Data are mean values (n=3)±S.D.

Composition of SNEDDS formulations is given in table (1).

3.2.3. Effect of dilution on emulsion characteristics

Visual inspection declared that all the diluted self- emulsifying systems (formulas I – X) were translucent and showed no phase separation on storage for 12 hours.

Table (4) shows the mean globule size of the prepared systems 12 hours after being diluted to either 10 times or 100 times their volume with phosphate buffer of pH 6.8 or pH 7.4. As shown in table (4), the mean globule size of all the investigated formulas did not exceed 20 nm after dilution under the aforementioned conditions.

Table (4): Effect of dilution on mean globule size of the selected SNEDDS

Formula	10-times dilution with		100-times dilution with	
	Phosphate buffer (pH=6.8)	Phosphate buffer (pH=7.4)	Phosphate buffer (pH=6.8)	Phosphate buffer (pH=7.4)
I	19.86±0.93	19.42±0.70	19.76±1.05	19.26±1.61
II	18.05±1.11	15.23±0.98	16.54±0.71	15.36±1.14
III	18.33±1.13	16.53±0.91	16.22±1.55	16.14±1.49
IV	19.95±1.11	19.81±0.84	19.85±2.04	19.53±1.20
V	17.93±0.88	16.25±0.77	17.37±0.99	15.73±1.76
VI	16.52±1.56	13.54±1.06	15.83±1.23	13.22±1.10
VII	19.14±1.63	19.45±0.78	19.25±1.49	19.46±0.63
VIII	18.02±1.41	18.37±1.34	19.33±1.00	17.84±1.64
IX	17.94±1.27	16.35±1.62	16.26±0.98	16.23±0.91
X	17.16±1.35	15.14±1.48	15.43±1.40	14.82±1.06

Data are mean values (n=3) ±S.D.

Composition of the prepared formulas is shown in table (1).

Table (5) includes the PDI values determined for the prepared systems after dilution. From this table it is obvious that the tested formulas showed PDI values ranging from 0.109±0.010 to 0.622±0.032. Among the investigated formulas, only formulas IV and VIII showed PDI values less than 0.3. The differences in the PDI values determined for formulas IV and VII and those determined for other investigated formulas were statistically significant as the p- values were less than 0.01.

3.3. Characterization of olanzapine- loaded SNEDDSs

None of the tested systems (formulas IV and VII) showed any evidence of phase separation or drug precipitation after a storage period of 1 week at ambient temperature.

Moreover, from the results presented in table (6), it was obvious that all the prepared olanzapine- loaded systems had globule size smaller than 20 nm and most of them showed PDI values less than 0.3. Only formula IV loaded with 15mg drug and formula VIII loaded with 5mg drug showed PDI values exceeding 0.3.

Table (5): Effect of dilution on polydispersity index (PDI) of the selected SNEDDS globules

Formula	10-times dilution with phosphate buffer		100-times dilution with Phosphate buffer	
	pH=6.8	pH=7.4	pH=6.8	pH=7.4
I	0.524±0.032	0.493±0.010	0.486±0.020	0.311±0.034
II	0.620±0.028	0.622±0.032	0.591±0.027	0.551±0.027
III	0.436±0.022	0.520±0.033	0.418±0.010	0.452±0.025
IV	0.254±0.013	0.228±0.011	0.127±0.011	0.109±0.010
V	0.420±0.021	0.447±0.025	0.337±0.038	0.427±0.020
VI	0.462±0.017	0.432±0.030	0.411±0.030	0.428±0.039
VII	0.415±0.022	0.438±0.025	0.384±0.022	0.398±0.017
VIII	0.211±0.010	0.287±0.024	0.216±0.007	0.215±0.027
IX	0.416±0.026	0.439±0.043	0.400±0.028	0.417±0.015
X	0.442±0.030	0.570±0.029	0.414±0.021	0.533±0.044

Data are mean values (n=3) ±S.D. Composition of the prepared formulas is shown in table (1).

Table (6): Mean globule size (G size) and polydispersity index (PDI) values of olanzapine-loaded self- nanoemulsifying formulas IV and VIII

Olanzapine (mg/ gm system)	Formula IV		Formula VIII	
	G size (nm)	PDI	G size (nm)	PDI
5	15.52±1.30	0.236±0.033	18.32±1.13	0.594±0.014
10	14.21±1.27	0.161±0.011	19.93±1.41	0.225±0.018
15	16.93±0.99	0.333±0.013	18.74±1.35	0.213±0.011
20	17.42±1.31	0.180±0.010	15.75±1.30	0.154±0.012

Composition of the formulas is shown in table (1). Data are mean values (n=3) ±S.D.

3.4. Transmission Electron Microscope (TEM)

The TEM pictures representing self- nanoemulsifying formulas IV and VII loaded with 20 mg olanzapine are shown in figure (3). From the presented figure, it was apparent that globules of both formulas were well dispersed and no globule aggregation took place.

(a) **(b)**

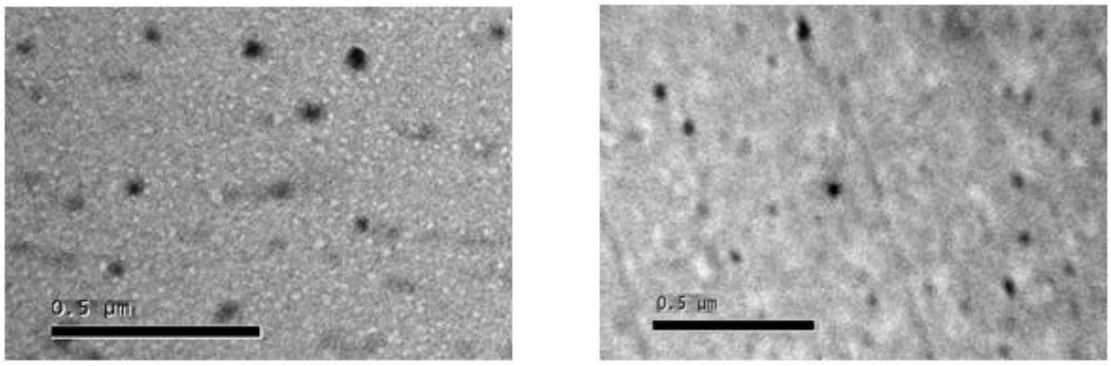


Fig.(3): Transmission Electron Microscope photographs of olanzapine- loaded self-nanoemulsifying formulas, IV (a) and VIII (b)

Composition of SNEDDS formulations is given in table (1).

4. DISCUSSION

To design a self-nanoemulsion with acceptable physicochemical characteristics the components of the system, including oil phase, surfactant and co- surfactant must be carefully chosen. The four investigated oils were fatty acids commonly utilized in SNEDDS formulation that differ in nature and chain length [13]. Amphiphilic, long chain fatty acids were investigated (Maisine™ 35-1 and Peceol™) [14] as well as a medium chain one (Capryol 90®) and a lipophilic long chain one (IPP) [15]. All the investigated surfactants in this study were non- ionic hydrophilic ones. Being non- ionic, the investigated surfactants are considered safe and biocompatible [16& 17] and being hydrophilic (with HLB values > 10), they are superior in forming fine, uniform emulsion droplets which can empty rapidly from the stomach [18] and provide large surface area that facilitates rapid drug release and absorption [19]. In addition, the chosen surfactants were reported for their bioactive properties that increase the intracellular concentration of the co-applied drug resulting in absorption enhancement [13& 20-27]. Investigated co- surfactants were the commonly-used ones in the preparation of SENDDSs, namely (Transcutol® HP, Lauroglycol™ FCC and Labrafil® M 2125 CS). The selection of excipients in which the drug has high solubility is a precondition to SNEDDS formulation in order to ensure high drug- loading ability of the developed systems. Therefore, the first step in designing the SNEDDS was to study olanzapine solubility in different oils, surfactants and co- surfactants.

The choice of the oil phase depended mainly on the drug solubility in the investigated oils to ensure the ability of the prepared emulsion to be efficiently loaded with the drug. As revealed by the results of drug solubility in single oils, the highest drug solubility was noticed in Capryol 90®. This may be attributed to the medium chain length (eight carbons) and the amphiphilic nature of Capryol 90® which provide it with surfactant properties and therefore, enhance drug solubilization, as explained by Balakrishnan et al [28]. Besides its high drug solubilization power, Capryol 90® -being a saturated medium chain fatty acid with HLB value = 6- is known for its efficient self- emulsification properties which aids the formation of the self- emulsifying system containing the drug [4]. Despite these potential advantages of Capryol 90® as an oil phase in the designed self- emulsifying system, it could not be used alone for the preparation of such delivery system because the chain length of such fatty acid does not allow for complete targeting to the lymphatic tissue. Holm et al [29] and Caliph et al [30] studied the distribution of different fatty acids in the body and they found a significant correlation between the chain length of the fatty acid and its mechanism of intestinal uptake. They stated that medium chain fatty acids are directly absorbed to the blood circulation through intestinal capillaries. On the other hand, long chain fatty acids, being too large to be taken by the tiny intestinal capillaries, are absorbed into the fatty walls of the intestinal villi where they enter the blood stream via the lymphatic system. This absorption pathway avoids the hepatic first-pass metabolism of drugs dissolved in such long chain lipids resulting in increased bioavailability.

For this reason mixtures of Capryol 90® (a medium- chain fatty acid with the highest drug solubility) and IPP (long chain fatty acid) were prepared at different ratios and the drug solubility in such mixtures was studied. These oil mixtures were expected to combine the advantages of efficient drug solubilization and lymphatic targeting.

Statistically non- significant differences were found in the results of drug solubility in the prepared oil mixtures (1:1, 2:1 and 3:1 w/w IPP: Capryol 90®). This indicates that the ratio between IPP and Capryol 90® in the oil mixture had no significant effect on drug solubility. Consequently, the oil mixture containing the highest proportion of the long chain fatty acid (IPP) was preferred to be used in the preparation of the self-emulsifying system as it ensures lymphatic uptake of the incorporated drug.

Although being a major parameter in choosing the ingredients of the SNEDDS, drug solubility is not the only parameter governing the choice of the surfactant in the prepared systems. The emulsifying efficiency of the surfactant is rather a much more important factor [31] and therefore, the emulsifying efficiency of different surfactants was screened regarding the tested oils. The

ability of the surfactant to form an emulsion was assessed by the number of flask inversions needed for emulsion formation, while the stability of the formed emulsion was expressed by its percent UV transmition, two hours after preparation. The results of the emulsification efficiency tests showed that the emulsification efficiency of an investigated surfactant differed according to the tested oil. For all the tested oils, the largest number of flask inversions was reported for Acconon MC8-2, indicating the most difficulty in emulsion formation. In addition, emulsions formed by Acconon MC8-2 had the least stability as indicated by the least percent UV transmission reported for them. On the other hand, relatively few numbers of flask inversions were needed for emulsion formation using Cremophor RH40® or Tween® 80 as emulsifying agents, moreover, the percent UV transmission of the formed emulsions (two hours after preparation) approached 100% indicating an accepted stability of the formed emulsions. These observed differences in the emulsification efficiency of the investigated surfactants were attributed to the difference in their chain length and structure as explained by Lawrence in his study on microemulsions as drug delivery vehicles [32].

Because they easily formed stable emulsions with the investigated oils, especially the components of the selected oil mixture (IPP and Capryol 90®), Cremophor RH40® and Tween® 80 were considered as excellent emulsifiers for the designed oil phase and they were chosen among the investigated surfactants to be used in the preparation of the self- emulsifying systems. Olanzapine's solubility in both Cremophor RH40® and Tween® 80 was significantly lower than that in both Acconon MC8-2 and Labrasol® (p- values < 0.05), nevertheless, the higher emulsification efficiency shown by the former surfactants motivated their selection in the prepared systems. The selection of the best co-surfactant among the screened ones (Transcutol® HP, Lauroglycol™ FCC and Labrafil® M 2125 CS) should depend on the emulsification power regarding the chosen oil mixture of 3:1 (w/w) IPP and Capryol 90® in the presence of the selected surfactants (Cremophor RH40®, Tween® 80 and their 1:1 w/w mixture). However, the statistically non- significantly different emulsification powers shown by the investigated co-surfactants resulted in the predominance of drug solubilization power as the key parameter in selection of the most suitable co-surfactant for the designed self- emulsifying system. Consequently, Transcutol® HP was selected as co-surfactant in the prepared self- emulsifying systems as it possessed the highest solubilizing power for olanzapine compared to the other investigated co-surfactants. Accordingly, the designed self- emulsifying systems contained IPP/ Capryol90® mixture (at a w/w ratio of 3:1) as the oil phase, Cremophor RH40®, Tween® 80 or their 1:1 w/w mixture as the surfactant and Transcutol® HP as the co- surfactant.

For these three self- emulsifying systems, ternary phase diagrams were constructed in order to optimize the composition of the prepared system by identifying the most appropriate ingredients for the preparation of a stable one and their optimum proportions. As concluded by Shen and Zhong [25] in their study on self- emulsifying drug delivery systems containing atorvastatin, Cremophor RH40® was a better emulsifier than Tween® 80. This was evident in the present study as the nanoemulsion region in the ternary phase diagram constructed for emulsions prepared using the former surfactant was wider. The widest nanoemulsion region was identified in the ternary phase diagram of emulsion systems prepared using 1:1 w/w mixture of Cromophor and Tween® 80. This indicates that the use of such surfactant mixture enhanced the emulsification power compared to that produced by each single surfactant. The better emulsification provided by a mixture of surfactants, compared to pure ones was previously described and explained by Huibers and Shah[33]. Moreover, none of the systems which gave clear nanoemulsions after dilution showed phase separation, 24 hours after its preparation indicating acceptable stability of the prepared systems.

The study of the constructed ternary phase diagrams led to the conclusion that the most appropriate surfactant for the preparation of stable self- emulsifying was the mixture of Cremophor RH40® and Tween® 80 at an equal w/w ratio. To determine the optimum proportion of each ingredient in the designed self- emulsifying, ten emulsions were prepared having the same components at different proportions. All the prepared systems contained IPP/ Capryol 90® (3:1 w/w mixture) as the oil phase, Cremophor RH 40®/ Tween® 80 (1:1 w/w mixture) as the surfactant and Transcutol® HP as the co- surfactant. The preferences of the prepared systems were judged according to their self- emulsification time and characters upon dilution.

The self-emulsification time was previously used by Lia et al [34] to evaluate the ability of the prepared systems to be easily and rapidly emulsified. The short self- emulsification time reported for all the investigated systems indicate their ability for easy and rapid emulsification. This ability is very important for efficient SNEDDS as emulsification process is considered the rate limiting process for drug absorption. Rapid emulsification influences globule formation and accessibility of interface for the loaded drug to partition. The rapid self-emulsification of the investigated systems can be attributed to their low oil content (only10% and 20%, w/w).

The effect of dilution on the characteristics of the prepared systems is a very important parameter for evaluation of the quality of the investigated systems. When applied orally the prepared systems are expected not only to be diluted in the gastrointestinal tract but also to be exposed to different pH values along it. These in- vivo conditions may lead to phase separation

of the prepared emulsion systems resulting in their failure as a drug delivery system [35]. They may also affect the globule size of the emulsion and its globular size distribution. For this reason, the prepared systems were diluted with different volumes of diluent with pH values of the small intestine (pH 6.8) and the blood (pH 7.4) to simulate their in vivo dilution before and after absorption, respectively. The diluted systems were visually inspected and evaluated for their mean globule size and the polydispersibility index of the globules.

Visual inspection of the diluted systems showed no signs of phase separation even after 12 hours of dilution. This gave a good indication about the suitability of such systems for oral application where they have a great chance to pass along the gastrointestinal tract as emulsified oil globules without phase separation. When happens early in the gastrointestinal tract, phase separation prevents globule formation, as a result the drug is no more encapsulated in the oil globules and consequently, its absorption is negatively affected [36].

Determination of globule size is important in the formulation of SNEDDSs. Systems with mean globule size below 200 nm fulfill the criteria of SNEDDSs [10] by enhancing the drug release and bioavailability. All the investigated systems had mean globule size less than 20 nm indicating their efficiency as SNEDDSs. The small globule size of the diluted systems can be attributed to the use of the proper surfactant/co-surfactant mixture. This provided adequate reduction in the free energy of the system which in turn resisted the thermodynamic instabilities on changing the environment pH and volume. Also, the surfactant/co-surfactant mixture provided a strong mechanical barrier to protect the formed globules from being aggregated as explained by Nepal et al [37] and Singh et al[38].

The mean globule size is not the only parameter to be considered in the formulation of SNEDDSs. The globule size distribution is another parameter of equal if not much importance. The globule size distribution is expressed by a dimensionless number called the polydisperity index (PDI) [39 & 40]. High value of PDI (> 0.3) indicates a wide globule size distribution. This was the case with all the investigated systems except formulas IV and VIII. The small values of PDI shown by these two formulas indicate homogenous globule population and narrow globule size distribution. This in turn indicates more uniform emulsions with higher physical stability [41]. Although formula VIII contained less proportion of the surfactant mixture (50% w/w) than that contained in formula IV (60% w/w) and both formulas contained the same proportion of the co-surfactant and the drug, the former formula showed an acceptable PDI value. The higher proportion of oil phase in formula VIII compared to formula IV (20% and 10% w/w, respectively) indicates higher Capryol90® content of the former formula. Capryol90® was

reported to have some surfactant properties [14 & 28] which may explain the enhancement of globule polydispersity of formula VIII. For this reason, formulas IV and VIII were chosen as optimum self- nanoemulsifying systems to be loaded with the drug. The chosen formulas were loaded with different amounts of olanzapine (5, 10, 15 and 20 mg) in order to specify the highest possible drug loading that maintains system stability. The loaded systems were further inspected for drug precipitation to ensure that the loaded drug is borne inside the oil/surfactant globules after the emulsification process. No drug precipitation was noticed with any of the prepared olanzapine- loaded formulas indicating that the prepared systems can keep up to 20 mg of the incorporated drug in solution.

Furthermore, changing the amount of loaded drug didn't negatively affect the mean globule size of the formed nano-emulsions after being diluted with phosphate buffer (6.8) , where the mean globule size values remained below 20 nm. In addition, despite of formula IV loaded with 15 mg drug and formula VIII loaded with 5 mg drug, all the prepared formulas had PDI values less than 0.3 indicating well dispersed globules on dilution. Accordingly formulas IV and VIII loaded with 20 mg olanzapine were chosen as optimum olanzapine- loaded self-nanoemulsifying systems as they had satisfactory globule size and PDI value although loaded with the highest investigated drug proportion (20 mg/ gm. of the system). The transmission electron microscope images of these formulas showed perfect emulsification.

5. CONCLUSION

The results of this study led to the conclusion that olanzapine- loaded SNEDDS with satisfactory physical stability can be prepared using the proper oil phase, surfactant and co-surfactant at adequate proportions. The oil phase of choice was the 3:1 mixture of IPP and Capryol 90®, in addition to 1:1 Cremophor RH40® : Tween® 80 surfactant mixture and Transcutol® HP co-surfactant. The self- emulsifying formula containing 10%, 60% and 30% of the aforementioned components, respectively as well as that containing 20%, 50% and 30%, in respective way was loaded with 20 mg olanzapine per gram formula. The drug- loaded systems were found to fulfill the criteria of adequate SNEDDS. They had rapid self-emulsification time, adequate mean globule size (< 20 nm), good dispersion characteristics (PDI values < 0.3) as well as marked stability on dilution.

Accordingly, the prepared olanzapine- loaded SNEDDSs are promising carriers for the oral delivery of the drug aiming to solve its major oral delivery problem which is first-pass metabolism. For this reason, formulating the prepared olanzapine-loaded SNEDDSs in oral solid dosage forms is currently studied.

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