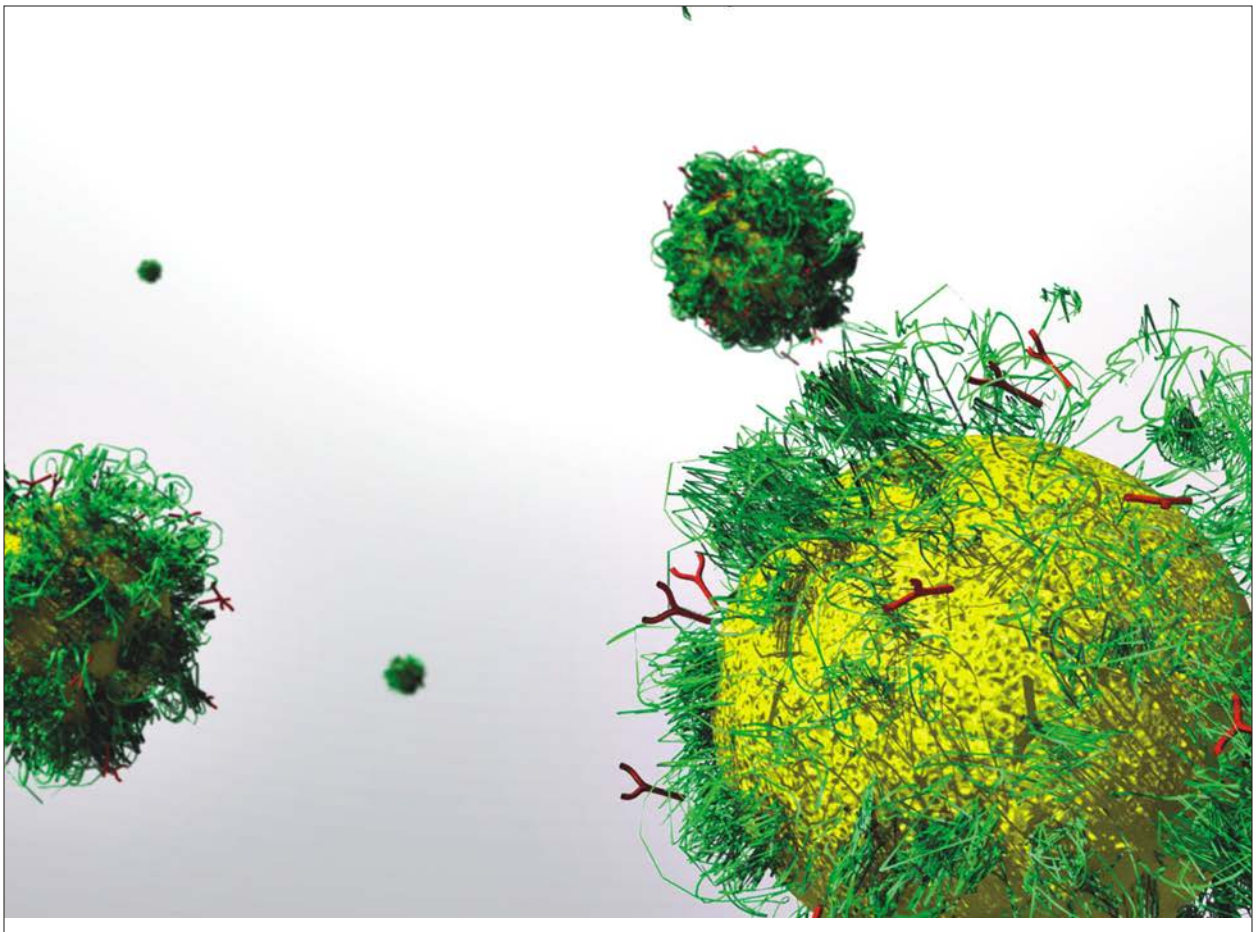

American Journal of Drug Discovery and Development

ISSN 2150-427X

Academic Journals Inc.

www.academicjournals.com

American Journal of Drug Discovery and Development 2 (1): 1-16, 2012
ISSN 2150-427x / DOI: 10.3923/ajdd.2012.1.16
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In vivo Evaluation and Application of Central Composite Design in the Optimization of Amisulpride Self-Emulsifying Drug Delivery System

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ABSTRACT

Amisulpride is practically insoluble in water and suffers from irregular and low bioavailability (48%). This could be due to low solubility and being a substrate for P-glycoprotein efflux. The aim of this study is to develop and statistically optimize Self-Emulsifying Drug Delivery System (SEDDS) formulation containing bio-enhancers and P-glycoprotein inhibitors components, for the improvement of dissolution and oral absorption of amisulpride; using Central Composite Rotatable Design (CCRD). Preliminary screening was carried out to determine amisulpride solubility in various oils and surfactants. Formulations were prepared using oil (Capryol-90®), two surfactants (Cremophor EL® and Labrasol®, "S_{mix}") and co-surfactant (Transcutol HP®). CCRD was applied for optimization. Oil percentage, S_{mix}: co-surfactant ratio and Cremophor EL: Labrasol ratio in S_{mix}, were selected as independent variables while mean droplet size, drug loading and light absorbance of diluted SEDDS as dependent variables. Second-order polynomial equations were fitted to data. Optimized formulation, containing 10% oil, 1.31 as S_{mix}: co-surfactant ratio and 2 as ratio of Cremophor ®EL: Labrasol® in S_{mix} was prepared according to software determined levels using desirability function and overlay plot. It provided drug loading of 50 mg mL^{-1} and released amisulpride completely within 15 min irrespective of type or pH of dissolution medium. Optimized SEDDS showed significant ($p<0.01$) increase *in vivo* bioavailability compared to drug suspension. CCRD could be considered as an efficient approach for the optimization of SEDDS. Also, the optimized SEDDS formulation demonstrated a great potential as a possible alternative to traditional oral formulations of amisulpride.

Key words: Amisulpride, self-emulsifying, central composite, rabbits, P-glycoprotein

INTRODUCTION

Amisulpride, is a benzamide derivative selectively block cerebral dopamine D_2 and D_3 receptors (Lecrubier *et al*., 1997). When administered at an oral daily dose of 50 mg, it improves the dopaminergic neurotransmission with a D_2 dopaminergic receptors presynaptic inhibition and it is used in the treatment of dysthymia (Zanardi and Smeraldi, 2005). It suffers from low bioavailability (48%) with high inter-individual variability. It is metabolized in liver only to a minor degree (Nirogi *et al*., 2008). 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 (Amidon *et al*., 1995).

This low and irregular bioavailability could be attributed to several factors. Amisulpride is practically insoluble in water and is a weakly basic drug (pKa = 9.37). It shows pH dependent solubility because it has one ionizable amino group which can be charged at acidic pH values, making the molecule more soluble. However, the solubility is low at neutral pH values (Musenga *et al.*, 2008). 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 (Ghai and Sinha, 2011; Badawi *et al.*, 2011; Tapas *et al.*, 2011).

In addition, amisulpride was recently recognized as a substrate of P-glycoprotein (Ela *et al.*, 2004; Hartter *et al.*, 2003; Linnet and Ejsing, 2007). Intestinal P-glycoprotein efflux system in the intestine is a major physiological obstacle for the bioavailability of many orally administered drugs. P-glycoprotein is a plasma membrane glycoprotein. Due to its action in the apical membranes of epithelial cells in the intestine, drugs can be pumped back into the lumen of the intestine after absorption, leading to reduced bioavailability of many orally administered drugs (Zhu *et al.*, 2009). It was also reported that the co-administration of Cyclosporine A, a P-glycoprotein efflux inhibitor, with amisulpride, resulted in a change in the pharmacodynamics and pharmacokinetics of amisulpride and its levels in blood and within the CNS were increased (Schmitt *et al.*, 2006). However, this couldn't consider being a practical solution to solve the problem of low bioavailability because of the possible side effects.

On the other hand, Self-Emulsifying Drug Delivery System (SEDDS) is a class of emulsions that have been used as a mean of enhancing oral bioavailability of poorly absorbed drug (Obitte *et al.*, 2008). SEDDS is an isotropic mixture of oils, surfactants, co-surfactants and drug that form fine oil-in-water (o/w) emulsion when introduced into aqueous phases under gentle agitation. SEDDS disperses to form fine emulsion when it is released into the lumen of the gut. Thus, the drug remains in solution, avoiding the dissolution step which frequently limits the rate of absorption of poorly soluble drugs (Gursoy and Benita, 2004).

Furthermore, certain types of surfactants and co-surfactants such as Cremophor®, Labrasol® and Transcutol® that are used extensively in SEDDS are considered to be bioenhancers. They are reported to improve the bioavailability of drugs by facilitating transcellular and paracellular absorption. Also, they act as p-glycoprotein inhibitors decreasing intestinal efflux (Chen, 2007; Yin *et al.*, 2009). Accordingly, it could be suggested that SEDDS is an attractive choice for amisulpride oral delivery.

Central composite design is a type of response surface methodology which is an effective statistical technique for optimizing multifactor experiments in formulation processes (Agarry *et al.*, 2010). It is more reliable, more accurate as well as less laborious and time consuming than the "change one factor at a time" conventional optimization method (Babu *et al.*, 2008). It can be used to derive equations relating the response and the independent variables while taking the direct, pair wise interaction and curvilinear effects of the variables into consideration (Xiong *et al.*, 2009; Ye *et al.*, 2006).

The current study is aimed at developing and optimizing a self-emulsifying formulation of amisulpride in order to improve oral absorption of amisulpride. To achieve this goal efficiently, with smaller number of trials, computer-aided optimization technique using central composite design was employed to statistically optimize the levels of the selected components of SEDDS using mathematical equations and response surface plots. Finally, *in vivo* oral bioavailability of the

optimized SEDDS was evaluated in rabbits and was compared to that of aqueous suspension of amisulpride.

MATERIALS AND METHODS

Materials: Amisulpride was supplied by Al Andalous for pharmaceutical industries (Egypt), Glycerol monolinoleate (Maisine 35-1®), propyleneglycol monocaprylate (Capryol 90®), medium chain triglycerides (Labrafac lipophile® WL 1349), PEG-8caprylic/capric glycerides (Labrasol®), oleoyl macrogol 6-glycerides (Labrafil® M1944CS) and diethylene glycol monoethyl ether (Transcutol HP®), glyceryl oleate (Peceol®), propylene glycol monolaurate (Lauroglycol 90) and lauroyl macrogol glycerides (Gelucire 44/14®) were kindly donated by Gattefosse Co. (Lyon, France). Polyoxy 40 hydrogenated castor oil (Cremophor RH 40®), polyoxy 35 castor oil (Cremophor EL®) and macrogol-15-hydroxystearate (Solutol® HS 15) were supplied by BASF Co. (Germany). Triglycerides of caprylic/capric acid (Captex 355) were donated by Abitec Corp. (Janesville, WI). caprylic/capric triglyceride (Neobee® M-5) was obtained from Stepan (Northfield, Illinois, USA). Polyethyleneglycol 400 (PEG 400), propylene glycol (PG), Tween 20 and Tween 80 were obtained from Al-Nasr Pharmaceutical Co. (Egypt). All other chemicals used were of analytical grade.

Methods

Solubility studies: Solubility of amisulpride in different surfactants, co-surfactants and oils was determined as follows; known excess of amisulpride (200 mg) was added to 2 g of each vehicle. Mixtures were vortexed for 30 sec (GEMMY vortex mixer; VM-300, Germany) and shaken for 48 h at 25°C±0.5 in a thermostatically controlled water bath (Model 1083, GLF Corp., Germany), then centrifuged at 4000 rpm for 5 min. Concentration of the dissolved drug was quantified spectrophotometrically (UV-1601 PC, Shimadzu, Japan) via a validated method at 279.5 nm. Each experiment was carried out in triplicates.

Preparation of SEDDS of amisulpride: Homogenous blends of the selected ingredients; Capryol-90®, Cremophor EL®, Labrasol® and Transcutol HP® were prepared in the specified weight ratios. Amisulpride (200 mg) was added to each mixture followed by vortex mixing for 30 sec and shaking for 48 h at 25±0.5°C in a thermostatically controlled water bath. Mixtures were then centrifuged at 4000 rpm for 5 min and the supernatants was separated and kept at room temperature for further study.

Drug loading: Concentration of the dissolved amisulpride in each of the prepared SEDDS formulation was determined spectrophotometrically as mentioned under solubility studies section.

Droplet size measurement: Mean droplet size (nm) was determined after dilution (1:100) with buffers of pH 1.2, 4.5 and 6.8 using particle size analyzer (Malvern Zetasizer 3000, UK). The samples were measured 24 h after the dilution process.

Optical clarity: In order to test the optical clarity of aqueous dispersions of the prepared SEDDS formulations at different pH values, SEDDS were diluted 100 times with buffers of pH 1.2, 4.5 and 6.8 and left for 24 h at room temperature (25°C). The absorbance was measured at 400 nm using UV spectrophotometer (UV-1601 PC, Shimadzu, Japan) (Basalious *et al.*, 2010).

Experimental design: In order to reduce the number of trials needed in the optimization of amisulpride SEDDS formulation, five-level, three-factorial, circumscribed, Central Composite Rotatable Design (CCRD) (Design Expert®, Version 7, Stat-Ease Inc., Minneapolis, MN) was applied. In this design a 2^3 full factorial design was expanded to a CCRD by replicating 3 times a center point and adding 6 axial points (total run = 17) resulting in 5 levels for each factor. The factorial points contribute to estimating the linear terms and two factor interactions. The axial points contribute to estimating the quadratic terms. The repeated center points contribute to the estimation of the pure experimental uncertainty at the factor levels (Lo *et al.*, 2009; Narendra *et al.*, 2005; Nazzal and Khan, 2006; Zhang *et al.*, 2010). The following polynomial equation was fitted to the data:

$$Y = b_o+b_1X_1+b_2X_2+b_3X_3+b_{12}X_1X_2+b_{23}X_2X_3+b_{13}X_1X_3+b_{11}X_1^2+b_{22}X_2^2+b_{33}X_3^2 \quad (1)$$

where, Y is the measured response resulted from each factor level combination; b_o is the intercept; b_1 to b_{33} are the regression coefficients; and X_1, X_2 and X_3 are the tested factors (independent variables). The terms X_iX_i and X_i^2 (i = 1, 2 or 3) are the interaction and quadratic terms, respectively (Gannu *et al.*, 2010). A positive sign in front of the term in the polynomial equations indicates synergistic effect while negative sign denotes antagonistic effect of the factors (Yue *et al.*, 2010). The independent variables were; oil percentage (X_1), S_{mix}/co-surfactant ratio (X_2) and Cremophor®/Labrasol® ratio in S_{mix} (X_3). The levels of each factor were designated as -1.68, -1, 0, +1 and +1.68 (Dash and Gummadi, 2007). Seven responses were determined; droplet size at pH 1.2, 4.5 and 6.8, assigned Y_1, Y_2 and Y_3, respectively, drug loading (Y_4) and optical clarity at pH 1.2, 4.5 and 6.8 assigned Y_5, Y_6 and Y_7, respectively. The corresponding actual values for each variable, the constrains and the importance for each response are given in Table 1.

***In vitro* release studies:** One gram of the optimized formula containing 50 mg amisulpride and 50 mg amisulpride powder were filled into soft gelatin capsules. The study was performed using USP dissolution tester, apparatus II (VK 700, Vankel, USA) at 37±0.5°C, at a rotating speed of 50 rpm, in 900 mL of distilled water and buffers of pH 1.2, 4.5 and 6.8. At selected time intervals, aliquots each of 3 mL were withdrawn from the dissolution medium through a Millipore membrane

Table 1: Variables in the central composite experimental design

	Levels				
Factors	-1.68	-1	0	+1	+1.68
X_1: Oil percentage	6.59	10.00	15.00	20	23.41
X_2: S_{mix}/cosurfactant ratio	0.66	1.00	1.50	2	2.34
X_3: Chremophore®/Labrasol® ratio in S_{mix}	0.32	0.75	1.38	2	2.43
Response	Constraints				Importance
Y_1: Droplet size at pH 1.2 nm	Minimize				3
Y_2: Droplet size at pH 4.5 nm	Minimize				3
Y_3: Droplet size at pH 6.8 nm	Minimize				5
Y_4: Drug loading	Target = 50				5
Y_5: Optical clarity at pH 1.2	Minimize				3
Y_6: Optical clarity at pH 4.5	Minimize				3
Y_7: Optical clarity at pH 6.8	Minimize				5

filter (0.45 μm) and compensated with an equivalent volume of the fresh dissolution medium. Concentrations of amisulpride were determined spectrophotometrically at 279.5 nm using the regression equation of a standard curve developed in the same medium. The dissolution experiments were carried out in triplicate.

***In vivo* bioavailability study in rabbits:** Bioavailability of the optimized formulation of amisulpride SEDDS was compared with amisulpride aqueous suspension (50 mg mL^{-1}). The protocol of the study was approved by the Research Ethics Committee in Faculty of Pharmacy, Cairo University, Egypt. The study was conducted in accordance with EC Directive 86/609/EEC for animal experiments. Male rabbits (weighing approximately 3 ±0.3 kg) were fasted for 12 h prior to the experiment and water was available *ad libitum*. Six rabbits were allocated at random into two equal treatment groups. The animals were kept in individual cages and administered the optimized SEDDS formulation and amisulpride suspension in latin square crossover design. The washout period between the two treatments was 7 days. After oral administration of amisulpride dose (50 mg), about 2 mL of blood sample was collected through retro-orbital plexus into heparinized tubes at 0 (predose), 0.5, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 8 and 12 h. Blood samples were centrifuged at 5,000 rpm for 10 min using a high-speed centrifuging machine and plasma samples were withdrawn and stored at -20°C until analysis by HPLC. The quantitative determination of amisulpride in plasma was performed by a reverse-phase High-Performance Liquid Chromatography (HPLC) procedure as described by Malavasi *et al*. (1996).

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 (C_{max}, ng mL^{-1}) and the time to reach C_{max} (T_{max}, h) were directly obtained from individual plasma concentration time curve. The area under the curve AUC_{0-24} (ng.h mL^{-1}) was determined as the area under the plasma concentration-time curve up to the last measured sampling time and calculated by the trapezoidal rule. The area under the curve from zero to infinity $AUC_{0-\infty}$ (ng.h mL^{-1}), was calculated as:

$$AUC_{0-\infty} = AUC_{0-24} + \frac{C_t}{k}$$

where, C_t is the last measured concentration at the time t and k (h^{-1}) is the terminal elimination rate constant. The relative bioavailability F was calculated using the following equation:

$$F\ (\text{Relative bioavailability}) = \frac{(AUC)SEDDS}{(AUC)\ \text{suspension}} \times 100$$

The data obtained were analyzed by one-way analysis of variance (ANOVA) using Excel® software (Microsoft®, USA). Statistically significant differences were assumed when $p<0.01$. All values are expressed as their Mean±SD.

RESULTS AND DISCUSSION

Solubility studies: The requested criteria for the selection of components (oil, surfactants and co-surfactants) in SEDDS formulation are; drug solubility (Shafiq *et al*., 2007), emulsification efficiency (Date and Nagarsenker, 2007) and their capability to promote the intestinal transport

Table 2: Solubility of amisulpride in various vehicles (n = 3)

Vehicle	Solubility (mg g^{-1})±SD
Oils	
Maisine 35-1®	10.75±0.35
Caproyl 90®	24.53±0.60
Labrafac lipophile® WL 1349®	8.56±0.51
Neobee M-5®	16.48±0.60
Captex 355®	4.43±0.32
Lauroglycol 90®	10.33±0.25
Peceol®	4.35±0.35
Surfactant	
Labrasol®	30.28±0.32
Cremophor EL®	13.33±0.39
Cremophor RH40®	7.69±0.16
Solutol HS15®	11.5±0.420
Gelucire 44/14®	28.28±0.32
Tween 80®	15.65±0.35
Tween 20®	20.26±0.23
Cosurfactant	
Transcutol HP®	49.31±0.31
Labrafil M1944CS®	2.47±0.58
PEG 400	40.41±0.27
PG	21.74±0.23

of drug (O'Driscoll, 2002). The solubility of amisulpride in various oils, surfactants and co-surfactants is presented in Table 2.

The solubilizing efficiency of the oily phase for the drug is the key determining factor for oil selection (Elnaggar *et al.*, 2009). Amongst the various tested oils, Capryol 90® had the largest solubilizing capacity for amisulpride (24.53±0.60 mg g^{-1}), so it was chosen for further investigation.

Taking in consideration that the target dose of amisulpride (50 mg) is relatively high, the solubility of the drug in the surfactants will be an important factor in surfactant selection beside its emulsification efficiency. Cremophor EL® and Cremophor RH 40® are reported to be efficient surfactants especially with Capryol 90® (Date and Nagarsenker, 2007; Elnaggar *et al.*, 2009; Rao *et al.*, 2008).

The solubility of amisulpride in Cremophor EL® (13.33±0.39 mg g^{-1}) was higher than in Cremophor RH 40® (7.69±0.16 mg g^{-1}). Consequently, the later was excluded. In addition, amisulpride exhibited the highest solubility in Labrasol® among the tested surfactants (30.28±0.32 mg g^{-1}). Hence, a mixture of Cremophor EL® and Labrasol® was chosen as surfactant mixture "S_{mix}" for further investigation.

Regarding co-surfactant selection, the solubility of the drug will be the perspective criteria particularly due to the substantially high dose of amisulpride. Transcutol HP® showed the maximum solubility of amisulpride (49.31±0.31 mg g^{-1}) and so it was the co-surfactant of choice in the present study. It is worthy to note that, Cremophor®, Labrasol® and Transcutol® are known to have inhibitory effect on P-glycoprotein efflux (Shen and Zhong, 2006; Yin *et al.*, 2009), that is responsible for the low bioavailability of many drugs.

Central composite rotatable design (CCRD) and analysis: Seventeen experiments were carried out in one block, formed of eight factorial points, six axial points and three central points. Composition and the observed responses for the CCRD are given in Table 3.

Table 3: Compositions and observed responses for the central composite rotatable design

	Factors levels in actual values			Responses						
Run	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6	Y_7
Factorial points										
1	10.00	1.00	0.75	31.42	244.10	232.20	49.00	0.03	0.21	0.48
2	20.00	1.00	0.75	43.51	264.40	240.80	48.00	0.05	0.49	0.65
3	10.00	2.00	0.75	31.73	249.60	216.50	49.00	0.04	0.38	0.30
4	20.00	2.00	0.75	38.77	253.30	251.50	47.00	0.04	0.70	0.67
5	10.00	1.00	2.00	25.98	136.10	144.70	55.50	0.08	0.02	0.01
6	20.00	1.00	2.00	29.85	193.20	350.30	55.50	0.13	0.37	0.31
7	10.00	2.00	2.00	25.80	76.80	55.79	43.50	0.02	0.00	0.02
8	20.00	2.00	2.00	28.62	156.10	263.50	43.50	0.08	0.14	0.57
Axial points										
9	6.59	1.50	1.38	35.33	175.80	127.00	40.20	0.02	0.20	0.25
10	23.41	1.50	1.38	45.10	218.50	390.40	43.00	0.06	0.31	0.57
11	15.00	0.66	1.38	33.40	236.10	275.20	54.00	0.06	0.31	0.44
12	15.00	2.34	1.38	30.68	198.10	255.30	42.00	0.04	0.19	0.44
13	15.00	1.50	0.32	40.50	300.00	225.20	53.50	0.07	0.84	0.92
14	15.00	1.50	2.43	25.79	134.20	66.87	53.23	0.11	0.17	0.16
Centre points										
15	15.00	1.50	1.38	29.94	197.40	264.80	47.50	0.07	0.30	0.29
16	15.00	1.50	1.38	28.68	205.50	231.50	46.50	0.06	0.39	0.38
17	15.00	1.50	1.38	33.79	215.30	241.80	47.50	0.06	0.33	0.33

Table 4: The analysis of variance for responses Y_1, Y_2, Y_3, Y_4, Y_5, Y_6 and Y_7

	Model	X_1	X_2	X_3	X_1X_2	X_1X_3	X_2X_3	X_1^2	X_2^2	X_3^2	Lack of fit
Y_1 F-value	7.4358	16.8903	1.0262	33.9697	0.6010	2.4995	0.1473	9.5818	0.1544	0.0062	1.1288
P>F	0.0075	0.0045	0.3448	0.0006	0.4636	0.1579	0.7125	0.0174	0.7060	0.9396	0.5315
Y_2 F-value	28.3281	20.9843	10.7117	206.2696	0.0208	8.3930	5.4772	2.3510	0.0373	0.0373	2.8787
P>F	0.0001	0.0025	0.0136	<0.0001	0.8893	0.0231	0.0518	0.1691	0.8523	0.8523	0.2777
Y_3 F-value	14.0746	65.6981	3.7215	12.5294	0.1126	18.9301	4.0360	0.1914	0.4848	16.1144	3.9473
P>F	0.0011	<0.0001	0.0950	0.0095	0.7471	0.0034	0.0845	0.6749	0.5087	0.0051	0.2144
Y_4 F-value	22.6017	0.1204	84.1309	0.8517	0.0704	0.6332	37.2178	18.4204	1.9820	38.2644	7.0622
P>F	0.0002	0.7388	<0.0001	0.3868	0.7985	0.4523	0.0005	0.0036	0.2020	0.0005	0.1287
Y_5 F-value	13.9846	29.1130	15.8433	27.7105	0.0290	8.7868	14.3034	8.9458	4.7968	8.4136	4.9838
P>F	0.0011	0.0010	0.0053	0.0012	0.8695	0.0210	0.0069	0.0202	0.0647	0.0230	0.1755
Y_6 F-value	7.2920	11.7059	0.0295	39.8062	0.3869	0.1372	4.8154	1.8427	2.0725	2.5196	7.4736
P>F	0.0079	0.0111	0.8685	0.0004	0.5537	0.7220	0.0643	0.2168	0.1932	0.1565	0.1222
Y_7 F-value	8.5434	25.5804	0.0979	42.5148	2.4507	1.0972	2.1512	0.0535	0.3026	2.9030	7.5298
P>F	0.0049	0.0015	0.7635	0.0003	0.1615	0.3297	0.1859	0.8238	0.5994	0.1322	0.1214

To estimate the significance of the model and terms, analysis of variance (ANOVA) was performed (Table 4). The relatively low probability p-value ($p<0.05$) denoted that the model was significant (Amid *et al.*, 2010). Polynomial equations and the resultant R^2 values are listed in (Table 5).

There was a strong correlation between the experimentally observed and predicted values indicated by R^2 values greater than 0.9 (Jaiswal *et al.*, 2011). The resulting equations were left with their full model form, without removing any terms, to avoid loss of any important information (Ricci *et al.*, 2006).

Table 5: Regression equations of the fitted quadratic model in terms of coded factors for responses Y_1, Y_2, Y_3, Y_4, Y_5, Y_6 and Y_7 and the corresponding R^2-values

$Y_1 = 31.02+3.09\ X_1-0.76\ X_2-4.39\ X_3-0.76\ X_1X_2-1.56\ X_1X_3+0.38\ X_2X_3+2.56\ X_1^2-0.33\ X_2^2+0.065\ X_3^2$, $R^2 = 0.9053$
$Y_2 = 206.97+17\ X_1-12.15\ X_2-53.31\ X_3+0.70\ X_1X_2+14.05\ X_1X_3-11.35\ X_2X_3-6.26\ X_1^2+0.79\ X_2^2+0.79\ X_3^2$, $R^2 = 0.9733$
$Y_3 = 246.20+65.89\ X_1-15.68\ X_2-28.78\ X_3+3.56\ X_1X_2+46.21\ X_1X_3\ -21.34\ X_2X_3+3.91\ X_1^2+6.23\ X_2^2\ -35.92\ X_3^2$, $R^2 = 0.9476$
$Y_4 = 47.09+0.13X_1-3.31\ X_2+0.33\ X_3-0.13\ X_1X_2+0.37\ X_1X_3-2.88\ X_2X_3-1.7\ X_1^2+0.56\ X_2^2+2.46\ X_3^2$, $R^2 = 0.9667$
$Y_5 = 0.066+0.015\ X_1-0.011\ X_2+0.015\ X_3+0.001\ X_1X_2+0.011\ X_1X_3-0.014\ X_2X_3-0.009\ X_1^2-0.007\ X_2^2\ +0.009\ X_3^2$, $R^2 = 0.9473$
$Y_6 = 0.34+0.094\ X_1-0.005\ X_2-0.173\ X_3-0.022\ X_1X_2-0.013\ X_1X_3-0.079\ X_2X_3-0.041\ X_1^2-0.043\ X_2^2+0.048\ X_3^2$, $R^2 = 0.9036$
$Y_7 = 0.328+0.14\ X_1+0.009\ X_2-0.181\ X_3+0.057\ X_1X_2+0.038\ X_1X_3+0.053\ X_2X_3+0.007\ X_1^2+0.017\ X_2^2+0.052\ X_3^2$, $R^2 = 0.9116$

The mean droplet size is a critical factor to evaluate a SEDDS. The droplet size is reported to have an impact on drug absorption. As the droplet size decreases, the interfacial surface area provided for drug absorption becomes larger (Gershanik and Benita, 2000). The effect of pH on the droplet size was studied to assess the effect of pH of the gastrointestinal tract on the mean droplet size and stability of emulsion. Table 3 shows that the optical clarity (Y_5, Y_6 and Y_7) was decreasing (indicated by increase in light absorption) by increasing the droplet size (Y_1, Y_2 and Y_3). In addition, the droplet size increased as the pH of the dilution medium increased. Wang *et al.* (2009) suggested that dug could be precipitated in the oil-water interface at higher concentrations, leading to a more compact interfacial film which hinder the spontaneous emulsification of SEDDS, hence larger droplet size are obtained. In addition, Park and Kim (1999) suggested that the increase in droplet size with increasing drug content could be due to formation of drug aggregate on the surface of oil droplets due to the excess amount of undissolved drug. Accordingly, the observed increase in droplet size at higher pH could be attributed to the reduced drug solubility at this pH.

According to the regression equations (Table 5), three-dimensional response surfaces and contour plots were presented in Fig. 1-3. These plots show the effects of two factors on the response at any time. In all the presented pictures, the third factor was kept at level zero.

Figure 1 reveals that the droplet size increased by increasing the percentage of oil in the formulation irrespective of the pH of the medium. This came in accordance with Dixit *et al.* (2010) who reported that high concentration of oil in valsartan SEDDS forms poor emulsion with entrapment of very low amount of water upon dilution and that the percentage of oil should not exceed 10%.

It was also found that the increase in Cremophor®/Labrasol® ratio from 0.75 to 2 imparted significant decrease in particle size in all tested pH (Fig. 1). Moreover, droplet size decreased when S_{mix}/co-surfactant ratio increased from 1 to 2 at all tested pH values as depicted by the negative coefficient (b_2) as shown in Table 5. Accordingly, it could be concluded that that increasing surfactant proportion led to a more favorable formation of nanoemulsion. This is consistent with Xi *et al.* (2009) who found that droplet size decreased significantly in oleanolic acid SEDDS if the concentration of Cremophor® EL increased from 20-40% (w/w). They also found that droplet size decreased with increasing surfactant to co-surfactant ratio. This decrease in droplet size could be the result of the more availability of surfactant to stabilize the oil-water interface and the formation of a better close-packed film of surfactant at the oil-water interface (Wei *et al.*, 2005). It was reported that the addition of SEDDS into the aqueous media under gentle agitation results in spontaneous microemulsion formation because the free energy required to form an emulsion is very low (Craig *et al.*, 1995). Surfactants form a film around the emulsion droplets and reduce the interfacial energy as well as provide a mechanical barrier to coalescence (Pouton, 1985). On the other hand, co-surfactants cause expansion of that film (Patel and Vavia, 2007).

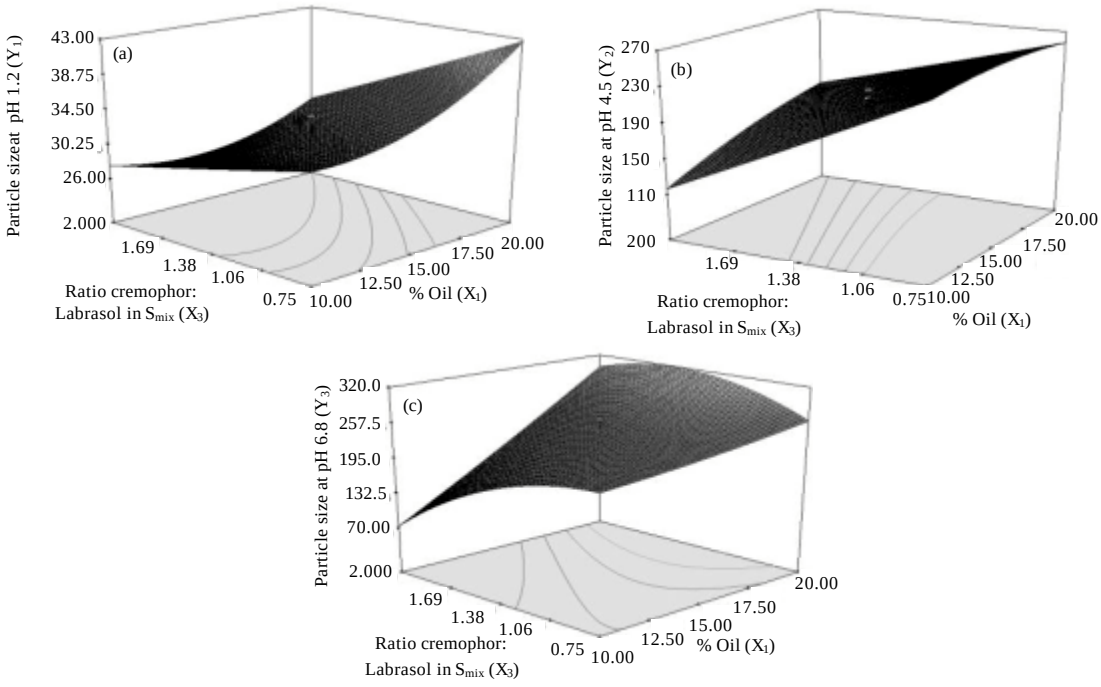

Fig. 1(a-c): Response surface plots for particle size at pH 1.2, 4.5 and 6.8 (Y_1, Y_2 and Y_3) as a function of % of oil (X_1) and ratio of cremophor to labrasol in S_{mix} (X_3)

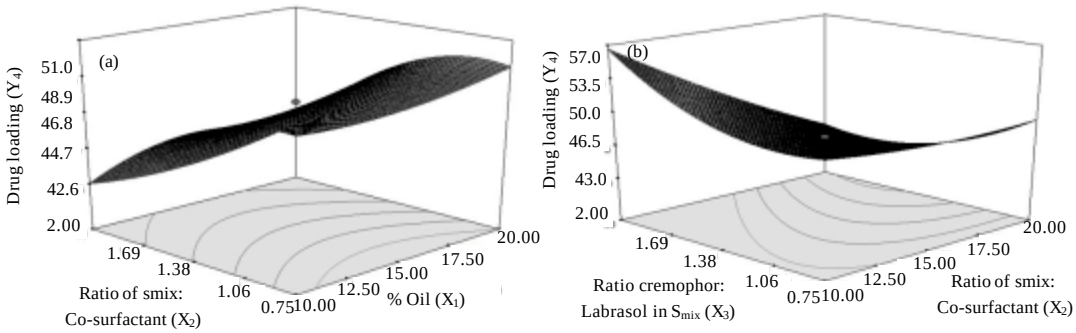

Fig. 2(a-b): Response surface plots for drug loading (Y4) as a function of: (a) % of oil (X_1) and ratio of Smix: co-surfactant (X_2) and (b) ratio of Smix: co-surfactant (X_2) and ratio of cremophor to labrasol in S_{mix} (X_3)

Figure 2 shows that drug loading decreased when S_{mix}/co-surfactant ratio increased from 1 to 2, especially at Cremophor EL®/Labrasol® ratio of 2. This might be explained by the low solubility of amisulpride in Cremophor EL® (13.33±0.39 mg g^{-1}) compared to Transcutol® (49.31±0.31 mg g^{-1}) or Labrasol® (30.28±0.32 mg g^{-1}).

The optical clarity of the prepared SEDDS was studied by measuring light absorption at different pH to ensure that neither drug precipitation nor emulsion separation could occur in the different pHs along the GIT. Generally, as shown in Fig. 3, the optical clarity followed the same pattern shown and discussed in studying the effect of pH on particle size. In general, maximum clarity

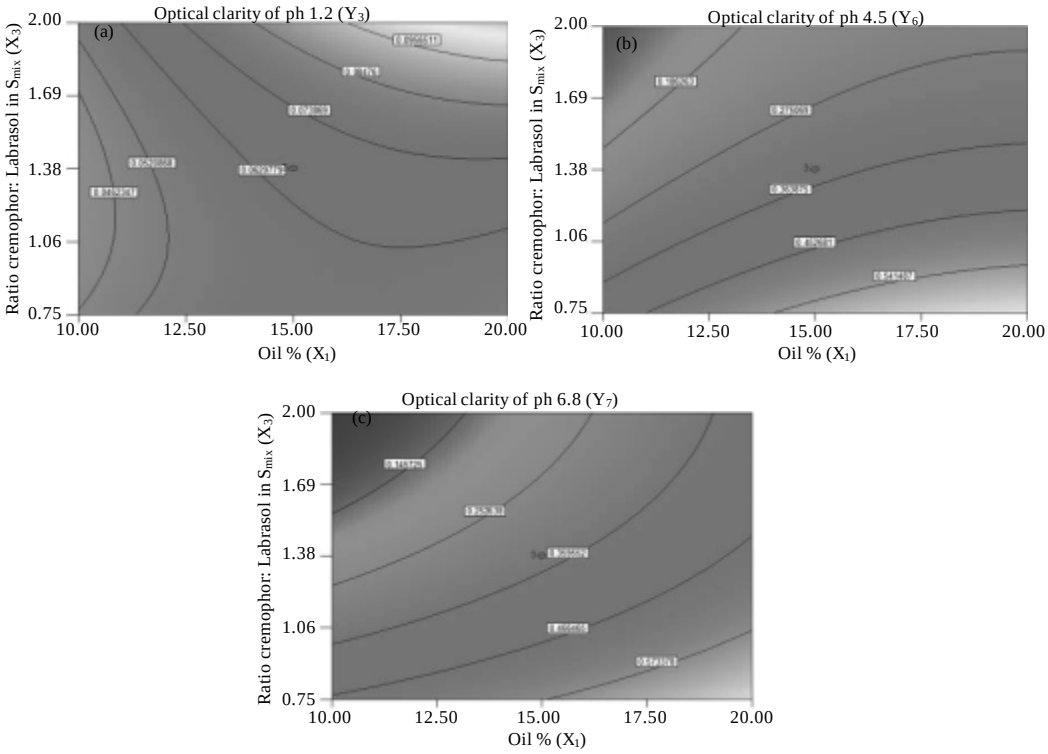

Fig. 3(a-c): Contour plots for optical clarity at pH 1.2, 4.5 and 6.8 (Y_5, Y_6 and Y_7) as a function of % of oil (X_1) and ratio of cremophor to labrasol in S_{mix} (X_3)

indicated by the smallest light absorption can occur by decreasing oil percentage and increasing the ratio of Cremophor to Labrasol in S_{mix}.

Optimization: After generating the polynomial equations relating the dependent and independent variables, the optimum formulation was selected based on the criteria for attaining the minimum droplet size and optical clarity at pH 1.2, 4.5 and 6.8, (Y_1, Y_2, Y_3, Y_5, Y_6 and Y_7, respectively). The drug loading was constrained with a target of 50 mg (Y_4), as shown in Table 1. The optimization procedure was conducted automatically by the Design Expert® and based on utilizing the desirability function. The individual desirability function d_i was calculated for each response. It can be varied over the range 0 to 1 (least to most desirable, respectively). After that, an Overall Desirability (OD) is obtained by combining the individual desirability using the geometric mean as shown in the following equation:

$$D = (d_1.d_2.d_3.....d_m)^{1/m}$$

where, m is the number of responses. The formulation variables are then selected to maximize the overall desirability (Mallipeddi *et al.*, 2010).

Figure 4 represents an overlay plot showing the optimized formulation variables chosen by the software to get the responses in the required range. The optimized formulation was achieved with 10% oil, 1.31 as Smix: co-surfactant ratio and 2 as the ratio of Cremophor EL® : Labrasol ®in S_{mix}.

Table 6: The observed and predicted values for the optimized formulation

Factor			Optimized level
X_1: Oil percentage			10.000
X_2: S_{mix}/cosurfactant ratio			1.310
X_3: Chremophore/Labrasol ratio in S_{mix}			2.000
Overall desirability			0.868
Response	Expected	Observed	Residual
Y_1: Droplet size at pH 1.2 (nm.)	27.548	28.910	1.362
Y_2: Droplet size at pH 4.5 (nm.)	126.395	113.610	-12.785
Y_3: Droplet size at pH 6.8 (nm.)	89.602	98.200	8.598
Y_4: Drug loading	50.000	50.000	0.000
Y_5: Optical clarity at pH 1.2	0.063	0.071	0.008
Y_6: Optical clarity at pH 4.5	0.114	0.090	-0.024
Y_7: Optical clarity at pH 6.8	0.039	0.043	0.004

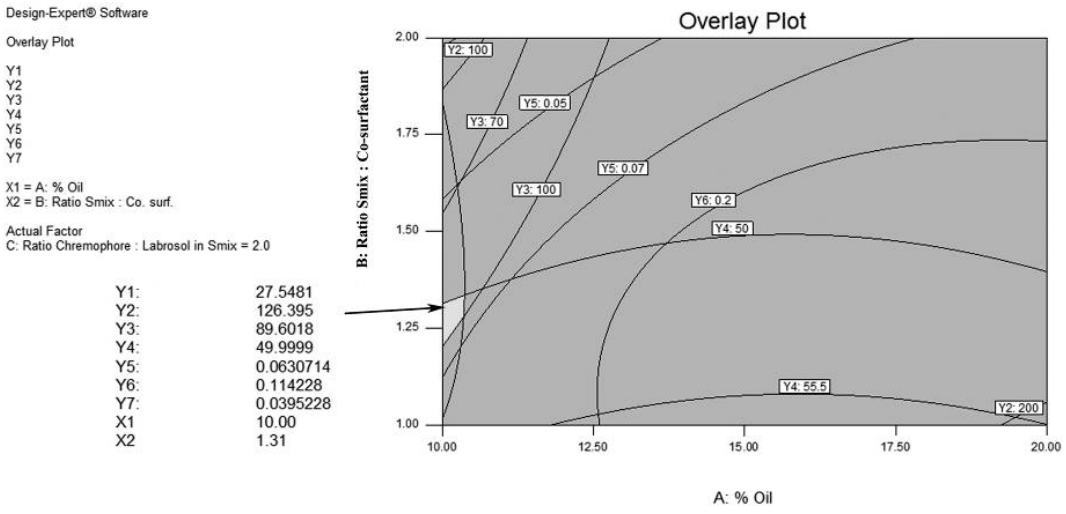

Fig. 4: Overlay plot for optimized variables

The Overall Desirability (OD) is 0.868 which is close to 1 (Table 6). To check the validity of the calculated optimal formulation variables and predicated responses, a new batch with the optimized formula was prepared and evaluated. Table 6 shows the predicated and observed responses for the optimum formulation. There is small residual between the predicated and observed responses. Accordingly, these results demonstrate the validity and reliability of the optimization procedure used in prediction of the formulation variables.

***In vitro* release studies:** Figure 5 shows the dissolution profiles of the optimized formula and aqueous drug suspension in distilled water and buffers of pH 1.2, 4.5 and 6.8. It was found that the optimized formula released 100% of the drug in less than 15 min irrespective of the pH of the dissolution medium. On the other hand, aqueous drug suspension exhibited different dissolution behavior dependent on medium pH. The dissolution of the drug became slower by the increase in pH from 1.2 to 6.8 which could be related to the decrease in drug solubility at high pH range.

Table 7: Comparison of Mean±SD for pharmacokinetic parameters of optimized SEDDS formulation and pure drug suspension after a Single 50 mg oral dose in rabbits (N = 6)

Parameters	Pure drug suspension	Optimized SEDDS formulation
C_{max} (ng mL^{-1})	268.87±6.32	288.85±17.62
T_{max} (h) (median)	4.00	4.00
$AUC_{0-\infty}$ (ng.h mL^{-1})	800.02±18.95	1105.45±24.18*
F (%)	-	138.00

*Significant at $p<0.01$ from drug suspension

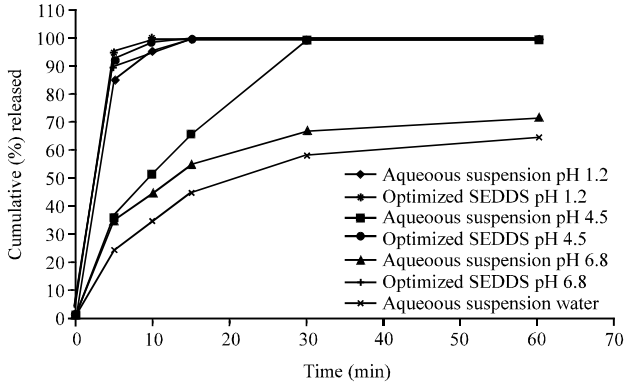

Fig. 5: *In vitro* dissolution profile for amisulpride from optimized SEDDS formulation compared to that from aqueous suspension (error bars have been omitted for clarity)

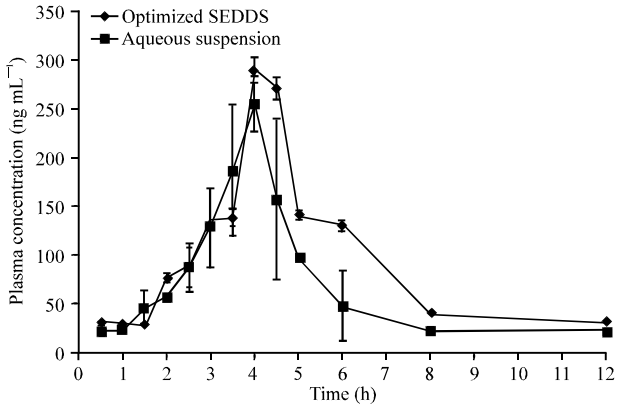

Fig. 6: Mean amisulpride plasma concentration (ng mL^{-1}) following the administration of optimized SEDDS and aqueous suspension in rabbits (n = 6)

***In vivo* bioavailability study in rabbits:** Figure 6 shows the mean amisulpride plasma concentration vs. time profiles obtained after single oral administrations of both the optimized SEDDS formulation and the aqueous suspension (50 mg mL^{-1}). The plasma profile of the optimized SEDDS formulation exhibits a lower variability as indicated by narrower error bars compared to that of the aqueous suspension. The mean pharmacokinetic characteristics are summarized in Table 7.

There is no statistically significant difference between Cmax and Tmax ($p>0.01$) of SEDDS and aqueous suspension were observed. However, the AUC of amisulpride from the optimized SEDDS

formulation was found to be significantly increased compared with orally administrated pure drug suspension (1105.45 ng.h mL^{-1} vs. 800.02 ng.h mL^{-1}, $p<0.01$). Higher drug concentration in blood indicates better systemic absorption of amisulpride from SEDDS. The oral relative bioavailability of amisulpride from optimized SEDDS exhibited a 1.38-fold increase compared with the orally administrated drug suspension. This improved oral bioavailability of amisulpride formulation could be explained by the combination of the following effects: (1) dispersion of amisulpride in the SEDDS that could keep the drug the drug in solution and overcome the barrier of solubility-limited absorption at higher neutral pH presents in the lower GIT. (2) the reported inhibitory effect of non-ionic surfactants e.g., Cremophor® and Labrasol® and the co-solvent Transcutol® on p-glycoprotein mediated efflux. (3) the absorption enhancing effects of the used components of SEDDS (Cornaire *et al.*, 2004; Yin *et al.*, 2009).

CONCLUSION

Central composite design is demonstrated to be more efficient approach for the optimization of SEDDS in shorter time using smaller number of experiments than conventional "change one factor at a time" formulation methods, especially for such a complex system of SEDDS. Amisulpride was successfully formulated as SEDDS formulation. The optimized SEDDS formulation composed of 10% Capryol 90®, 1.31 as a ratio of surfactant mixture to Transcutol® (co-surfactant) and 2 as a ratio of Cremophor® to Labrasol® in the surfactant mixture. It exhibited faster and more complete dissolution of amisulpride than aqueous drug suspension regardless of the type and pH of the dissolution medium. Also, it showed a significant improvement of the bioavailability of amisulpride in rabbits. Thus, the developed SEDDS could be considered as a promising oral delivery system that could solve the low and variable bioavailability of amisulpride if used in human trials.

ACKNOWLEDGMENTS

The authors are deeply grateful to Al Andalous for pharmaceutical industries, Gattefossé, Basf, Abitec and Stepan for providing the required chemicals for our research.

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