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Preparation, characterization and optimization of probucol self-emulsified drug delivery system to enhance solubility and dissolution

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The main purposes of this work were to prepare, characterize and optimize a self-emulsified drug delivery system of probucol (PBSEDDS) with enhanced dissolution and better chance for oral absorption. The methods included determination of the solubility of probucol in different oils, surfactants and co-surfactants using saturation solubility method and HPLC for drug analysis. The ingredients showing high drug solubility were used to prepare PBSEDDS after being tested for physical and chemical compatibility with the drug using DSC and FTIR. The prepared formulations were evaluated for droplet size, turbidity, spontaneity of emulsification and dissolution in water. Optimization was performed using a three-factor, three-level Box-Behnken experimental design. The results showed high drug solubility and compatibility with soybean oil (solvent), Labrafil M1944CS (surfactant) and Capmul MCM-C8 (co-surfactant). Oil to surfactant/co-surfactant ratio showed large influence on the characteristics of PBSEDDS. Several fold improvement of drug dissolution was observed compared to drug solution in soybean oil alone. Optimization study showed that observed and predicted values of cumulative percent drug dissolution after 60 min were in reasonable agreement. The experimental design applied helped in understanding the effects and the interaction effects between the independent factors. The prepared PBSEDDS may have the potential to enhance the therapeutic bioavailability of probucol.

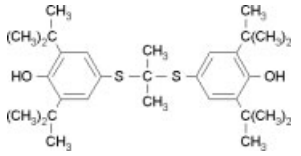
1. Introduction

A limiting factor for *in vivo* performance of poorly water-soluble drugs, following oral administration, is their resistance to being wetted by and dissolved in the fluid of the gastrointestinal tract. Increasing the solubility and dissolution rate of such drugs are thus important for optimizing bioavailability (Kocbek et al. 2006). In addition to that, the dissolution rate of these compounds is greatly influenced by the diet and the flow of bile secretion. Therefore, even if these drugs have powerful pharmacological activities, their oral delivery is frequently associated with low bioavailability, high intra- and inter-subject variability, and lack of dose proportionality (Robinson 1996).

Self-emulsifying drug delivery systems (SEDDS) represent efficient vehicles for the *in vivo* administration of lipophilic drugs. SEDDS are not microemulsions, although they may be considered to be a closely related system. The key difference between the two preparations is the optical clarity and the finer droplet size achieved when SEDDS are dispersed into the aqueous phase. Additionally, in O/W microemulsion, the outer phase is an aqueous phase while SEDDS do not contain water, they are more stable and more easy to transport. A SEDDS typically comprises a mixture of surfactant(s), oil and drug which when introduced into the body is rapidly dispersed to form droplets of micrometer or nanometer size. The dispersed systems would be expected to self-emulsify rapidly in the aqueous contents of the stomach or the upper small intestine and form a thermodynamically stable O/W microemulsion. These small fine oil droplets have the capability to empty rapidly from the stomach and promote wide distribution of the drug throughout the gastrointestinal tract (Devani et al. 2004). Additionally, these oil droplets provide large surface area for pancreatic lipases to hydrolyze the glycerides and the fatty constituents of the emulsion and the subsequent drug containment in mixed micelles formed from biliary extracts (Shah et al. 1994). For the aforementioned reasons, SEDDS may offer an improvement in both the rate and extent of absorption for drugs subject to dissolution rate limited absorption. Although many studies have been carried out in this area, there are few drug products on the pharmaceutical market formulated as SEDDS confirming the difficulty of formulating hydrophobic drug compounds into such formulations. At present, there are four drug products, Sandimmune® and Sandimmun Neoral® (cyclosporine A), Norvir® (ritonavir), and Fortovase® (saquinavir) on the pharmaceutical market, the active compounds of which have been formulated into specific SEDDS. Significant improvement in the oral bioavailability of these drug compounds has been demonstrated for each case. The fact that almost 50% of new drug compounds are hydrophobic in nature implies that studies with SEDDS should continue, and more drug compounds formulated as SEDDS will reach the pharmaceutical market in the future (Neslihan and Benita 2004). The

efficiency of oral absorption of the drug compound from the SEDDS depends on many formulation related parameters, such as surfactant concentration, oil/surfactant ratio, polarity of the emulsion, droplet size and charge, all of which in essence determine the self-emulsification ability. Only very specific pharmaceutical excipient combinations will lead to efficient self-emulsifying systems. In most cases, the relationship between design variables and responses is complicated and requires statistically designed experiments. Experimental design can be used to underline the relationship between formulation or process variables and their influence in obtaining the optimized formulation. One of the well known experimental design is Response Surface Methodology (RSM). The goal of the RSM is to obtain a regression model and to find a suitable approximation for the true functional relationship between the responses and the set of independent variables. The general optimization procedures may be preceded by screening experiments to reduce the set of factors to those that are most influential to the response(s) being investigated. The statistical optimization designs have been documented for the formulation and optimization of many pharmaceutical solid and liquid dosage forms (Zaghloul et al. 2001, 2006).

Probucol (PB) is an extremely lipophilic drug. Its water solubility is 0.002–0.005 μg/ml and its log P is 11 (Yagi et al. 1996).



Probucol

It is used as a lipid regulating agent to treat hyperlipidaemias particularly type IIa hyperlipoproteinaemia. In addition, it has pronounced antioxidant properties and has been shown to protect and amend heart and vascular disorders in addition to its effects on neural and synaptic plasticity in brain aging (Champagne et al. 2003) and combating common Alzheimer disease through its cholesterol-lowering effect (Poirier 2003). The absorption of PB from the gastrointestinal tract is limited and variable, and is stated to be at a maximum if taken with food. Arachis oil showed greater increase in PB absorption compared to coconut and paraffin oils (Palin and Wilson 1984). The increase in pharmacokinetics and relative bioavailability of PB inclusion complex capsule in healthy dogs is attributed to the improvement of its water-solubility by the inclusion process (Zhang et al. 2002). These findings suggested that enhancing the solubility and dissolution of this compound through SEDDS may improve absorption and bioavailability.

The objectives of this study were to prepare, characterize and optimize a PBSEDDS with enhanced dissolution and better chance for oral absorption. To achieve these goals, the following procedures were followed: 1. The solubility of PB in different oils, surfactants and co-surfactants was demonstrated using saturation solubility method and HPLC for drug analysis. 2. Ingredients showing high drug solubility were used to prepare PBSEDDS after being tested for physical compatibility (miscibility) and chemical compatibility with the drug using DSC and FTIR. 3. Different formulations were characterized for droplet size, turbidity, spontaneity of emulsification and dissolution in water. 4. Optimization of PBSEDDS using Box-Behnken experimental design to evaluate the effect of formulation ingredients on the drug release and get the optimized formulation. As part of the optimization process, the main effects, interaction effects, and quadratic effects of the formulation ingredients on drug release were investigated.

2. Investigations, results and discussion

2.1. Solubility and compatibility of PB different oily and aqueous solutions

The results of solubility study were shown in Table 1. As shown, soybean oil, Labrafil M1944CS and Capmul MCM-C8 demonstrated the highest solubility among all the investigated reagents. The mixture also showed physical compatibility where no phase separation was observed. For these reasons they were used to prepare PBSEDDS. Soybean oil is an unmodified edible oil and provides the most natural bases for lipid formulations in addition to increasing the fraction of lipophilic drugs transported via the intestinal lymphatic system. Unfortunately, because of its poor ability to dissolve large amounts of hydrophobic drugs, it needs to be mixed with one or more surfactant. Addition of Labrafil increased the drug uptake but showed low emulsification efficiency when the preparation was diluted with water. Capmul addition improved the emulsification and increased the drug uptake and hence the drug loading concentration was increased. Labrafil is a polyoxylglyceride and Capmul is a mono and diglyceride of medium chain fatty acids. Both of them have solubilizing and emulsifying properties and they are generally recognized as safe (GRAS). Since alcohol and other volatile cosolvents comprised in the conventional SEDDS are known to migrate into the shells of soft gelatin or hard gelatin capsules, resulting in drug precipitation (Gershanika and Benita 2000), they were excluded from our formulations.

It has been reported that a drug may interact with one or more of SEDD formulations leading to change in the droplet size, decrease emulsification efficiency and reduce the stability of SEDDS (Craig et al. 1993). For this reason, pre-formulation compatibility studies were necessary in order to design an optimal self emulsifying drug delivery vehicle. The chemical compatibility of the oily ingredients with PB was performed using DSC and FTIR. PB has been reported as having at least two polymorphs with onset melting points of 126 °C (Form I) and 116 °C (Form II), where the Form I polymorph is the thermodynamically stable form. The material as received was found to have

Table 1: Solubility study of PB in different oils, surfactants and co-surfactants

Reagent	Solubility (% w/v)
Corn oil	4.39
Peanut oil	4.09
Soybean	6.23
Olive oil	3.77
Cremophor 2%	0.76
Labrafil M1944CS 2%	4.44
Labrasol 2%	2.39
Lauroglycol 2%	0.20
Tween 20, 2%	0.74
Tween 40, 2%	0.74
Tween 60, 2%	0.48
Tween 80, 2%	0.50
Capmul MCM-C8, 2%	5.01
Propylene glycol, 2%	0.01

melting point of 126 °C as measured by DSC and is thus form I. DSC thermograms of PB and PBSEDDS showed the major endotherm at 126 °C representing the melting point of PB. The two spectra resemble one another, indicating that the molecular species are identical (thermograms are not shown).

PB IR spectrum showed several characteristic peaks. The spectra of PB and PBSEDDS (not shown) had the same features with the expected peak broadening due to the lower concentration of PB in the formulation and/or the change of some crystalline structure of PB into the amorphous form. These investigations indicated that there was no chemical interaction in the mixture and the molecular structure of PB remained intact.

2.2. Preparation and characterization of different PBSEDDS for screening studies

For the development of a self-emulsified formulation, the correct blend of oil with surfactant(s) is necessary for the formation of a stable microemulsion. Based on solubility and compatibility studies, soybean oil, Labrafil M1944CS and Capmul MCM-C8 were selected. The screening study was performed using oil concentration ranging from 10–90%, surfactant concentration ranging from 8–72% and co-surfactant concentration ranging from 2–18%. The prepared formulations were characterized for droplet size, turbidity and spontaneity of emulsification. Table 2 shows the design and results of characterization of different PBSEDDS for screening study. Few techniques have been mentioned in the literature to categorize and characterize the self-emulsifying performance. This performance is based on type and content of lipid phase, surfactant(s) and any other ingredients in the preparation such as cosolvent. So far the techniques for the evaluation and characterization of SEDDS have primarily based on equilibrium phase behavior studies of systems mixed with water. Among these techniques is the visual inspection where the emulsification rate and resultant emulsion are qualitatively described. Spontaneity or the rate of self-emulsification can also be assessed by monitoring the turbidity change and/or the droplet size analysis of the dispersion by appropriate instrumental method. All measurements were done in duplicate and the mean values were reported in Table 2. The results showed that there was a correlation between spontaneity of emulsification and surfactant concentration. Increasing the concentration of surfactant(s) improved the shape and stability of the emulsion. Out of the 9 formulations, F5–F9 showed no separation or precipitation of the drug when kept at room temperature for more than 2 months. High droplet size of some of these formulations (F5, F6 and F8) may be attributed to the interfacial disruption elicited by enhanced water penetration into the oil droplets mediated by the increased surfactant concentration and leading to ejection of oil droplets into the aqueous phase (Robinson 1996). The results of droplet size are in agreement with the conclusion that decreased surfactant to lipid ratio of the vehicle, gives rise to a decreased surface to volume ratio of the formed nanoemulsion droplets and therefore an increase of the droplet size (Nielsen et al. 2007). The turbidity of the prepared PBSEDDS increased initially with lipid content, but declined slightly when the particle size decreased. In general, formulations exhibiting better dispersion showed better results of droplet size and turbidity and vice versa.

Table 2: Design and characterization of different PBSEDDS for screening study

Form. #	Oil (%)	S (%)	CoS (%)	Mean droplet size (μm)	Turbidity (NTU)	Spontaneity of emulsification
1	90	8	2	1.65	9.00	bad
2	80	16	4	1.68	8.00	bad
3	70	24	6	1.59	9.53	bad
4	60	32	8	1.57	9.22	fair
5	50	40	10	1.11	7.93	good
6	40	48	12	1.02	7.10	good
7	30	56	14	0.91	6.51	good
8	20	64	16	1.01	6.62	good
9	10	72	18	0.60	6.30	good

2.3. Dissolution studies

The primary mechanism of action which leads to improved bioavailability from SEDDS is usually avoidance, or partial avoidance, of the slow dissolution process which limits the bioavailability of hydrophobic drugs from solid dosage forms. Ideally the formulation allows the drug to remain in a dissolved state throughout its transit through the gastrointestinal tract. HPMC capsules were selected because of their chemical inactivity, low moisture content, short disintegration time and not coloring the dissolution medium. The dissolution profiles of different PBSEDDS (screening runs) and blank formulation were shown in Figs. 1, 2. The highest percent drug dissolution were 63.2, 73.9, 64.3 and 77.7 for formulations 5, 6, 7 and 8 respectively and the lowest were 20.9, 3.5, 12.1, 12.2 and 14.2 for formulations 1, 2, 3, 4 and 9 respectively. PB in oil (blank) showed only 0.018% drug release after 30 min. The results indicted that SEDDS increased the release of PB tremendously compared to blank formulation. With the

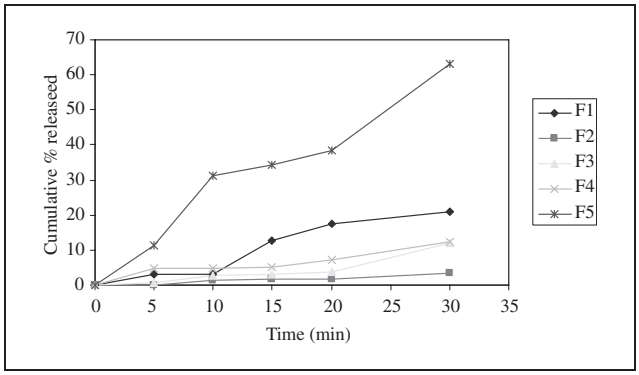


Fig. 1: Dissolution profiles of PBSEDDS (F1–F5) in distilled water

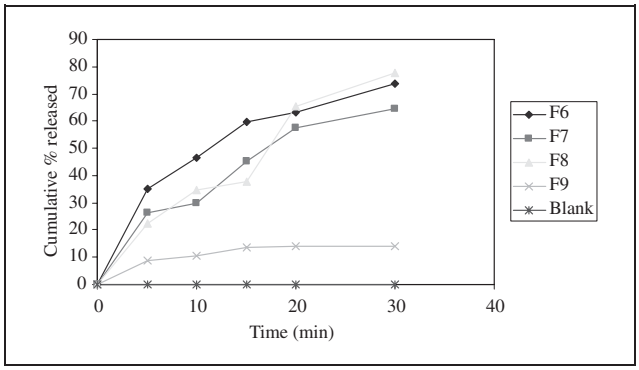


Fig. 2: Dissolution profiles of PBSEDDS (F6–F9) and blank formulation in distilled water

exception of F9, there was a good correlation between the results of dissolution and the rate of emulsification of formulations 5–9.

From the previous results of characterization and the results of dissolution, it was obvious that good PBSEDDS emulsion could be obtained with oil concentration ranging from 10–50%, Labrafil concentration ranging from 40–72% and Capmul concentration ranging from 10–18% (F5–F9). Other formulations showed either turbidity and/or drug precipitation after two months stay at room temperature. These findings were in agreement with the report stated that the usual surfactant concentration in self-emulsifying formulations required to form and maintain a stable microemulsion *in vitro* and *in vivo* is ranging from 30 to 60% (Constantinides 1995).

2.4. Response surface methodology for optimization of PBSEDDS

From the results in this work and others (Karim et al. 1994; Chanana and Sheth 1995), it was revealed that the self-emulsification process is specific to the nature of the oil/surfactant(s) pair, the surfactant concentration and oil/surfactant ratio. In this study, Box-Behnken statistical design was used to optimize and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the *in vitro* performance of PBSEDDS.

Fifteen experiments were required for the response surface methodology based on this design. Constraints on the formulas were placed so that the oil phase ranged from 10–50% and surfactant ranged from 40–72% and co-surfactant ranged from 10–18%. The different variables with their constraints, experimental runs and the results of characterization were given in Tables 3 and 4. Based on the experimental design, the factor combinations resulted in different PBSEDDS characteristics and drug release. The dissolution profiles were shown in Figs. 3–5. The range of the drug release was 98.8% in formulation No 4 (maximum) and 9.5% in formulation No 11 (minimum). None of the 15 formulations showed phase separation or drug precipitation after 2 months stay on shelf at room temperature. The confidence that the regression equation would predict the observed values better than the mean for Y_1 was 92.8%. The polynomial equation relating the response Y_1 and independent variables was: $Y_1 = 313.25 - 9.32X_1 - 3.45X_2 - 3.31X_3 + 0.009X_1X_2 + 0.035X_1X_3 + 0.057X_2X_3 + 0.128X_1^2 + 0.014X_2^2 - 0.096X_3^2$.

The above equation represents the quantitative effect of process variables; X_1 through X_3; and their interactions on the response Y_1. The values of the coefficients X_1–X_3 are related to the effect of these variables on the response Y_1. Coefficients with more than one factor term and those with higher order terms represent interaction terms and

Table 3: Variables in the Box-Behnken experimental design

Independent variables	Dependent variables
X_1 = % of oil (10, 30, 50)	Y_1 = Drug dissolved after 60 min (%)
X_2 = % of surfactant (40, 56, 72)	Y_2 = Turbidity (NTU)
X_3 = % of co-surfactant (10, 14, 18)	Y_3 = Droplet size (um)

Table 4: Box-Behnken design and results of characterization (optimized runs)

Form. #	Oil (part)	S (part)	CoS (part)	Droplet size (um)	Turbidity (NTU)	Spontaneity of emulsification
1	50	72	14	1.849	11.93	bad
2	50	40	14	0.554	10.8	good
3	10	72	14	0.874	6.76	good
4	10	40	14	0.982	8.76	good
5	50	56	18	0.825	7.4	good
6	50	56	10	1.176	7.94	good
7	10	56	18	1.114	10.7	good
8	10	56	10	0.706	10.6	good
9	30	72	18	0.693	8.03	good
10	30	72	10	0.439	10.4	good
11	30	40	18	1.03	7.78	good
12	30	40	10	0.57	10.3	fair
13	30	56	14	0.731	8.5	fair
14	30	56	14	0.616	9.2	fair
15	30	56	14	0.695	8.9	fair

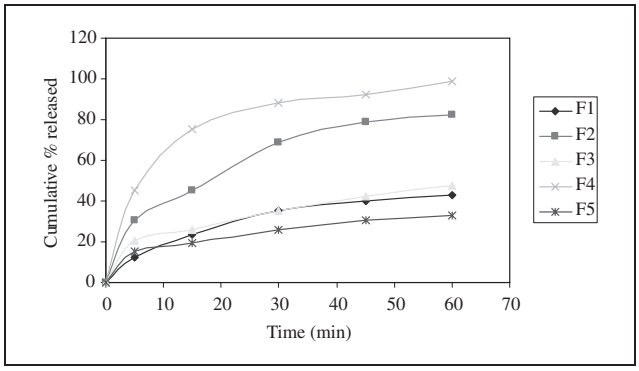


Fig. 3: Dissolution profiles of PBSEDDS optimized formulations (F1–F5) in distilled water

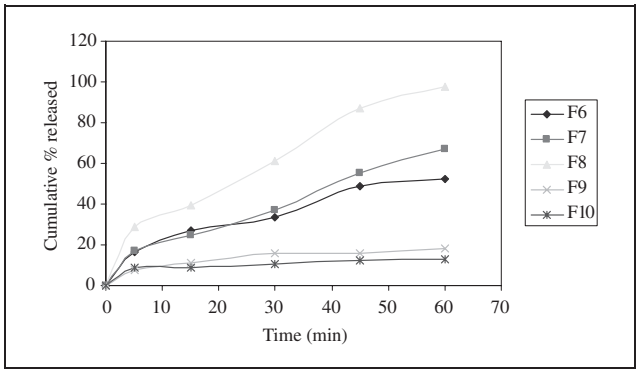


Fig. 4: Dissolution profiles of PBSEDDS optimized formulations (F6–F10) in distilled water

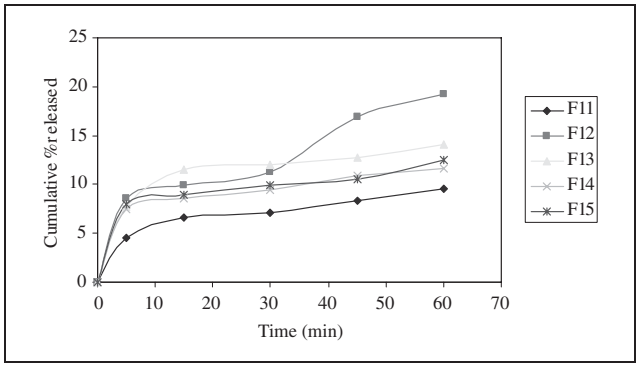


Fig. 5: Dissolution profiles of PBSEDDS optimized formulations (F11–F15) in distilled water

Table 5: Observed and predicted values of the response (Y_1)

Form. #	Observed (%)	Predicted (%)	Residuals
1	42.8	47.3	4.5
2	82.2	63.38	18.8
3	47.5	66.3	18.8
4	98.8	94.3	4.5
5	33.0	45.8	12.8
6	52.6	54.0	1.4
7	66.7	65.2	1.4
8	97.5	84.6	12.8
9	18.2	0.81	17.3
10	13.1	7.16	5.9
11	9.5	15.4	5.9
12	19.2	36.5	17.3
13	14.1	12.7	1.4
14	11.5	12.7	1.2
15	12.5	12.7	0.2

quadratic relationships respectively. A positive sign represents a synergistic effect, while a negative sign indicates an antagonistic effect. The values of X_1–X_3 were substituted in the equation to obtain the theoretical values of Y_1. The theoretical (predicted) values and the observed values were in reasonably good agreement as seen from Table 5. The significance of the ratio of mean square variation due to regression and residual error was tested using analysis of variance (ANOVA). The ANOVA indicated a significant ($P < 0.05$) effect of the tested factors on response Y_1 as seen from Table 6. The relationship between the dependent and independent variables was further elucidated using contour and response surface plots. The effects of X_1, X_2 and X_3 and their interaction on Y_1 are given in Figs. 6–8.

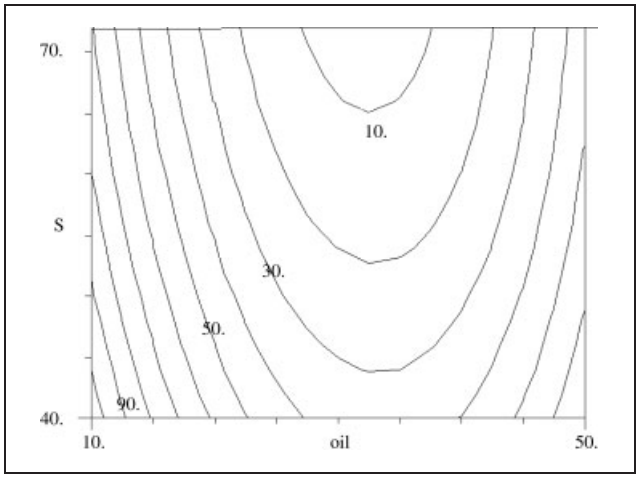


Fig. 6: Contour plot showing the effect of oil and surfactant concentrations on drug release

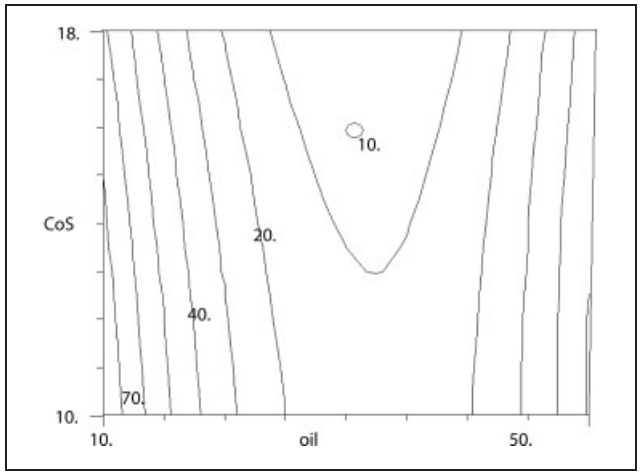


Fig. 7: Contour plot showing the effect of oil and co-surfactant concentrations on drug release

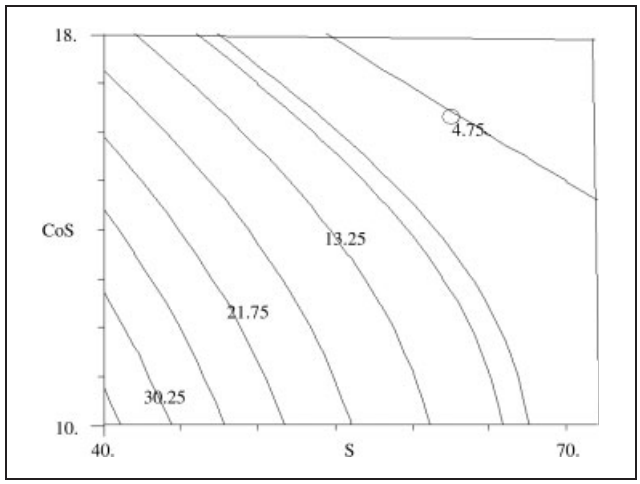


Fig. 8: Contour plot showing the effect of surfactant and co-surfactant concentrations on drug release

2.5. Optimization of the formulation ingredients

After generating the polynomial equations relating the dependent and independent variables, the process was optimized for the response Y_1. Optimization was performed to obtain the levels of X_1–X_3, which maximize Y_1 at constrained conditions of Y_2 through Y_3. The predicted levels for X_1 through X_3 were 10, 40 and 10% for X_1, X_2 and X_3 respectively. To verify these values, a new formulation was prepared according to the predicted levels of X_1- X_3. The obtained Y_1 was in a close agreement with the predicted value. The dissolution profile for the optimized formulation was shown in Fig. 9. The given results demonstrated the reliability of the optimization procedure in predicting the output properties of PBSEDDS.

Table 6: Analysis of variance for Y_1

Source	DF	SS	MS	F-Ratio
Total (corrected)	14	14306		
Regression	9	12542	1394	3.94
Residual	5	1764	352.8	
Standard deviation about the regression	= 18.7			
Explained variation about the mean	= 87.7%			
Confidence that the regression equation predict the observed values better than the mean	= 92.8%			

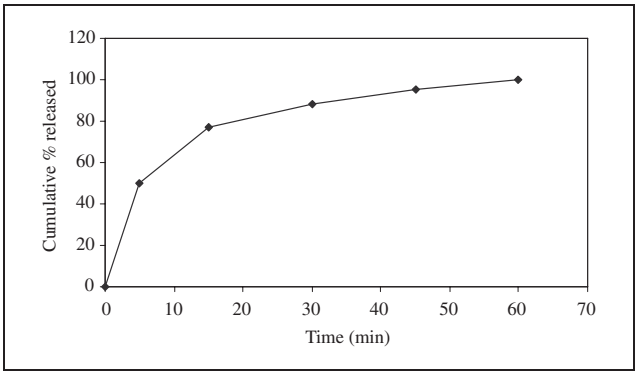


Fig. 9: Dissolution profile of PBSEDDS optimized formulation in distilled water

In conclusion, PBSEDDS could be prepared without interaction or incompatibility between the ingredients. The prepared PBSEDDS improved the characteristics, increased the dissolution rate several folds compared to the oily solution of PB. The findings also indicated that the quantitative RSM experimental design applied in this study helped in understanding the effects and the interaction effects between the three factors applied. The produced PBSEDDS may have the potential to enhance the therapeutic bioavailability of PB. The priority for future work will focus to conduct in vivo bioavailability studies, and to do more basic studies on the mechanisms of action of this fascinating and diverse group of formulations.

3. Experimental

3.1. Materials

Probucol, soy been oil, olive oil, corn oil, peanut oil, Cremophor EL, Pluronic F-68, Tween 20, Tween 40, Tween 60 and Tween 80 were purchased from Sigma Chemicals Co. (NJ, USA). Labrafil M 1944 CS, Labrasol, Lauroglycol 90, were obtained as a gift from Gattefosse Corporation (Paramus, NJ, USA). Capmul MCM-C8 was gifted from Abitec Corp. (Jamesvills, WI, USA). Hydroxypropyl methylcellulose (HPMC) capsules were gifted from Qualicaps (Whitsett, NC, USA). All other chemicals were of analytical grade and were used as received.

3.2. Determination of the solubility of PB in different oily and aqueous solutions

The solubility of PB in the following oils was investigated: soya bean oil, olive oil, corn oil and peanut oil. The solubility of PB in 2% aqueous solution of the following surfactants and co-surfactants was investigated: Cremophor EL, Labrafil M 1944 CS, Labrasol, Lauroglycol 90, propylene glycol, Tween 20, Tween 40, Tween 60, Tween 80 and Capmul MCM-C8. Solubility determination was carried out applying saturation solubility method. This was done by preparing a saturated solution of the drug in these reagents, shaking for 48 h at 20 °C, and 50 rpm. After centrifugation, samples from the supernatant layer containing the dissolved drug were withdrawn and analyzed using HPLC.

3.3. Chromatography

PB was analyzed according to Nourooz-Zadeh method with some modification (Nourooz-Zadeh et al., 1993). The method included determination of drug concentration at ambient temperature utilizing a hypercil ODS-Agilent column 4×125 mm, 5 µm, mounted on a HPLC Agilent cartridge holder (Agilent Technologies Inc. Santa Clara, CA, USA). The mobile phase was acetonitrile:water (distilled de-ionized) 85 : 15 and was pumped at a flow rate of 1 ml/min. The injection volume was 50 µl and the retention time was 18 min. The HPLC instrument consisted of a 2690 Waters separation module and a 996 photodiode array detector set at a wavelength of 242 nm. The chromatographic data were managed using Waters empower 2 software (all from Waters Corporation, Milford, MA, USA).

3.4. Compatibility study of PBSEDDS components

Physical and chemical compatibility between different ingredients of PBSEDDS were investigated. The physical compatibility between drug vehicle ingredients (miscibility) was observed visually while the chemical compatibility between these ingredients and PB was demonstrated using Differential scanning calorimeter (DSC) and Fourier transform infrared spectroscopy (FTIR).

3.5. DSC and FTIR

Differential scanning calorimetric measurements were carried out using Setaram DSC 141 (Setaram group SFIM, Caluire, France). Approximately 10 mg of PB powder and PBSEDDS were analyzed in sealed aluminum pans under nitrogen purge. Thermal analysis was carried out between 40 °C and 150 °C at a heating rate of 10 °C/min. An empty aluminum pan was used as a reference and indium was used as instrument calibration standard.

FTIR spectra of PB and PBSEDDS were investigated using FTIR spectroscopy (Nicolet, AVATAR 360 FT-IR ESP, Nicolet instrument corp., Madison, USA). The method included dissolving PB or PBSEDDS in suitable volatile solvent to form a thin film on the spectroscopy plate and leave to dry.

3.6. Preparation and characterization of PBSEDDS: screening study

The results of solubility showed that soybean oil (solvent), Labrafil M1944CS (surfactant) and Capmul MCM-C8 (co-surfactant) demonstrated the highest solubility and compatibility among the investigated reagents.

3.6.1. Preparation of PBSEDDS

A series of self-emulsifying systems were prepared with varying concentrations of the soybean oil (10–90%), Labrafil M1944CS (8–72%) and Capmul MCM-C8 (2–18%). The oil was accurately weighed into a screw-capped glass vial. Labrafil and Capmul were mixed and added while stirring with a magnetic bar until a clear mixture was obtained. The drug was added at a final loading of 250 mg/4 ml and stirred to dissolve. Table 2 shows the concentration of different ingredients in the formulations.

3.6.2. Characterization of PBSEDDS

The prepared formulations were characterized for the following:

3.6.2.1. Emulsion droplet size analysis

The mean droplet size distribution of the resultant emulsions was determined by a Coulter N4 plus submicron particle sizer and the data obtained were analyzed using N4 Plus software (Coulter Corporation, Miami, FL, USA). Each formulation (1 ml) was diluted with pure water, pre-equilibrated at room temperature to 1000 ml in an Erlenmeyer flask and gently mixed by hand. The data were collected for 60 s and the droplet size was calculated from the volume size distribution.

3.6.2.2. Turbidity measurements

Turbidity of the resultant emulsions given in nephlometric turbidity units (NTU) was measured using HACH 2100N IS turbidimeter (HACH Company, Loveland, CL, USA). The turbidimeter was first calibrated with Formazin Standards Kit. The kit includes sealed vials of turbidity range from < 0.1 NTU to 4000 NTU The accuracy as specified by the manufacturer and based on instrument calibration, is approximately $\pm$ 0.01 NTU with stray light less than or equal to 0.01 NTU. The procedures included diluting 1 ml of the formulation with pure water, pre-equilibrated at room temperature to 1000 ml in an Erlenmeyer flask and gently mixed by hand. Turbidity measurements were performed on 30 ml of the emulsion stored in a clear screw-capped sample vials.

3.6.2.3. Visual observation

To assess the self-emulsification properties and spontaneity of the emulsification, 1 ml of the formulation was introduced into 900 ml of pure water in a glass Erlenmeyer flask at room temperature and the contents were gently stirred. The preparations were observed visually. The tendency to spontaneously form a transparent emulsion was judged as 'good', and it was judged 'bad' when there was poor or no emulsion formation. "Fair" preparation is something in between. Phase diagram was constructed identifying the good self-emulsifying region. All studies were repeated in duplicate.

3.7. Dissolution studies

Dissolution studies of HPMC capsules (size 00) filled with PBSEDDS or PB dissolved in soybean oil without any other additives (blank) were determined using USP rotating paddle apparatus (Erweka® Gmbh, Type DT80, Germany) at 37 $\pm$ 0.5 °C and a rotating speed of 50 rpm in 900 ml of distilled water. Capsules were held to the bottom of the vessel using copper sinkers. Samples of 2 ml were withdrawn after 5, 10, 15, 20 and 30 min (5, 15, 30, 45, and 60 min in optimization study), filtered (hydro-

philic syringe filter PTFE 0.45 μm, Millipore Millex – LCR), properly diluted and assayed for the drug by the HPLC method previously mentioned.

3.8. Optimization of PBSEDDS applying experimental design

RSM was applied for the optimization of PBSEDDS. A three factor, three level Box-Behnken experimental design was used for the optimization process. This design is suitable for exploring quadratic response surfaces and constructing second order polynomial models. The design consists of replicated center points and the set of points lying at the midpoint of each edge of the multidimensional cube that defines the region of interest. The nonlinear quadratic model generated by the design is of the form: $Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_1X_2 + A_5X_2X_3 + A_6X_1X_3 + A_7X_1^2 + A_8X_2^2 + A_9X_3^2 + E$, where Y is the measured response associated with each factor level combination; A_0 is an intercept; A_1–A_9 are the regression coefficients; X_1, X_2 and X_3 are the factors studied and E is the error term.
For this purpose, a series of PBSEDDS were prepared as previously mentioned. The concentrations of different ingredients were as per the design (Table 4). The concentration of oil, surfactant and co-surfactant represented the independent factors X_1, X_2 and X_3 respectively. The prepared formulations were visually inspected and tested for cumulative percent drug dissolution after 60 min, turbidity and droplet size and were represented by the dependent variables Y_1, Y_2 and Y_3 respectively. The independent and the dependent variables used in the design are listed in Table 3. Response surface plots were used to demonstrate the effects of factors X_1 through X_3 on the response Y_1. Optimization was performed to obtain the levels of X_1, X_2 and X_3, which maximize Y_1 at constrained conditions of Y_2 through Y_3. The optimized (observed) and predicted values of Y_1 are shown in Tables 5, 6.

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