Studies on self-nanoemulsifying drug delivery system of flurbiprofen employing long, medium and short chain triglycerides

Junaid Daar[1], Ahmad Khan[1], Jallat Khan[2], Amjad Khan[3] and Gul Majid Khan[1*]

[1]Department of Pharmacy, Quid I Azam University, Islamabad, Pakistan
[2]Basic Science Department, Khuwaja Fareed University of Engineering and Information Technology, Rahim Yar Khan, Pakistan
[3]Discipline of Clinical Pharmacy, School of Pharmaceutical Sciences, University Sains Malaysia, Penang, Malaysia

Abstract: The aim of the study was to successfully design, formulate and evaluate self-nanoemulsifying drug delivery system (SNEDDS) of poorly aqueous soluble drug viz. flurbiprofen using long (LCT), medium (MCT) and short chain triglycerides (SCT). The SNEDDS are thermodynamically stable lipid based drug delivery systems which consist of mixture of oil, surfactant and co-surfactant. Upon aqueous dilution, this mixture produces nano-emulsion spontaneously on slight agitation. The excipients intended to be used were screened for their potential to dissolve the drug and to form clear dispersion upon aqueous dilution. Labrafil M 1944 CS, capryol-90 and triacetin were selected as long, medium and short chain triglycerides, respectively, as lipids while tween-80 and polyethylene glycol-400 (PEG-400)/ethanol (3:1 ratio) were selected as surfactant and co-surfactant, respectively. The excipients were studied at every possible combination ratios using pseudo-ternary diagram. The LCT, MCT and SCT-SNEDDS were optimized using thermodynamic studies, percentage transmittance value, viscosity, refractive index (RI), electrical conductivity, globule size analysis and *in-vitro* drug release studies. The drug release profiles of optimized SNEDDS were then compared with market product at different pH mediums. The LCT-SNEDDS was considered to be superior for enhancement of the drug bioavailability when compared with other SNEDDS formulations and market product.

Keywords: Self-nanoemulsifying drug delivery system, Flurbiprofen, Triglycerides, bioavailability enhancement.

INTRODUCTION

Drugs could be administered to the body through a number of means but oral route is considered the most appropriate and convenient because of the advantages it has got (Shahiwala, 2011). It is estimated that approximately 60% of the marketed drugs are administered orally (Masaoka *et al.*, 2006). SNEDDS are isotropic and thermodynamically stable mixture of lipid, surfactant, co-surfactant and drug which on mild agitation in aqueous media forms nano-emulsion with droplet size ranging from 20-200 nm (Basalious *et al.*, 2010).

Flurbiprofen is a non-steroidal anti-inflammatory drug (NSAID) and is a propionic acid derivative, prescribed majorly for pain management (Panusa *et al.*, 2007). Flurbiprofen display poor aqueous solubility that is about 5-10µg/ml (Anderson and Conradi, 1985). It shows weakly acidic properties having pKa value of 4.2, is highly lipophilic with short half-life of about 3.3-3.5 hours (Greenblatt, 1985). The absorption of the flurbiprofen via oral route is dependent on the release of the drug from a dosage form in the GIT. There fore, rapid onset of action could be achieved by modifying the dissolution rate of the drug (Otagiri *et al.*, 1983).

The basic aim of the study was to enhance and compare the dissolution profile of a poorly water soluble drug by formulating it in the form of SNEDDS using long, medium and short chain triglycerides which may lead to enhanced solubility and bioavailability.

**Corresponding author:* e-mail: gmkhan@qau.edu.pk

MATERIALS AND METHODS

Materials

Capryol-90 (propylene glycol monocaprylate), Labrafac™ Lipophile WL 1349 (caprylic/capric triglycerides), Labrafil® M 1944 CS (oleoyl polyoxyl-6 glycerides), Labrasol® (caprylocaproyl polyoxyl-8 glycerides) were supplied gratis by Gattefosse Co. (Saint Priest, France). Coconut oil and soybean oil was purchased from local market. Ethanol, Propylene glycol (PG), Tween®20 (polysorbate 20) and Tween®80 (polysorbate 80) were purchased from Sigma Aldrich (Saint Louis, USA). Flurbiprofen was generously provided by Global Pharmaceuticals (Islamabad, Pakistan). Glycerol was purchased from BDH Chemicals (Poole, England). Polyethylene glycol-400 (PEG-400) was generously gifted by Bio-Labs Pharmaceuticals (Islamabad, Pakistan). Triacetin (glyceryl triacetate) was purchased from Alfa Aesar (Karlsruhe, Germany).

Establishment of pseudo–ternary phase diagram

The pseudo-ternary phase diagrams were established in the absence of the drug to check the phase behavior of SNEDDS over the possible concentration range of selected oils, surfactant and co-surfactant. The ratio of oil: S_{mix}(surfactant/co-surfactant) was varied from 1:9 to 9:1. The K_m (surfactant:co-surfactant) ratios 1:1, 1:2, 2:1 were also assessed for 27 formulations.

Preparation of SNEDDS

The drug loaded SNEDDS were prepared by a method as previously reported (Quan *et al.*, 2012).

Thermodynamic stability studies

After successful formulation, the SNEDDS were then subjected to several thermodynamic tests. Effect of variation in temperature on the stability of emulsion was assessed through these tests to identify the unstable, metastable or biphasic SNEDDS (Bali *et al.*, 2011).

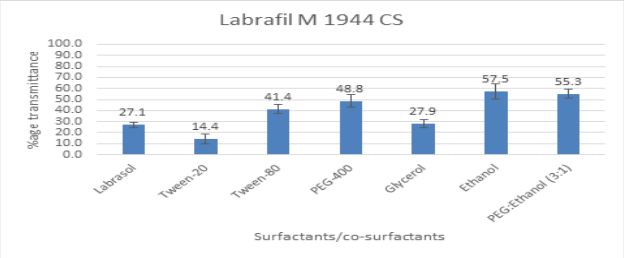

Fig. 1: mean percentage transmittance values of LCT dispersions with surfactants and co-surfactants ±S.D.

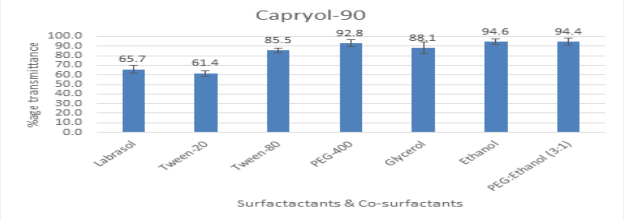

Fig. 2: mean percentage transmittance values of MCT dispersions with surfactants and co-surfactants ±S.D.

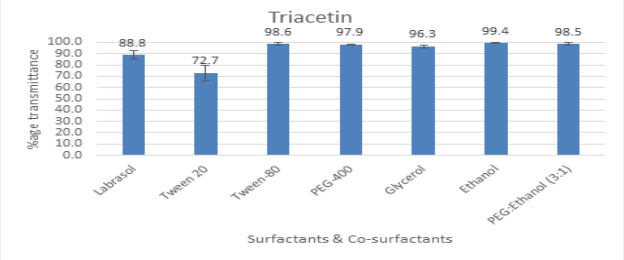

Fig. 3: Graphi mean percentage transmittance values of SCT dispersions with surfactants and co-surfactants ±S.D.

Centrifugation test

The SNEDDS formulations were subjected to centrifugation at 5000 rpm for almost 30 min using centrifugation machine (Hermle labortechnik, Z-206A, Germany).

Heating and cooling cycle

The stable formulations were subjected to six heating and cooling cycles in which samples were placed at 4°C and 45°C for 48 h using constant climate chamber (Memmert, HPP 260, Germany).

Freeze thaw cycle

The stable SNEDDS were then subjected to three freeze thaw cycles by keeping SNEDDS at temperatures ranging from -21°C to 25°C, for not less than 48 h by using Biomedical freezer (Panasonic, MDF-137, Japan) and Pharmaceutical refrigerator (Panasonic, MPR-161D H, Japan).

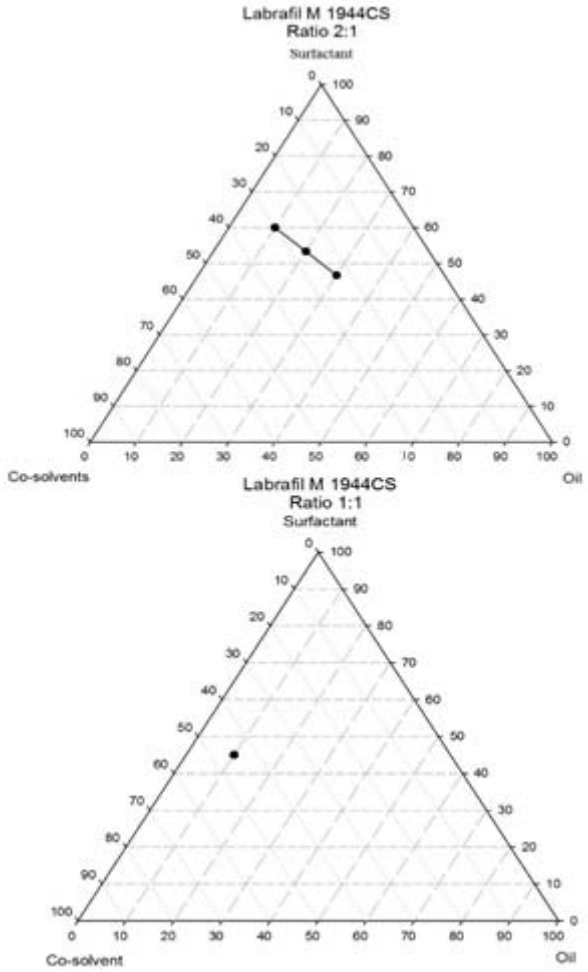

Fig. 4: Pseudo-ternary diagram of LCT-SNEDDS with 2:1 and 1:1 K_m ratio.

Percentage transmittance

The percentage transmittance of the selected LCT, MCT and SCT-SNEDDS were then evaluated for clarity (Furlanetto *et al.*, 2011).

Determination of cloud point temperature

The SNEDDS were diluted with distilled water in conical flask in 1:250 ratio and placed in water bath. The temperature was increased gradually, visually observed for turbid appearance and drop in percent transmittance was evaluated spectrophotometrically (Elnaggar *et al.*, 2009).

Viscosity

The viscosity of undiluted SNEDDS were established by Brookfield viscometer (Brookfield engineering laboratories, LVDV-I prime, USA) at 25 ±0.5°C and rotation speed was maintained at 20 rpm. The spindle used was of 50 mm diameter (Gupta *et al.*, 2011).

Refractive index (RI)
The RI of undiluted SNEDDS was determined by using digital refractometer (Bellingham & Stanley Ltd, RFM 330 plus, Kent, U.K) for confirmation of its isotropic nature (Niederquell *et al.*, 2012).

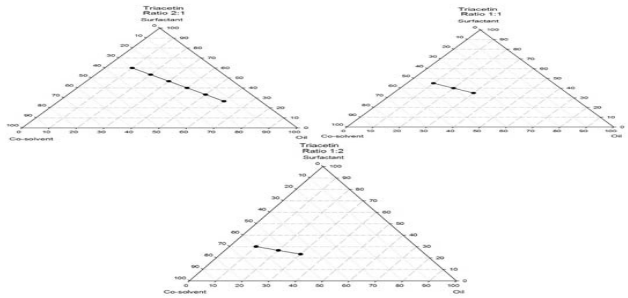

Fig. 5: Pseudo-ternary diagram of MCT-SNEDDS

Electrical conductivity
The digital conductivity meter (Bante instruments, DDS-11AW, Chicago, USA) was used to determine the phase nature of the resultant nano-emulsion formed (Mustafa *et al.*, 2009).

Determination of globule size
Fifty microliter (50µl) of the SNEDDS were added in 50 ml of distilled water in a stoppered conical flask and gently stirred. The resultant emulsion was placed for 2 h and droplet size was then determined by dynamic light scattering (Brookhaven instrument corporation, BI-200SM, New York, USA).

In-vitro release study
In-vitro drug release studies from the selected long, medium, short chain SNEDDS and the marketed tablet dosage form (Froben® 50 mg) were performed in 900 ml release medium (pH 1.2, 4.5 and 6.8) based on USP 24 dissolution apparatus II (Erweka, DT-820, Heusensatamm, Germany) at 37°C with rotation speed of 100 rpm, using the dialysis bag method.

STATISTICAL ANALYSIS

The drug release data was interpreted by one way ANOVA test and significant difference between groups was then evaluated by applying Tukey-Kramer multi-comparison test using Microsoft Excel 2013. Significance level selected was $p<0.001$. Results were repeated in triplicate and expressed as mean ±S.D.

RESULTS

Establishment of pseudo-ternary phase diagram
The dispersion obtained after dilution could form a visually stable clear/transparent nano-emulsion or slightly turbid/milky emulsion which may or may not tend to separate immediately after formation as shown in figs. 4 and 5.The pseudo-ternary diagram represents simple visual representation of the nano-emulsion region when certain concentration range of excipients are mixed to form the SNEDDS. Fig 4 and 5 represent the pseudo-ternary diagrams of all the possible combinations of excipients that yielded nano-emulsions. So, the formulations with K_m ratio 2:1 were selected for further testing and composition is shown in table 1.The pseudo-ternary phase studies were also accomplished in drug loaded SNEDDS.

Thermodynamic studies
The kinetic stability of LCT, MCT and SCT-SNEDDS were established by thermodynamic studies which included centrifugation, heating-cooling and freeze thaw tests.

Percentage transmittance
The clarity of the resultant nano-emulsion was established by measuring transparency in terms of the percentage transmittance. The appearances of the selected three formulations are shown in fig. 1-3.

Viscosity
For physical characterization and stability of the SNEDDS, viscosity of optimized SNEDDS was determined. The mean viscosity of LCT_2, MCT_3 and SCT_4-SNEDDS were 158.8±4.4, 145.9±3.2 and 112.8 ±2.3 cP, respectively.

Refractive index (RI)
The mean refractive index values of long, medium and short chain triglycerides were 1.468, 1.436 and 1.429, respectively. The RI of undiluted LCT_2, MCT_3 and SCT_4-SNEDDS were 1.471, 1.468 and 1.466, respectively.

Electrical conductivity
The electrical conductivity of diluted LCT_2, MCT_3 and SCT_4-SNEDDS were 241, 219 and 187 µS, respectively, which indicated that the resultant emulsions were o/w in nature.

Determination of globule size
The globule size of the resulting nano-emulsion is important for the stability and fate of nano-emulsion. The droplet size of LCT_2, MCT_3 and SCT_4-SNEDDS were 66.94, 127.58 and 189.67 nm, respectively. The droplet size of all nano-emulsions was less than 200 nm.

In-vitro release study
The *In-vitro* drug release study from optimized LCT_2, MCT_3, SCT_4-SNEDDS and the market product of flurbiprofen was established in the dissolution mediums of pH 1.2, 4.5 and 6.8 (figs. 6-8).

STATISTICAL ANALYSIS

Statistical investigations like one way ANOVA followed by Tukey-Kramer test revealed that there was no significant difference in drug release from LCT_2, MCT_3

Table 1: LCT, MCT and SCT-SNEDDS with K_m ratio (2:1)

Long Chain Triglycerides	Labrafil M 1944CS	Tween-80	Co-surfactant	
			PEG-400	Ethanol
LCT_1	10	60.00	22.50	7.50
LCT_2	20	53.33	20.00	6.67
LCT_3	30	46.67	17.50	5.83
Medium Chain Triglycerides	Capryol-90	Tween-80	Co-surfactant	
			PEG-400	Ethanol
MCT_1	10	60.00	22.50	7.50
MCT_2	20	53.33	20.00	6.67
MCT_3	30	46.67	17.50	5.83
Short Chain Triglycerides (SCT)	Triacetin	Tween-80	Co-surfactants	
			PEG-400	Ethanol
SCT_1	10	60.00	22.50	7.50
SCT_2	20	53.33	20.00	6.67
SCT4	40	40.00	15.00	5.00

and SCT_4-SNEDDS ($p>0.001$) in pH 6.8 medium, however, the drug release data from LCT_2-SNEDDS differed significantly than that of the market product ($p<0.001$). The other two types of SNEDDS released drug with no significant difference as compared to the market product. The drug release from all types of SNEDDS differ significantly from that of the market product in pH medium 4.5 and 1.2. The SNEDDS were able to enhance the drug release significantly as compared to that of the market product. There was also significant difference in the release values between LCT_2, MCT_3 and SCT_4-SNEDDS in pH 4.5 and 1.2 mediums which indicated that LCT_2-SNEDDS expressed good results at lower pH mediums as compared to other types of the SNEDDS.

DISCUSSION

The concept of SNEDDS is an emerging field in the pharmaceutical research and development area to address the solubility and dissolution related issues of the drugs. It has been proved that the oral bioavailability of many drugs with low aqueous solubility was improved remarkably when formulated in the form of SNEDDS (Gursoy and Benita, 2004).

Non-ionic surfactants were considered for use in present study because of their non-toxicity(Constantinides, 1995). It was explained that there was an increase in the bioavailability of digoxin when administered with tween-80 (Zhang *et al.*, 2008) which could serve as an additional character to enhance bioavailability of the drug, apart from its role as surfactant. In our study tween-80 displayed higher percentage transmittance value as compared to the other surfactants. In a previous study, tween-80 was shown to have more percentage transmittance than Labrasol (Date and Nagarsenker, 2007). Use of alcohols solely as co-surfactant could result in the precipitation of drug when subjected to aqueous dilution, probably due to its partitioning in the aqueous phase and evaporation during storage (Lawrence and Rees, 2000).

Wider nano-emulsion region was obtained when surfactant in higher ratio was used i.e. S_{mix} (2:1) ratio. This finding correlates with a study where wider nano-emulsion region was obtained when S_{mix} was 2:1 and same ratio was used for the formulation of SNEDDS (Miao *et al.*, 2014). It might be possible that the increased concentration of surfactant at the interface leads to its enhanced adsorption and drop in the interfacial tension, resulting in the formation of nano-emulsion (Anton and Vandamme, 2009). No significant effect of the drug on nano-emulsion region was observed in present study which was in line as observed previously (Kim *et al.*, 2012). The formulations having higher oil concentration showed phase separation which might be due to coalescence of oil droplets as explained previously (Fahmy *et al.*, 2015). Irreversible phase separation of the SNEDDS could occur when the temperature crosses 37°C limit. The might be due to the separation of surfactant because of dehydration of the polyethylene oxide part of the surfactant. If the cloud point temperature of the SNEDDS is low, there is possibility of phase separation which could raise questions on stability of the SNEDDS (Zhang *et al.*, 2008). It was observed in our studies that increase in the concentration of oil leads to increase in droplet size. Similar results were reported where increase in oil concentration was shown to increase the droplet size (Kim *et al.*, 2013). It was noted that drug release from SNEDDS were decreased with decrease in the pH of the medium but still significantly higher than the marketed product. (Li and Zhao, 2003). It was also noticed that the LCT_2-SNEDDS which was more efficient in release of the drug have 10% less oil concentration as compared to MCT_3-SNEDDS and 20% less than SCT_4-SNEDDS. similar finding were reported in a study where drug release was enhanced in formulations having 10% less oil concentration as compared to others (Wu *et al.*, 2006).

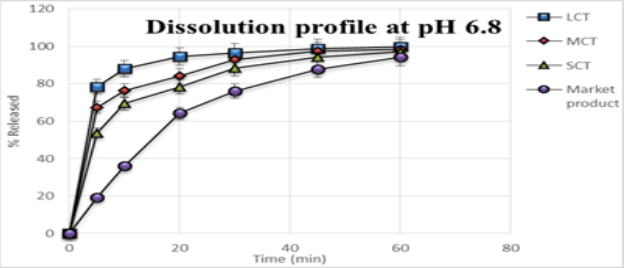

Fig. 6: The drug release profiles in pH 6.8 medium.

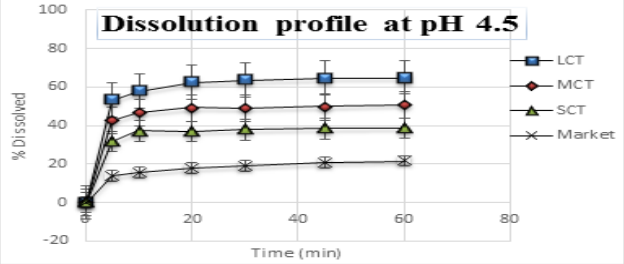

Fig. 7: The drug release profiles in pH 4.5 medium.

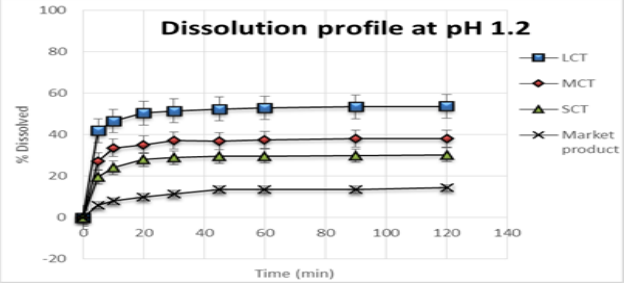

Fig. 8: The drug release profiles in pH 1.2 medium.

Long chain triglycerides were used successfully in the formulation of SNEDDS (Gursoy and Benita, 2004). In this connection LCT-SNEDDS have potential to enhance the systemic availability of the drugs with low aqueous solubility and high first pass metabolism.

CONCLUSION

The present study was designed to successfully formulate, evaluate and compare the SNEDDS of flurbiprofen using LCT, MCT and SCT as oil options. The SNEDDS were optimized based on thermodynamic stability, higher cloud point temperature, low viscosity, isotropic in nature, better percentage transmittance, small droplet size and enhanced dissolution rate. The SNEDDS having LCT were considered to be most efficient in enhancing the release of the drug.

REFERENCES

Anderson BD and Conradi RA (1985). Predictive relationships in the water solubility of salts of a nonsteroidal anti-inflammatory drug. *J. Pharm. Sci.*, **74**: 815-820.

Anton N and Vandamme TF (2009). The universality of low-energy nano-emulsification. *Int. J. Pharm.*, 377: 142-147.

Bali V, Ali M and Ali J (2011). Nanocarrier for the enhanced bioavailability of a cardiovascular agent: *In vitro*, pharmacodynamic, pharmacokinetic and stability assessment. *Int. J. Pharm.*, **403**: 46-56.

Basalious EB, Shawky N and Badr-Eldin SM (2010). SNEDDS containing bioenhancers for improvement of dissolution and oral absorption of lacidipine. I: Development and optimization. *Int. J. Pharm.,* **391**: 203-211.

Constantinides P (1995). Lipid Microemulsions for Improving Drug Dissolution and Oral Absorption: Physical and Biopharmaceutical Aspects. *Pharm. Res.*, **12**: 1561-1572.

Elnaggar YSR, El-Massik MA and Abdallah OY (2009). Self-nanoemulsifying drug delivery systems of tamoxifen citrate: Design and optimization. *Int. J. Pharm.*, **380**: 133-141.

Fahmy U, Ahmed OA and Hosny K (2015). Development and evaluation of avanafil self-nanoemulsifying drug delivery system with rapid onset of action and enhanced bioavailability. *AAPS Pharm. Sci. Tech.*, **16**: 53-58.

Furlanetto S, Cirri M, Piepel G, Mennini N and Mura P (2011). Mixture experiment methods in the development and optimization of microemulsion formulations. *J. Pharm. Biomed. Anal.*, **55**: 610-617.

Greenblatt DJ (1985). Elimination half-life of drugs: value and limitations. *Annu. Rev. Med.*, **36**: 421-427.

Gupta S, Chavhan S and Sawant KK (2011). Self-nanoemulsifying drug delivery system for adefovir dipivoxil: Design, characterization, *in vitro* and *ex vivo* evaluation. *Colloid Surface A*, **392**: 145-155.

Gursoy RN and Benita S (2004). Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomed. Pharmacother.*, **58**: 173-182.

Kim DW, Kang JH, Oh DH, Yong CS and Choi H-G (2012). Development of novel flurbiprofen-loaded solid self-microemulsifying drug delivery system using gelatin as solid carrier. *J. Microencapsul.*, **29**: 323-330.

Kim GG, Poudel BK, Marasini N, Lee DW, Hiep TT, Yang KY, Kim JO, Yong CS and Choi H-G (2013). Enhancement of oral bioavailability of fenofibrate by solid self-microemulsifying drug delivery systems. *Drug. Dev. Ind. Pharm.*, **39**: 1431-1438.

Li P and Zhao L (2003). Solubilization of flurbiprofen in pH-surfactant solutions. *J. Pharm. Sci.*, **92**: 951-956.

Masaoka Y, Tanaka Y, Kataoka M, Sakuma S and Yamashita S (2006). Site of drug absorption after oral administration: Assessment of membrane permeability and luminal concentration of drugs in each segment of gastrointestinal tract. *Eur. J. Pharm. Sci.*, **29**: 240-250.

Mustafa G, Khan ZI, Bansal T and Talegaonkar S (2009). Preparation and Characterization of Oil in Water Nano-

Reservoir Systems for Improved Oral Delivery of Atorvastatin. *Curr. Nanosci.,* **5**: 428-440.

Niederquell A, Völker AC and Kuentz M (2012). Introduction of diffusing wave spectroscopy to study self-emulsifying drug delivery systems with respect to liquid filling of capsules. *Int. J. Pharm.,* **426**: 144-152.

Panusa A, Multari G, Incarnato G and Gagliardi L (2007). High-performance liquid chromatography analysis of anti-inflammatory pharmaceuticals with ultraviolet and electrospray-mass spectrometry detection in suspected counterfeit homeopathic medicinal products. *J. Pharm. Biomed. Anal.,* **43**: 1221-1227.

Quan Q, Kim D-W, Marasini N, Kim DH, Kim JK, Kim JO, Yong CS and Choi H-G (2012). Physicochemical characterization and *in vivo* evaluation of solid self-nanoemulsifying drug delivery system for oral administration of docetaxel. *J. Microencapsul.,* **30**: 307-314.

Shahiwala A (2011). Formulation approaches in enhancement of patient compliance to oral drug therapy. *Expert. Opin. Drug. Deliv.,* **8**: 1521-1529.

Wu W, Wang Y and Que L (2006). Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. *Eur. J. Pharm. Biopharm.,* **63**: 288-294.Zhang P, Liu Y, Feng N and Xu J (2008). Preparation and evaluation of self-microemulsifying drug delivery system of oridonin. *Int. J. Pharm.,* **355**: 269-272