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Self nano emulsifying solid of clopidogrel - developement, characterization, evaluation and effect on bioavailability

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ABSTRACT

The aim of the present study is development and characterization of self-nanoemulsifying drug delivery systems (SNEDDSs) to improve the rate of dissolution of poorly water soluble anti-platelet agent clopidogrel bisulfate. The solubility was estimated in various vehicles to select proper components and compositions. Cinnamon oil (as oil), Tween 80 (as surfactant), polyethylene glycol 400 (as co-surfactant) and Water is used to construct pseudo-ternary phase diagrams. Stability, dispersibility and robustness to dilution were performed to optimize formulations by using phase diagram. Formulations were prepared with different composed of cinnamon oil, Tween 80 and PEG 400 (S_{mix}) ratios. The globule size of the optimized system was less than 200 nm which could be an accepted nanoemulsion size range. The selected formulation F 7 SNEEDS of z-average size was 140.8 nm and zeta potential was -28.6. *In vitro* drug release studies showed significantly enhanced rate of dissolution of F 7 SNEDDS when compared to the marketed formulation.

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INTRODUCTION

Newly developed drug candidates (almost 40%) are practically water insoluble drugs (PWSD). These API's poses several problems while developing dosage forms and water insolubility implicates in low bioavailability (Dokania and Joshi, 2015; Patel *et al.*, 2010). Currently researchers are developing dosage form to increase the solubility in the gastrointestinal tract and support drug exposure after oral administration (Zanchetta *et al.*, 2009). These are the formulations developed to increase the bioavailability of PWSD salt formulation (Serajuddin, 2007), micronization (Vandana *et al.*, 2014) and inclusion in cyclodextrins (Wu *et al.*, 2006), encapsulation in micro/nanoparticles (Zanchetta *et al.*, 2009), and preparation of solid dispersion (Woo *et al.*, 2007). Lipids based formulations, including self-emulsifying formulations, are the most promising technique for PWSD and enhance the oral absorption drugs (Liu *et al.*, 2007). SNEDDS are defined as the isotropic mixture of oils, solid or liquid surfactants and co-solvent/ co-surfactant that have a unique ability of forming fine oil-in-water (o/w) emulsion upon mild agitation followed by dilution in aqueous media, such as gastrointestinal (GI) fluids (Khan *et al.*, 2015). Clopidogrel selectively inhibits the binding of adenosine diphosphate (ADP) to its platelet receptor and the subsequent ADP-mediated activation of the glycoprotein GPIIb/IIIa complex, thereby inhibiting platelet aggregation. Clopidogrel also inhibits platelet aggregation induced by agonists other than ADP, by blocking the amplification of platelet activation due to the released ADP. However, clopidogrel does not inhibit phosphodiesterase activity. Clopidogrel acts by irreversibly modifying the platelet

ADP receptor. Consequently, platelets exposed to clopidogrel are affected for the remainder of their lifespan; the oral bioavailability of clopidogrel is less than 50% due to poor water solubility. Hence the aim of this study is to be enhancing the solubility of clopidogrel by formulating into a SNEDDS (Patel *et al.*, 2010).

MATERIALS AND METHODS

Materials

Clopidogrel bisulfate was kindly get as a gift sample, cinnamon oil was purchased from S.D. Fine Chemicals, cold pressed sesame oil, olive oil, cotton seed oil corn oil. Tween 40, Tween 80 was purchased from Sigma Aldrich, span 80 was purchase from loba chemie, PEG 400 was purchased from sigma Aldrich, all other chemicals and materials used were of analytical grade.

Methods

Solubility studies

The solubility of clopidogrel bisulfate was determined in many oils (castor oil, cinnamon oil, sesame oil and sunflower oil), surfactants and co-surfactants (Tween 80, PEG 400, span 20 and Tween 20) an excess amount of 300 mg drug was added to each 2 ml Eppendorf tubes containing 1 ml each vehicles and the things were mixed using vortex mixer (REMI Equipment, Mumbai, India) to form a uniform mixing of drug for more than 10 mins. The tubes were centrifuged at 5000 rpm using (Eppendorf centrifuge 5415 R) for 20 mins. The supernatant was filtered through a 25 mm micron syringe filter. Form the filtered supernatant 0.1 ml was collected and diluted with methanol. The drug was quantified by using UV spectroscopy method (Balakumar *et al.*, 2013).

Selection of oil

The selection of oil was based on the solubility of clopidogrel bisulfate in oils. Higher solubility of drug will shows the high drug loading capacity and potential. In this study, the drug was more soluble in cinnamon oil. So that was chosen as oil phase for formulating the SNEDDS system (Mohd *et al.*, 2015).

Selection of surfactant

The screening of surfactant was based on the ability to solubilize the drug and ability to emulsify the oil phase. The selection of surfactants was determined from solubility studies. To determine their emulsification ability, 20μl of surfactant and 20μl of the selected oil phase was added together thoroughly mixed by using vortex in an Eppendorf tube. From this mixture 25μl of mixture was added to 25 ml distilled water in a standard flask. The formed emulsion were allowed to stand for 2 h and seen for phase separation. Then the transmittance was determined using UV spectrophotometer at 638 nm against distilled water as the blank (Kassem *et al.*, 2016).

Selection of co-surfactant

The screening of co-surfactant was based on both the ability to solubilize the drug in it and their efficacy to improve the nanoemulsification ability of the selected surfactants. The solubility studies same as in the case of oils and surfactants. Then to screen the efficacy of co-surfactant, 40μl of the selected surfactant and 20μl of co-surfactant to be screened and 60μl of selected oil phase were mixed together thoroughly in an Eppendrof tube. From this mixture, 25μl was added to 25 ml of distilled water in a standard flask. The ease of emulsification was checked in a similar fashion as described above (Kassem *et al.*, 2016).

Construction of pseudo-ternary phase diagram

The pseudo ternary phase diagram was constructed without using of clopidogrel bisulfate to identify the self-nanoemulsifying regions and to optimize the percentage of oil surfactant/ co-surfactant (S_{mix}) were mixed in different ratios and was added to oil phase in varying proportions (1:01-1:05). It was then titrated with water until the mixture turned clear. The values were then plotted in TRIDRAW1.0.0.0 software to identify the nanoemulsification region (Raavi *et al.*, 2014).

Preparation of L-SNEDDS

Twelve L-SNEDDS of clopidogrel bisulfate were formulated using oil (15, 20, 25, and 30) and S_{mix} in the ratio of 1:1, 2:1, 3:1. The drug loaded L-SNEDDS were prepared by adding the oil phase, containing accurately weighed quantity (97.875mg) of clopidogrel bisulfate, drop wise into the S_{mix} with constant stirring for 30 mins. It was equilibrated at ambient temperature for 48 h and investigated for signs of turbidity or phase separation. The compositions of prepared L-SNEDDS were represented inTable 1.

Emulsion droplet size and PDI analysis

The globule size of the emulsion determines the rate and extent of drug release. 1ml of the optimized SNEDDS was diluted with 100 fold of distilled water to assess the globule size using zetasizer (nano zs 90). The z-average diameter was derived from cumulated analysis by the auto-measure software (Mohd *et al.*, 2015).

Time for self-nanoemulsification

The time required for self-nanoemulsification of selected formulations was assessed on USP-II disso-

Table 1: Formulation table contains droplet size and PDI of liquid SNEDDS of clopidogrel

Formula	Cinnamc Oil (%)	Tween 80 (%)	PEG 400 (%)	Z-average size (d.nm)	PDI
F1	15	42.50	42.50	403.0	0.571
F2	20	40.00	40.00	160.5	0.669
F3	25	37.50	37.50	191.4	0.278
F4	30	35.00	35.00	181.6	0.663
F5	15	56.66	28.34	194.6	0.554
F6	20	53.33	26.66	94.4	0.491
F7	25	50.00	25.00	140.8	0.369
F8	30	46.66	23.33	229.7	0.349
F9	15	63.75	21.25	248.5	0.446
F10	20	60.00	20.00	196.3	0.531
F11	25	56.25	18.75	177.3	0.316
F12	30	52.50	17.50	281.6	0.422

lution apparatus. Each formulation was drop wise added to 500 ml of distilled water, mild agitation was required to form nanoemulsion. That gentle agitation was provided by standard stainless-steel which was rotated at 50 rpm. Self emulsification time was visually observed and graded (Khan *et al.*, 2015), Table 2.

Table 2: Observation for dispersibility test

S.No	Grade	Dispersibility and appearance	Time of self-emulsification (min)
1	A	Rapidly forming nanoemulsion, having a clear or bluish appearance.	< 1
2		Rapidly forming, slightly less clear nanoemulsion, having a bluish white appearance.	< 2
3	C	Fine milky emulsion.	< 2
4	D	Dull grayish white emulsion having slightly oily appearance that is slow to emulsify.	> 3
5	E	Formulation exhibiting either poor or minimal emulsification with large oil globules present on the surface.	> 3

Stability studies

The stability studies were conducted to determine the changes in *in-vitro* drug release studies, drug content, emulsion droplet size and PDI on storage. This stability studies were done according to the ICH guidelines by storing the optimized SNEDDS for 3 months. After that period of 3 months the samples collected and analyzed for the previously mentioned studies (Mohd *et al.*, 2015).

Preparation of S-SNEDDS from L-SNEDDS

The one of the simplest technique to convert L-SNEDDS to S-SNEDDS is freeze drier. Twenty grams of L-SMEF was added to 500 ml distilled water and stirred (100rpm) for 10 min, to form homogeneous fine emulsion. Further, 10g of Aerosil200 was added to the prepared emulsion and mixed by stirring at 100rpm for 10min.Obtained emulsion was deep freeze (Thermo scientific) for 24 h. The Aerosil suspension thus obtained was freeze dried to remove water by sublimation methods. No lyoprotectant for freeze drying process was used as Aerosil itself had inherent properties of lyoprotectant. The lyophilizer (MINI LYODEL, DELVAC PUMPS) was operated at condenser temperature -40°C and pressure below 15 Pascal (Singh *et al.*, 2013).

Solid state characterization prepared powder SNEDDS

The morphological characterization of clopidogrel bisulfate loaded SNEDDS was observed by SEM. The micrometer size photographs were taken at an excitation voltage of 20kV.

In vitro drug release studies

Marketed tablet (75 mg dose), dose equivalent amount of clopidogrel loaded S-SNEDDS were placed in a USPBI dissolution test apparatus (LABINDIA, Mumbai, India). The dissolution test was performed using 900 ml of acidic buffer pH 2.0 as a dissolution medium. Temperature and speed of the basket were adjust to $37\pm0.5^{\circ}$C and 50 rpm respectively, at predetermined time intervals (0, 5, 10, 15, 30, 45 and 60 min) an aliquot (5 ml) of the samples was collected and filtered through a membrane filter at each time 5 ml of fresh medium was replaced (Jassim and Hussein, 2017).

RESULTS AND DISCUSSION

Selection of oil

Solubility of clopidogrel in oils, surfactants and co-surfactants determination was the important principle for the selection of components for SNEDDS formulation. The solubility of clopidogrel in different oils was shown in the Figure 1. Among all these selected oils (castor oil, cinnamon oil, sesame oil and sunflower oil) in these cinnamon oil shows higher solubility for clopidogrel so this was selected as oil phase for SNEDDS formulation.

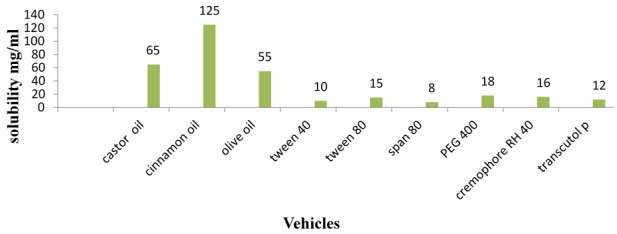

Figure 1: Solubility of clopidogrel in different vehicles

Selection of surfactant

Non-ionic surfactants were most preferred for oral ingestion and this was considered as safer than the ionic surfactants. These surfactants were screened based on the higher solubility of clopidogrel and also ability to emulsify the selected oil. Tween 80 showed highest solubilization capacity of clopidogrel Figure 1 so Tween-80 there chosen for further investigations.

Selection of co-surfactant

The PEG 400 shows good solubility of clopidogrel Figure 1 and also it form nanoemulsification efficiency of the selected surfactant. PEG 400 was selected as the co-surfactant for further investigations.

Ternary phase diagram

The ternary phase diagram were constructed to identify the nanoemulsion region and constructed to identify the concentration selected oil, surfactant, co-surfactant. Phase diagram was constructed in the absence of clopidogrel. This phase diagram plays important role in studying the phase behavior of the formed nanoemulsion (Parmar *et al.*, 2011; Constantinides, 1995)Figure 2.

Optimization of L-SNEDDS

The amount of oil, surfactant, co-surfactant for the further formulation of clopidogrel SNEDDS was optimized on the basis of Z-average size and PDI. The results are shown in Table 1. All the formulation Z-average size was from 90 d.nm – 281.6 d.nm.

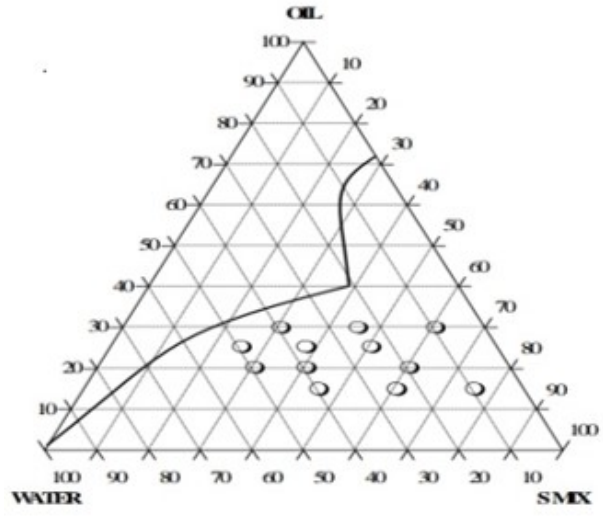

Figure 2: Ternary phase diagram of the selected system (cinnamon oil, Tween 80, and PEG 400)

Emulsion droplet size analysis

Droplet size of the emulsion defined the rate and extent of rug release. The z-average size of the nanoemulsion was determined by 100 times dilution with distilled water. Z-average size was determined to be 140.8 d.nm with PDI value of 0.369Figure 3 shows the narrow size distribution.

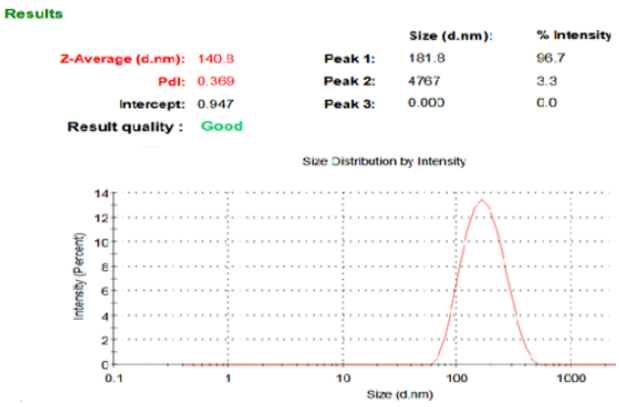

Figure 3: Z- average size (d.nm) of liquid SNEDDS of clopidogrel

Stability studies

Stability studies conducted to identify the temperature sensitivity of L-SNEDDS emulsion droplet size and PDI. The selected Liquid SNEDDS formulation F 7 were kept for stability testing at 30° ± 2° C / 65 ± 5% RH. There has no significant change in the emulsion droplet size and PDITable 3.

Time for self-nanoemulsification

SNEDDS formulation should have ability to disperse completely and quickly when subjected to dilution under mild agitation. Formulations F 3,F 4, F 7, F 8,

Table 3: F 7 SNEDDS stability study results

Time in days	z-average size (d.nm)	PDI
0	140.8	0.369
30	146.0	0.372
60	148.6	0.380
90	151.0	0.382

F 11, F 12, shows very less emulsification time (< 1 min) when compared to others Table 4.

Table 4: Self emulsification results

Formulation code	Self emulsification time (sec)
F 1	95 ± 3.5
F 2	97 ± 4.2
F 3	48 ± 3.2
F 4	54 ± 2.8
F 5	75 ± 4.6
F 6	80 ± 3.9
F 7	41 ± 2.6
F 8	57 ± 2.9
F 9	79 ± 4.0
F 10	82 ± 4.5
F 11	52 ± 3.1
F 12	60 ± 3.0

Characterization of solid SNEDDS

Morphological analysis solid SNEDDS (SEM)

The scanning electron microscopy picture of solid SNEDDS formulations were presented inFigure 4appeared as smooth surfaced, irregular shaped crystals.

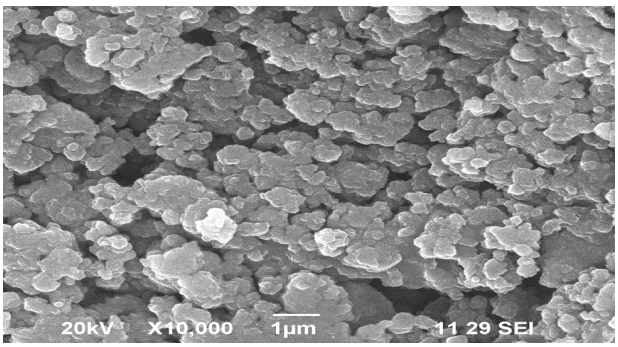

Figure 4: SEM picture of solid SNEDDS formulation (liquid SNEDDS F 7)

In vitro drug release studies

The dissolution profile of solid SNEDDS and marketed formulations were compared, using acetate buffer pH 2.0 as a dissolution medium. Marketed formulation shows 50% of the drug released within 20 mins. The solid SNEDDS shows 75% of drug released within 15 mins. This result indicates that the spontaneous nano emulsion was formed and drug has been released Figure 5.

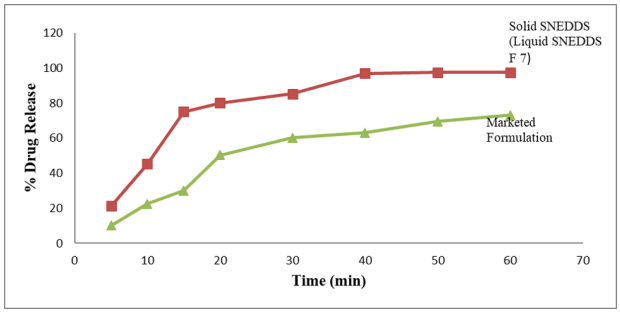

Figure 5: In vitro drug release in acetic buffer (pH2.0)

CONCLUSION

The present study concludes that the formulation of poorly water soluble drug clopidogrel in SNEDDS is useful for enhancing the *invitro* dissolution rate enhancement. The solid SNEDDS formulation F 7 shows good nanosize droplets, zeta potential and PDI values. Freeze dried F 7 formulation with Aerosil 200 as adsorbent shows 0.5 fold increased rate of dissolution when compare to marketed clopidogrel formulation.

REFERENCES

Balakumar, K., Raghavan, C. V., Selvan, N. T., Prasad, R. H., Abdu, S. 2013. Self nanoemulsifying drug delivery system (SNEDDS) of Rosuvastatin calcium: Design, formulation, bioavailability and pharmacokinetic evaluation. *Colloids and Surfaces B: Biointerfaces*, 112:337–343.

Constantinides, P. P. 1995. Lipid Microemulsions for Improving Drug Dissolution and Oral Absorption: Physical and Biopharmaceutical Aspects. *Pharmaceutical Research*, 12(11):1561–1572.

Dokania, S., Joshi, A. K. 2015. Self-microemulsifying drug delivery system (SMEDDS) – challenges and road ahead. *Drug Delivery*, 22(6):675–690.

Jassim, Z. E., Hussein, A. A. 2017. Dissolution Method Development and Enhancement of Solubility of Clopidogrel Bisulfate. *International Research Journal of Pharmacy*, 8(6):25–29.

Kassem, A. A., Mohsen, A. M., Ahmed, R. S., Essam, T. M. 2016. Self-nanoemulsifying drug delivery system (SNEDDS) with enhanced solubilization of nystatin for treatment of oral candidiasis: Design,

optimization, in vitro and in vivo evaluation. *Journal of Molecular Liquids*, 218:219–232.

Khan, A. W., Kotta, S., Ansari, S. H., Sharma, R. K., Ali, J. 2015. Self-nanoemulsifying drug delivery system (SNEDDS) of the poorly water-soluble grapefruit flavonoid Naringenin: design, characterization, in vitro and in vivo evaluation. *Drug Delivery*, 22(4):552–561.

Liu, X. S., Zhag, Y., Tang, Y. 2007. preparation and in vitro-in vivo evaluation of silybin lipid microspheres. *Asian Journal of Pharmaceutical Sciences*, 2(2007):204–210.

Mohd, A. B., Sanka, K., Bandi, S., Diwan, P. V., Shastri, N. 2015. Solid self-nanoemulsifying drug delivery system (S-SNEDDS) for oral delivery of glimepiride: development and antidiabetic activity in albino rabbits. *Drug Delivery*, 22(4):499–508.

Parmar, N., Singla, N., Amin, S., Kohli, K. 2011. Study of cosurfactant effect on nanoemulsifying area and development of lercanidipine loaded (SNEDDS) self nanoemulsifying drug delivery system. *Colloids and Surfaces B: Biointerfaces*, 86(2):327–338.

Patel, V., Kukadiya, H., Mashru, R., Surti, N., Mandal, S. 2010. Development of microemulsion for solubility enhancement of clopidogrel. *Iranian Journal of Pharmaceutical Research*, 9(4):327–334.

Raavi, S., Subramanian, S., Vuddisa, S. K. 2014. Supersaturated self nanoemulsifying tablets of atorvastatin. *World Journal of Pharmacy and Pharmaceutical Sciences*.

Serajuddin, A. T. M. 2007. Salt formation to improve drug solubility. *Advanced Drug Delivery*, 59(7):603–616.

Singh, S. K., Vuddanda, P. R., Singh, S., Srivastava, A. K. 2013. A comparison between use of spray and freeze drying techniques for preparation of solid self-microemulsifying formulation of valsartan and in vitro and in vivo evaluation. *Biomed Res Int*, pages 1–13.

Vandana, K. R., Raju, Y. P., Chowdary, V. H., Sushma, M., Kumar, N. V. 2014. An overview on in situ micronization technique – An emerging novel concept in advanced drug delivery. *Saudi Pharmaceutical Journal*, 22(4):283–289.

Woo, J. S., Kim, T. S., Park, J. H., Chi, S. C. 2007. Formulation and biopharmaceutical evaluation of silymarin using SMEDDS. *Archives of Pharmacal Research*, 30(1):82–89.

Wu, W., Wang, Y., Que, L. 2006. Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. *European Journal of Pharmaceutics and Biopharmaceutics*, 63(3):288–294.

Zanchetta, B., Teixeira, Z., Melo, B. A. 2009. Retinyl palmitate flexible polymeric nanocapsules: characterization and permeation studies. *Colloids and Surfaces B: Biointerfaces*, 81(1):374–380.