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Enhanced Solubility and Dissolution Rate of Clopidogrel by Nanosuspension: Formulation via High Pressure Homogenization Technique and Optimization Using Box Behnken Design Response Surface Methodology

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ABSTRACT

The present study was aimed to formulate nanosuspensions using high pressure homogenization (HPH), a top down technique for enhancement of dissolution rate and solubility of clopidogrel using Pluronic F127 as stabilizer. Clopidogrel is categorized as a BCS class II agent having oral bioavailability less than 50%. The formulation scheme was generated by Box- Behnken design of response surface methodology. The formulated nanosuspensions were assessed on particle size, polydispersity index, and zeta potential. Three formulations were selected based on different predicted particle size with manipulation of parameters using response optimizer. The selected formulations were checked on percentage of bias in between predicted value and observed value and evaluated based on drug content, drug entrapment efficiency and *in vitro* dissolution study. The formulation was optimized based on the smallest particle size and highest percentage of *in vitro* cumulative drug release. Formulation FF3 was selected as optimized formulation which attributed to the smallest particle size (478.1 nm) and the highest % CDR in both 0.1 N HCl (98.37%) and phosphate buffer (48.67%). The optimized formulation has shown a significant 2 folds enhancement in dissolution rate in 0.1 N HCl and 10 folds improvement in pH 6.8 phosphate buffers with 0.1% w/v Tween 80 compared to pure drug suspension.

INTRODUCTION

The ease of oral administration make it become a more preferential route for patients to consume medication (Kumar and Singh, 2013). However, most of the available active pharmaceutical ingredients are poorly water soluble which have dissolution limitation in gastrointestinal (GI) tract and eventually causing poor oral bioavailability issue that will greatly reduce therapeutic effectiveness of the drug pharmacologically, resulting in compromised patient adherence problem (Shid *et al.*, 2013; Chen *et al.*, 2011). Therefore, the improvement of dissolution rate and saturation solubility is essential to achieve optimum oral bioavailability. Based on the Noyes-Whitney equation, dissolution rate and saturation solubility of poor water soluble drug can be enhanced through size reduction into nano-range due to the increase of interfacial surface area available for wetting (Liu *et al.*, 2010). Hence, in recent years, nanotechnology has become a promising approach for the formulation of poorly water soluble drug (Attari *et al.*, 2015). Nanosuspension is one of the available nanoformulations that are effective to solve the solubility related bioavailability problems (Patel and Agrawal, 2011). It is defined as solid dispersion of active pharmaceutical ingredients in size of smaller than 1 μm in a liquid vehicle with addition of surfactant for stabilization purpose (Yadav and Singh, 2012). Nanosuspension can be prepared by either top-down technique which is more mechanical-effective and scalable or bottom-up technique which is more cost and time-saving in nanoparticles production (Liu *et al.*, 2010).

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High pressure homogenization (HPH) is a method categorised under top down technology by involving the reduction of particle size from larger size to the range of size in nm (Mane *et al*., 2014). HPH was selected in the nanosuspension production of this study due to its superiority in minimised variation between batch to batch after the process parameters are adjusted to optimum which can result in high reproducibility in the production of nanosuspension and its ability to produce nanosuspension in a wide range of concentration (Jagdale *et al*., 2010). In this study, clopidogrel was the selected drug to be prepared by HPH method for the production of nanosuspension. Clopidogrel is an anti-platelet agent which is widely used in prevention of thrombotic event in cardiovascular disease. Upon administered, clopidogrel is rapidly absorbed in upper GI tract, metabolized by cytochrome P450 to its active thiol metabolite, which has a short elimination half-life (1hour). Furthermore, bioavailability of clopidogrel is also predicted to be generally low, which is approximate to 50% according to volume of excreted unchanged drug and metabolite presented in urine and feces. According to the biopharmaceutics classification system (BCS), Clopidogrel is categorized as a class II agent having poor water solubility and high permeability, which is responsible for its poor oral bioavailability (Takagi *et al.*, 2006). Besides that, its highly pH dependent absorption characteristic at acidic pH is another factor of causing poor oral bioavailability as the drug gets precipitated when it reaches small intestine which has basic environment (Raghuvanshi and Pathak, 2014). The poor oral bioavailability and short elimination half-life of clopidogrel leads to the need of higher dose and more frequent dosing in order to achieve the desired therapeutic effect (Shahba *et al.*, 2012). However, extra doses increase the risk of drowsiness, dizziness, hypotension, and aggravation of extrapyramidal symptoms, lupus on skin and internal organs, and jaundice (Teva Pharmaceuticals Europe B.V., 2015; Takagi *et al.*, 2006). Nanosuspension has shown to have greater drug loading compared to other oral dosage form, and thus, lower dose is required to achieve intended therapeutic effect and patient compliance can be improved with the use of this drug delivery system (Yadav and Singh, 2012; Rabinow, 2004). The aim of this work is to formulate the clopidogrel nanosuspension by high pressure homogenization method to improve its solubility and to find out the effect of Drug:stabilizer mass ratio, number of homogenization cycles and pressure on the formulation.

METHODS

Materials

Clopidogrel was obtained as a gift sample from Fleming Labs (India). Pluronic F127 was obtained as a gift sample from BASF (United States). All other reagents used were of analytical grades.

Preparation of Nanosuspension

The nanosuspension of clopidogrel was prepared by HPH method. Clopidogrel (1mg/ml) was added into 70ml of mixed solution of Pluronic F-127 in varied stabilizer:drug mass ratio (20% to 40%w/w) with the assist of magnetic stirrer. The resulting mixture was further mixed using high speed homogeniser (Ultra-Turrax T 25 Digital, IKA, Germany) was applied at a speed of 10,000 rpm for 15 minutes to form pre-suspension. Then, the pre-suspension was forced through a high pressure homogeniser (Microfluidics M110P-20K, Newton, Massachusetts, USA) under pressure applied within range of 800 bars to 1300 bars for 10 cycles then continued with remaining cycles (5-15 cycles) under 1500 bar. Some of the formulations was repeated at which the pre-suspension was forced through the high pressure homogeniser under pressure applied within range of 800 bars to 1300 bars for 10 cycles then continued with the same pressure for further 15 cycles.

Experimental Design

The formulation scheme of clopidogrel nanosuspension was generated using 15 runs, 3 factors, unblocked Box-Behnken design of Response Surface Methodology (RSM) in Minitab software (Minitab® 17.3.1 trial version, Sydney) by varying 3 parameters which included stabilizer:drug mass ratio in range of 20% w/w to 40% w/w, homogenization pressure in range of 800 bar to 1300 bar in the first 10 cycles, and number of remaining homogenization cycle in the range of 5 to 15 cycles applied under 1500 bar as shown in Table 1.

The developed clopidogrel nanosuspensions were subjected for particle size analysis, polydispersity index (PDI), and zeta potential measurement.

Table 1: Formulations of clopidogrel nanosuspension generated using RSM.

Formulation	Stabilizer: Drug Mass Ratio (%)	Mass of Clopidogrel in 70 ml (mg)	Mass of Polymer in 70 ml (mg)	Homogenization Pressure in First 10 Cycles (bar)	Number of Remaining Cycles at 1500 bar
1.	40	70	28	800	10
2.	40	70	28	1050	15
3.	30	70	21	800	15
4.	20	70	14	1050	15
5.	30	70	21	1300	15
6.	40	70	28	1300	10
7.	30	70	21	800	5
8.	30	70	21	1050	10
9.	20	70	14	800	10
10.	20	70	14	1050	5
11.	30	70	21	1300	5
12.	30	70	21	1050	10
13.	20	70	14	1300	10
14.	30	70	21	1050	10
15.	40	70	28	1050	5

Response Analysis and Response Surface Analysis

The result obtained for the 3 responses was entered into the worksheet of RSM and analysed using response analysis. The response that fitted to the model was selected based on the regression coefficient (R^2) value above 80% and p-value below 0.05. Surface plot graphs were generated to determine the interaction effect of any 2 parameters on the selected response.

Selection and Optimization of Formulation

Three formulations were selected based on 3 different predicted particle size with manipulation of parameters by using response optimiser. The produced nanosuspensions of the 3 selected formulations were checked for the percentage of bias based on particle size result obtained with the equation of the following:

$$\text{Percentage of Bias (\%)} = \frac{\text{Predicted value - Observed value}}{\text{Observed value}} x\,100\%$$

The nanosuspensions of the 3 selected formulations were also evaluated on drug content, DEE, and *in vitro* dissolution study in order to find out the most optimized formulation in present study. The optimized formulation was further evaluated based on drug release kinetic study.

Evaluation Test of Nanosuspension

The evaluation tests were carried out to characterize the produced nanosuspension, including particle size and PDI analysis, zeta potential, drug content estimation study, DEE, *in vitro* dissolution study, and drug release kinetic study.

Particle size and PDI analysis

Particle size and PDI of the particles in the nanosuspension was measured by using Malvern Zetasizer (Model ZEN3600, Malvern, UK). A sample of nanosuspension was diluted with distilled water and subjected into disposable sizing cuvette for measurement at temperature of 25°C and scattering angle of 173˚ with setting of dispersant refractive index (RI) at 1.33, material RI at 1.58 and material absorption at 1.000 in triplicates (Chiang *et al.*, 2011; Agarwal and Bajpai, 2014).

Zeta potential measurement

The measurement of zeta potential was carried out using the additional electrode of Malvern Zetasizer that was used for particle size and PDI analysis. A sample of nanosuspension was diluted with distilled water and subjected into disposable sizing cuvette for measurement at temperature of 25°C with setting of dispersant RI at 1.33 and dielectric constant of dispersant at 78.5 in triplicates (Agarwal and Bajpai, 2014).

Drug content estimation study

An amount of 0.2 ml of nanosuspension was dissolved in 20 ml of 0.1 N HCl, filtered, and assayed using UV Spectrophotometer at 253 nm to determine the actual drug content in triplicates. The percentage of actual drug content was calculated based on the equation of the following:

$$\text{Percentage of actual drug content (\%)} = \frac{\text{Actual amount of drug}}{\text{Theoretical amount of drug}} x\,100\%$$

Drug entrapment efficiency (DEE)

An amount of 1.5 ml of freshly prepared nanosuspension was centrifuged at 10,000 rpm with for 30 min by using micro-centrifuge. Then, 0.2 ml of supernatant solution was diluted with 0.1 N HCl and subjected to UV Spectrophotometer to measure the amount of drug which was unincorporated. The DEE is calculated by deducting the amount of free drug present in supernatant from the actual amount of drug present in the formulation. This test was carried out in triplicates. The DEE can be calculated based on the equation of the following:

$$\text{Drug Entrapment Efficiency (DEE \%)} = \frac{\text{Actual amount of drug - Amount of free drug}}{\text{Actual amount of drug}} x\,100\%$$

***In vitro* dissolution study**

USP Type II dissolution apparatus was used to test the dissolution rate of the pure drug suspension of clopidogrel and nanosuspension by paddle method. The dissolution medium used for this study included 0.1 N HCl and pH 6.8 phosphate buffer with 0.1% w/v Tween 80. A volume of dissolution medium was transferred to every vessel of the dissolution apparatus. The stirring rate was set at 50 rpm and the temperature of dissolution media was set at 37 ± 0.5°C. Pure drug suspension and nanosuspension was transferred into one-end tied dialysis bag, then the open end of the bag was tied. The dialysis bag was attached on the paddle of dissolution apparatus. An amount of 5 ml of sample was withdrawn at regular time interval. For 0.1 N HCl, the time intervals for sampling were 5 min, 10min, 15 min, 20 min, 30 min, 40 min, and 60 min while for pH 6.8 phosphate buffer with 0.1% w/v Tween 80, the time intervals for sampling were 5 min, 10 min, 30 min, 50 min, 70 min, 90 min, and 120 min. An amount of 5 ml dissolution medium was added in order to remain the sink condition. Sample was filtered, diluted and subjected to UV Spectrometer to determine the amount of drug released. The procedure was carried out in triplicates.

Drug release kinetic study

The release kinetic study of nanosuspension in 0.1 N HCl and pH 6.8 phosphate buffer with 0.1% w/v Tween 80 was evaluated based on 4 models, including zero order, first order, Higuchi model, and Korsmeyer-Peppas model and graphs were plotted for each model. In zero order, the graph of percentage of cumulative drug release (% CDR) against time of dissolution was plotted. In first order, the graph of log % CDR against time of dissolution was plotted. In Higuchi model, the graph of % CDR against square root of time of dissolution was plotted. In Korsmeyer- Peppas model, the graph of log % CDR against log of time of dissolution was plotted. The best fitted model was selected based on the highest R^2 value. The n value in Korsmeyer-Peppas indicates the type of drug release mechanism. According to a review from Dash *et al*. (2010), n value below 0.5 indicates Fickian diffusion whereas n value greater than 0.5 indicates non-Fickian mechanism of drug release. In condition of n value equal

to 0.89, it indicates Case II transport and when n value greater than 0.89, it indicates super case II transport.

RESULTS AND DISCUSSION

Characterization of Formulated Nanosuspensions

The 15 formulated nanosuspensions were evaluated based on particle size, PDI, and zeta potential by using Malvern Zetasizer. The result of particle size, PDI and zeta potential of formulated nanosuspensions are shown in Table 2.

Table 2: Particle size, PDI and Zeta potential of formulated nanosuspensions

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	764.5 ± 209.1	0.419	-9.52
F2	554.1 ± 98.68	0.580	-8.57
F3	752.0 ± 239.6	0.522	-7.58
F4	509.0 ± 319.2	0.428	-9.81
F5	488.5 ± 271.7	0.660	-8.41
F6	500.4 ± 98.71	0.599	-10.00
F7	801.0 ± 193.3	0.421	-8.13
F8	614.1 ± 197.6	0.529	-8.55
F9	774.6 ± 213	0.431	-10.00
F10	836.6 ± 321.3	0.547	-5.04
F11	588.1 ± 168	0.520	-8.43
F12	583.9 ± 207.4	0.542	-8.12
F13	539.8 ± 240	0.595	-6.92
F14	593.5 ± 214.3	0.526	-9.08
F15	695.9 ± 19.05	0.619	-8.57

Basically, all 15 formulations had particle size within the nanosize range 400 nm to 900 nm. Formulation F5 that produced under 30% w/w stabilizer:drug mass ratio, 1300 bar in first 10 homogenization cycles and 15 cycles for 1500 bar of homogenization pressure had shown the smallest particle size with 488.5 nm. Formulation F7 with 30% w/w stabilizer:drug mass ratio, 800 bar in first 10 homogenization cycles and 5 cycles for 1500 bar of homogenization pressure had shown the greatest particle size with 801 nm. It was found that the increase in homogenization pressure and homogenization cycle resulted in smaller particle size. When there is increment of pressure and cycle in homogenization, it provides greater particles collision and higher shear force in cavitation for fining down the particles (Yadav and Singh, 2012).

According to the PDI study (Table 4.5), most of the formulations obtained value above 0.5, it indicates non-uniformity distribution of particles in nanosuspension. Formulations F1 (0.419), F4 (0.428), F7 (0.421) and F9 (0.431) obtained PDI below 0.5 which is acceptable in term of uniformity of particles distribution. Ideally, PDI value in between 0.1 to 0.25 is required to achieve better physical stability with the enhanced uniformity of particles distribution (Sabeti e*t al*., 2014).Thus, stabilizer type and concentration need to be researched further to develop the appropriate formulation with improved distribution.

Zeta potential analysis was carried out to study surface characteristics of nanosuspension in order to indicate the stability behaviour of each formulation. Generally, a more negative value than -30 mV or more positive value than +30 mV is required to obtain better stability of nanosuspension (Honary and Zahir, 2013). However, all formulations prepared have shown less negative zeta potential which indicates the instability of formulated nanosuspension. Then, in order to enhance the stability, further addition of ionic agent is suggested (Doymus, 2007).

Response Analysis and Response Surface Analysis

Based on the R^2 obtained for each response, it shown that particle size is the most affected response by the change in the parameters. Particle size also is the only response that fitted to the model as the R^2 value (93.15%) had exceeded 90% which indicates the variables are very highly correlated. Both PDI and zeta potential had R^2 below 80% which means that these 2 responses are not well fitted to the model (Table 3) Thus, the response optimizer was manipulated based on particle size only.

An equation was established based on the RSM analysis as stated in Figure 3.16 on particle size:

$$Y = 597.167 - 18.137X_1 - 121.913X_2 - 77.25X_3 + 19.579X_1^2 + 28.079X_2^2 + 32.154X_3 - 7.325X_1X_2 + 46.45X_1X_3 - 12.65X_2X_3$$

where Y indicated particle size; X_1 indicated stabilizer:drug mass ratio; X_2 indicated homogenization pressure in first 10 cycles; X_3 indicated number of remaining homogenization cycle under 1500 bar.

Based on Figure 1, the parameters that significantly affected the particle size of nanosuspension ($p<0.05$) include homogenization pressure in first 10 cycles with p-value of 0.001 and number of remaining homogenization cycle under 1500 bar with p-value of 0.009. stabilizer:drug mass ratio had p-value of 0.372 which indicates this parameter is not significantly affecting the particle size. The surface plot shown in Figure 2 and Figure 3 provided additional evidence to support insignificant effect of stabilizer:drug mass on particle size.

Response Surface Regression: Particle siz versus Stabiliser:D, Pressure (10,

The analysis was done using coded units.

Estimated Regression Coefficients for Particle size

Term	Coef	SE Coef	T	P
Constant	597.167	30.19	19.782	0.000
Stabiliser:Drug ratio / %	-18.137	18.49	-0.981	0.372
Pressure (10 cycle)/bar	-121.913	18.49	-6.595	0.001
No. of cycle at 1500bar	-77.250	18.49	-4.179	0.009
Stabiliser:Drug ratio / %* Stabiliser:Drug ratio / %	19.579	27.21	0.720	0.504
Pressure (10 cycle)/bar* Pressure (10 cycle)/bar	28.079	27.21	1.032	0.349
No. of cycle at 1500bar* No. of cycle at 1500bar	32.154	27.21	1.182	0.290
Stabiliser:Drug ratio / %* Pressure (10 cycle)/bar	-7.325	26.14	-0.280	0.791
Stabiliser:Drug ratio / %* No. of cycle at 1500bar	46.450	26.14	1.777	0.136
Pressure (10 cycle)/bar* No. of cycle at 1500bar	-12.650	26.14	-0.484	0.649

S = 52.2861 PRESS = 212160
R-Sq = 93.15% R-Sq(pred) = 0.00% R-Sq(adj) = 80.82%

Fig. 1: RSM analysis on response surface regression of particle size versus stabilizer:drug mass ratio, homogenization pressure in first 10 cycles, and number of remaining homogenization cycle under 1500 bar

Table 3: Response analysis by using RSM.

Response	Regression Coefficient (R^2) Percentage (%)
Particle Size	93.15
PDI	65.32
Zeta Potential	75.07

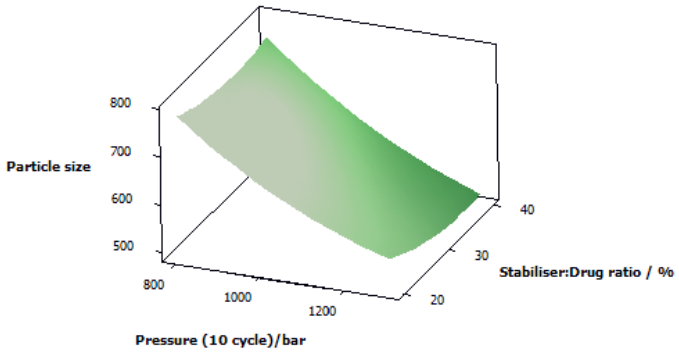

Fig. 2: Surface plot of particle size versus homogenization pressure in first 10 cycles and stabilizer:drug mass ratio.

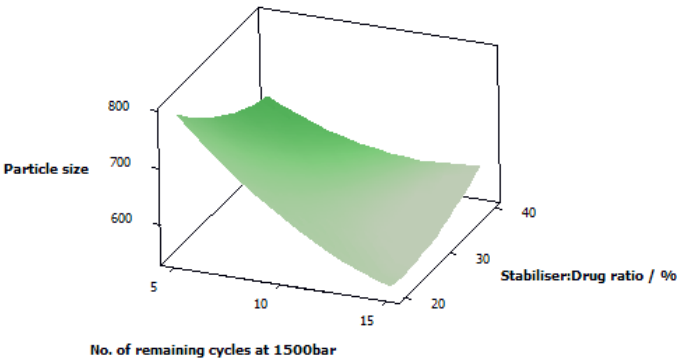

Fig. 3: Surface plot of particle size versus stabilizer:drug mass ratio and number of remaining homogenization cycle under 1500 bar.

Based on Figure 2, the surface plot shows that the increase of homogenization pressure in first 10 cycles able to reduce the particle size of drug proportionally whereas the increase of stabilizer:drug mass ratio didn't shown much effect in particle size reduction. According to Figure 18, the increase of number of remaining homogenization cycle under 1500 bar also able to reduce the particle size proportionally. However, the particle size became slightly bigger with the increase of stabilizer:drug mass ratio instead of giving reduction effect which may due excessive envelop of stabilizer surround the drug particles (Afifi *et al.*, 2015). In Figure 4, it shows that the increase of both homogenization pressure in first 10 cycles and number of remaining homogenization cycle under 1500 bar gave combination effect in the particle size reduction.

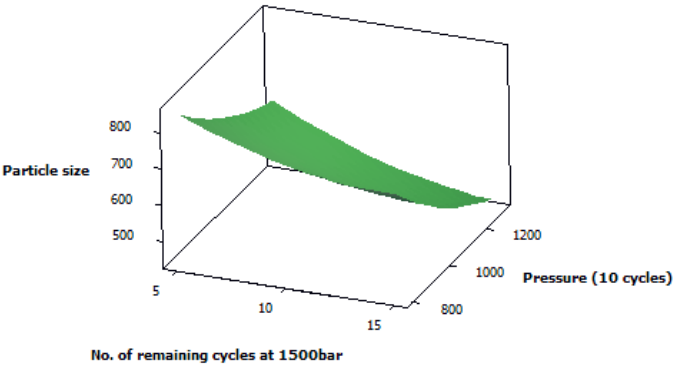

Fig. 4: Surface plot of particle size versus homogenization pressure in first 10 cycles and number of remaining homogenization cycle under 1500 bar.

Optimization of Process and Formulation by RSM

The study was continued with the optimization of the process and formulation parameters by using response optimizer of RSM to select 3 formulations. The selected formulations were evaluated on the basis of drug content, DEE, and *in vitro* dissolution study for selection of the most optimized formulation in present study.

The selection of formulations was based on 3 different predicted particle size in order to check the influence of particle size of drug on the dissolution rate. Thus, the formulation FF3 (440 nm) with smallest predicted particle size was selected and also predicted as the optimized formulation with expectation of better dissolution result due to its smaller particle size. In addition, another 2 formulations, formulation FF1 (701.9 nm) and formulation FF2 (542.8 nm) with bigger predicted particle size were selected for the purpose of comparison. These 3 formulations were prepared in according to the value of parameters shown in the response optimizer and the parameters values were listed in Table 4.

Table 4: Selected formulations based on response optimizer with the respective predicted particle size.

Formulation	Stabilizer: Drug Mass Ratio (%)	Homogenization Pressure in First 10 Cycles (bar)	Number of Remaining Cycles at 1500 bar	Predicted Particle Size (nm)
FF1	25	800	15	701.9
FF2	25	1050	15	542.8
FF3	25	1300	15	440.0

The selected formulations were characterized on particle size, PDI and zeta potential and the results were depicted in Table 5. The observed particle size of selected formulations was compared with the predicted particle size as shown in Table 6. The result shows that the observed values were quite closed to the predicted value as the percentage of bias was low, indicates the prediction of the response optimizer was trustworthy.

Table 5: Particle size, PDI and zeta potential of selected formulations.

Formulation	Particle Size (nm)	PDI	Zeta Potential (mV)
FF1	728.2 ± 248.1	0.456	-9.06
FF2	504.3 ± 213.8	0.625	-5.05
FF3	478.1 ±289.9	0.730	-6.61

Table 6: Comparison of predicted and observed experimental values of particle size the nanosuspension of selected formulations.

Formulation	Predicted Particle Size (nm)	Particle Size (nm)	Percentage of Bias (%)
FF1	701.9	728.2 ± 248.1	-3.61
FF2	542.8	504.3 ± 213.8	7.63
FF3	440.0	478.1 ±289.9	-7.97

Evaluation of Selected Formulations

The prepared formulations were sent for evaluation tests on drug content, DEE, and *in vitro* dissolution study. Drug content estimation study was carried out to determine the percentage of actual drug content in the nanosuspension prepared. Based on the

results listed in Table 7. All selected formulations had very good yield which indicates there was minimum loss of drug in the process of nanosuspension production and most of the drug added was conserved in the formulations. The DEE results of nanosuspension selected were depicted in Table 8. All selected formulations have very high efficiency in drug entrapment.

Table 7: Drug content of nanosuspension in selected formulations.

Formulation	Mean Absorbance	Actual amount of drug (mcg)	Theoretical amount of drug (mcg)	Percentage of actual drug content (%)
FF1	0.548	197.8	200	98.90
FF2	0.544	196.3	200	98.15
FF3	0.542	195.6	200	97.80

Table 8: DEE of nanosuspension in selected formulations.

Formulation	Mean Absorbance	Amount of free drug (mcg)	Actual amount of drug (mcg)	Drug entrapment efficiency (%)
FF1	0.042	1.479	197.8	99.25
FF2	0.060	2.130	196.3	98.91
FF3	0.049	1.732	195.6	99.11

In vitro dissolution study was carried out to evaluate the drug release rate of nanosuspension using dialysis bag. Dissolution study of the pure drug suspension and selected formulations was evaluated in two different dissolution media, includes 0.1 N HCl and pH 6.8 phosphate buffer with 0.1% w/v Tween 80. The % CDR of selected formulations was compared with the pure drug suspension to check for the improvement in drug dissolution rate.

Based on Figure 5, the selected formulations in 0.1 N HCl have shown abrupt increase in the drug release of almost 80% in the initial 10 min while the pure drug suspension, it took 20 min to reach peak drug release (47.51%). By comparing the % CDR of selected formulations with the pure drug suspension, nanosuspensions showed doubling-up effect in drug release compared to pure drug suspension in 0.1 N HCl. According to Figure 6, the selected formulations showed constant increase in drug release in phosphate buffer while for pure drug suspension, it also showed constant increase in drug release but in a slower manner throughout the 120 min dissolution test.

The nanosuspensions were found to have around 10-fold higher % CDR compared to pure drug suspension in phosphate buffer. By referring both Figure 5 and 6, formulation FF2 found to have greater % CDR than FF1 while FF3 had the highest % CDR among the selected formulations attributed to the smallest particle size obtained with formulation FF3. It can be further explained that the finer the drug particles, the greater the surface area which can provide the enhanced wetting on drug particles surface for better dissolution and resulting in improved drug release of nanosuspensions (Liu *et al.*, 2010). Thus, FF3 which had the smallest particle size (478.1 nm) and the highest % CDR in both 0.1 N HCl (98.37%) and phosphate buffer (48.67%) was confirmed as the optimized formulation as predicted.

However, nanosuspensions in 0.1 N HCl showed greater % CDR than phosphate buffer with 0.1% w/v Tween 80. Although Tween 80 was added in phosphate buffer to enhance solubility of clopidogrel in basic environment, the drug still showed comparatively better solubility and faster dissolution rate in 0.1 N HCl. This behaviour indicates pH-dependent solubility of clopidogrel in acidic environment.

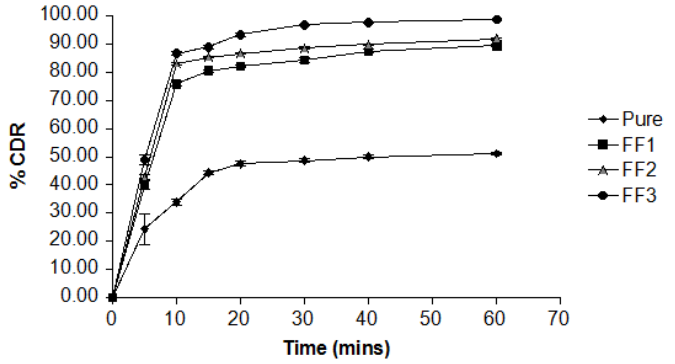

Fig. 5: Graph of percentage of CDR versus time for pure drug suspension, FF1, FF2 and FF3 nanosuspensions in 0.1 N HCl

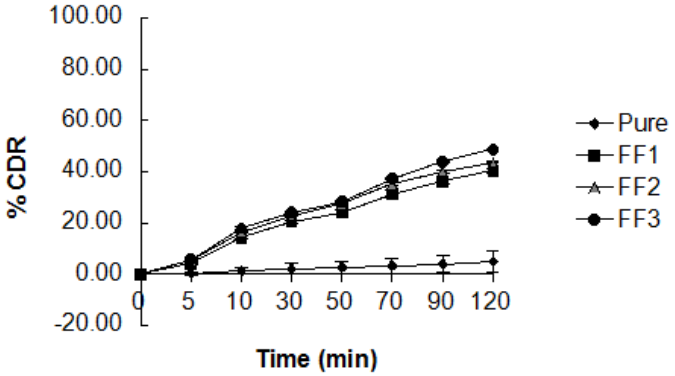

Fig. 6: Graph of percentage of CDR versus time for pure drug suspension, FF1, FF2 and FF3 nanosuspensions in pH 6.8 phosphate buffer with 0.1% w/v Tween 80.

Drug Release Kinetic Study of the Optimized Formulation

Drug release kinetic study was carried out to describe the release behavior of nanosuspension of the optimized formulation (FF3) based on the dissolution profile in 0.1 N HCl and pH 6.8 phosphate buffer. The release kinetic study was evaluated according to the model parameters. The model of release kinetic study that had been carried out include zero order, first order, Higuchi model, and Korsmeyer-Peppas model of drug release. Graphs of release kinetic study were plotted for each model and showed in Figure 7, 8, 9, and 10 for 0.1 N HCl while Figure 11, 12, 13 and 14 were showed for phosphate buffer.

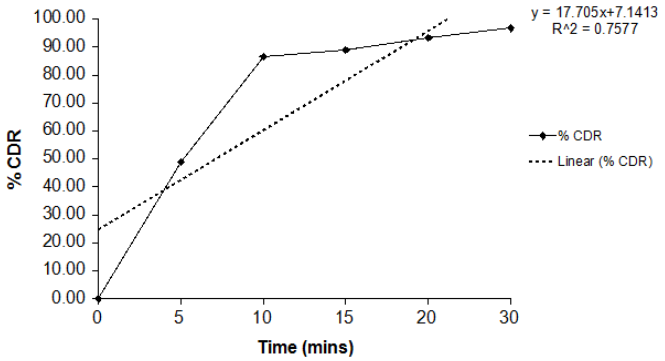

Fig. 7: Zero order release of clopidogrel nanosuspension formulation FF3 in 0.1 N HCl.

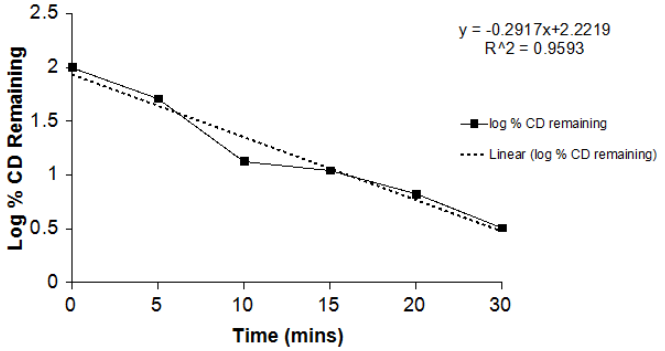

Fig. 8: First order release of clopidogrel nanosuspension formulation FF3 in 0.1 N HCl.

In 0.1 N HCl, the R^2 value obtained was 0.7577 in zero order (Figure 22), 0.9593 in first order (Figure 8), 0.7577 in Higuchi model (Figure 9), and 0.6488 in Korsmeyer-Peppas model (Figure 10) which indicates the drug release in 0.1 N HCl is best fitted with first order kinetic. First order kinetic states that the drug release is proportional to the concentration of drug remained (Dash *et al*., 2010). Thus, with the increase of dissolution time, the less concentration of drug remained in the dialysis bag, resulting in reduced rate of drug release after 10 min (Figure 9).

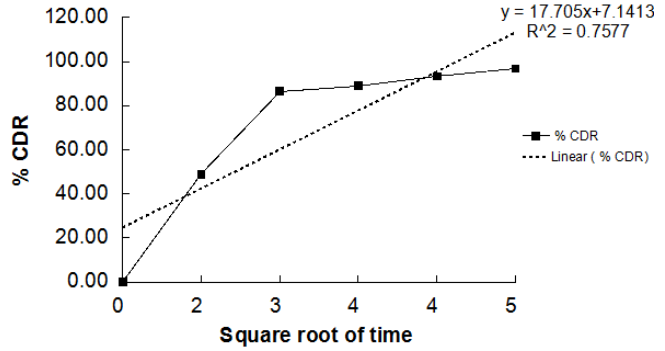

Fig. 9: Higuchi model release of clopidogrel nanosuspension formulation FF3 in 0.1 N HCl.

In phosphate buffer, the R^2 value obtained was 0.9888 in zero order (Figure 11), 0.9930 in first order (Figure 12), 0.9888 in Higuchi model (Figure 13), and 0.8317 in Korsmeyer-Peppas model (Figure 14). The drug release in phosphate buffer is best fitted with first order as this model produced the highest value in R^2 which indicates the concentration-dependent drug release. The n value was 0.1328 (n<0.5), indicates quasi-fickian diffusion behavior (Basak *et al*., 2008).

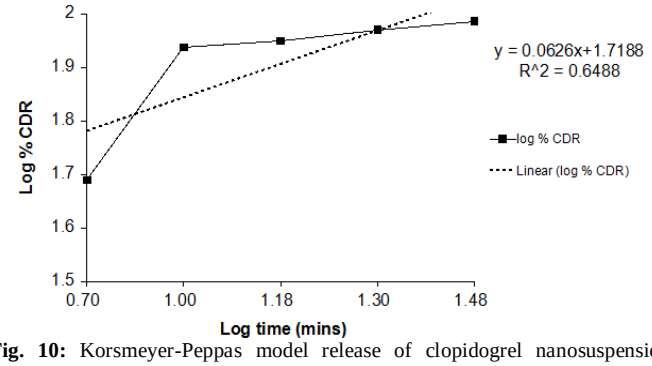

Fig. 10: Korsmeyer-Peppas model release of clopidogrel nanosuspension formulation FF3 in 0.1 N HCl.

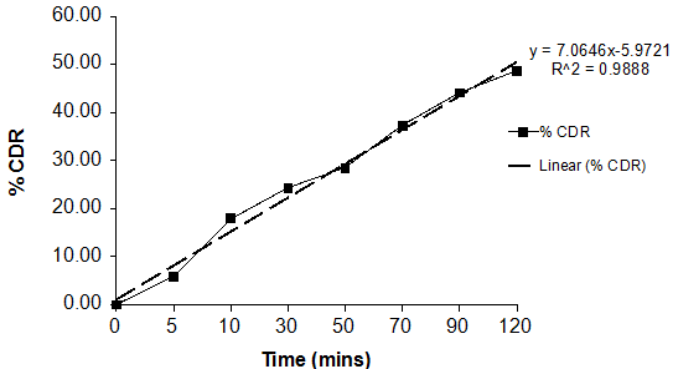

Fig. 11: Zero order release of clopidogrel nanosuspension formulation FF3 in pH 6.8 phosphate buffer with 0.1% w/v Tween 80.

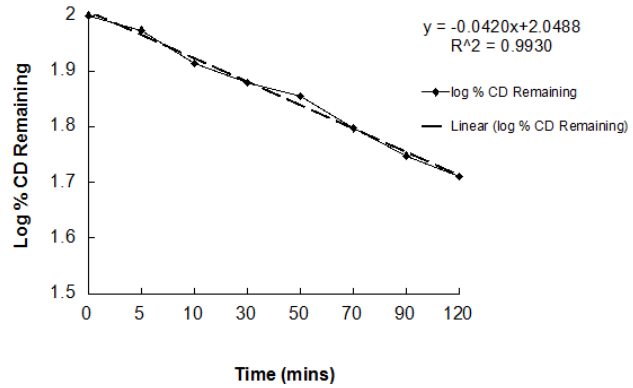

Fig. 12: First order release of clopidogrel nanosuspension formulation FF3 in pH 6.8 phosphate buffer with 0.1% w/v Tween 80.

CONCLUSION

High pressure homogenization method can be used as an effective tool for preparation of nanosized formulations.

Clopidogrel nanosuspension prepared by this method showed significant improvement in aqueous solubility as well as dissolution characteristics which may significantly improve its oral bioavailability. Smaller particle size was obtained in formulations with higher homogenization pressure and greater homogenization cycle.

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