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# FORMULATION AND EVALUATION OF CLOPIDOGREL BISULFATE PROLIPOSOMES

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## ABSTRACT

The objective of present work is to develop proliposomes of Clopidogrel bisulfate, to increase the bioavailability, increase aqueous solubility and to decrease the side effects like gastric bleeding. Proliposomes are prepared by Film deposition on carrier method using sorbitol as carrier powder. Lecithin and cholesterol were used as lipid phase and Clopidogrel bisulphate as drug. The results have shown no interaction between the drug and polymer through FTIR studies. Clopidogrel proliposomes with good flowability were obtained. The particle size of the proliposomes was from 20 -3 µm from scanning electron microscope photographs. Percent drug content and Percent drug entrapment efficiency of optimized formulation was found to be 82.6% and 75.6%.The *invitro* percent drug release of optimized F4 formulation has showed 88.65 % at 10 hours. Stability studies show that the formulation was stable at 40°C and at 75% RH. Proliposomes exhibited better stability when compared with liposomes. Clopidogrel bisulfate proliposomes with good flowability and sustained released characteristics can be obtained by controlling drug and lecithin concentration. Proliposomes prove to be efficient drug carriers for sustained drug delivery of Clopidogrel bisulfate.

**Keywords: Proliposomes, Clopidogrel bisulfate, lecithin, sorbitol *and in-vitro* release.**

## INTRODUCTION

Drug carrier is any substrate which is used for drug delivery. Drug carriers are generally used to control the release of drug into the systemic circulation [1]. Liposomes are drug carriers which are microscopic sealed vesicles in which the aqueous compartment is enclosed by one or more phospholipid bilayers [2]. They carry the drug and release it at the specific site of action.

The shelf life of liposomes is limited, and also exhibit poor stability, to overcome this problem proliposomes was discovered [3]. The proliposomes concept was first introduced by Payne et al, in 1986 [4]. Proliposomes can be defined as dry free flowing granular product which is composed of drug, phospholipids and water soluble porous powder [5]. Proliposomes form liposomal dispersion on hydration or when they come in contact with biological fluids after entering in to the body. Proliposomes show controlled release with high solubility, ease of handling and better stability compared to liposome's [6].

Proliposomes can be prepared by the different methods which include Film deposition on carrier method, Fluidized-bed method, Spray drying method and super critical anti-solvent method [5]. Proliposomes are applied in oral drug delivery, parentarel delivery, pulmonary delivery, mucosal delivery, transdermal delivery, and ocular drug delivery systems [7].

Clopidogrel bisulfate is an antiplatelet drug belonging to thienopyridine class of drug. It inhibits platelet aggregation which inhibits blood clotting to treat patients with myocardial infarction (MI) and stroke in patients suffering from atherosclerosis [8]. Clopidogrel prevents harmful clots by preventing aggregation of platelets. Clopidogrel is sold on the name of palvix [9]. The objective of present work is to develop proliposomes of Clopidogrel bisulfate, to increase the bioavailability, increase aqueous solubility and to decrease the side effects like gastric bleeding.

## MATERIALS AND METHODS

Clopidogrel bisulfate, lecithin (NICE chemicals Pvt ltd) used as a phospholipid, sorbitol (Qualikems Fine Chem Pvt. Ltd) used as carrier, cholesterol (NICE chemicals Pvt ltd), methanol (Merck life science Pvt ltd) as a solvent.

### Formulation of Proliposomes

Proliposomes are prepared using Film-deposition on carrier method. Various batches were prepared using different concentrations of lecithin given in Table 1.

**Table 1: Formulations of Clopidogrel bisulfate loaded proliposomes**

| S.No | Formulation | Drug(mg) | Lecithin(mg) | Cholesterol(mg) | Sorbitol(gms) |
|---|---|---|---|---|---|
| 1 | F1 | 100 | 100 | 100 | 5 |
| 2 | F2 | 100 | 200 | 100 | 5 |
| 3 | F3 | 100 | 300 | 100 | 5 |
| 4 | F4 | 100 | 400 | 100 | 5 |
| 5 | F5 | 100 | 500 | 100 | 5 |
| 6 | F6 | 100 | 600 | 100 | 5 |

Accurately weighed quantity of drug, lecithin and cholesterol are taken into a beaker. The mixture is dissolved using 5ml methanol and sonicated for 5 minutes to obtain a clear solution. 5g of sorbitol is placed in rotary evaporator and rotated at 80-90 rpm which is placed in water bath at 70 – $80^0$C. The powder is dried under vacuum for 30 minutes in the RBF while rotating, and then the temperature is decreased to 20- $30^o$C. Through solvent inlet tube 0.5 ml of drug and phospholipids solution is introduced, RBF is rotated until the solution is absorbed by sorbitol and it is dried under vacuum. After drying, when sorbitol attains good solubility again 0.5 ml of aliquot is added. The process is repeated until all the solvent is absorbed. The flask is rotated in water bath under vacuum until all the sorbitol appears dry further it is dried at room temperature for overnight. The obtained proliposomes were collected [10].

**Physicochemical characterization**

Fourier Transform Infrared Spectroscopy (FTIR) is used to determine the compatibility of drug with excipients.Flow properties of proliposomes were determined by carr's index, hausner's ratio and angle of repose. Bulk density and tapped density were determined to calculate Carr's index and hausners ratio.Scanning electron microscopy is used to determine the morphology of proliposome powder. Hydration study is done to determine the capacity of proliposomes to convert in to liposomes on hydration [11]. It is determined using a microscope.

**Percentage Yield of Proliposomes**

The proliposomes were dried and accurately weighed. The percentage yield was calculated using the following formula

$$\text{Percentage yield} = \frac{\text{Total weight of proliposomes}}{\text{Total weight of drug x weight of added materials}} \times 100$$

**Drug Content**

10 mg of proliposomes were dissolved in methanol and phosphate buffer (pH 7.4) in 9:1 ratio. 1ml of the above solution is taken and diluted with 10 ml methanol. The absorbance of the solution was determined at 272.5 nm by using UV spectrophotometer [12].

$$\% \text{ Drug content} = \frac{\text{Total drug concentration} - \text{unknown drug concentration}}{\text{Total drug concentration}} \times 100$$

**Drug Entrapment**

The drug entrapment efficiency of proliposomes is determined by hydrating the proliposomes to liposomes which is followed by separation of entrapped drug and un-entrapped drug. The un- entrapped drug is separated by ultracentrifugation [12].

10mg of proliposomes were dissolved in phosphate buffer (pH 7.4). The solution was centrifuged at 6000 x g for 30 minutes using a refrigerated centrifuge, to separate the non-entrapped drug. The concentration of free drug was determined by taking the supernatant solution and measuring the absorbance of solution at 272.5 nm by using UV spectrophotometer. The percent drug entrapment in proliposomes was calculated using the following formula

$$\% \text{ Drug entrapment} = \frac{\text{Total drug concentration} - \text{drug concentration in supernatant}}{\text{Total drug concentration}} \times 100$$

**In Vitro drug Release Studies**

Paddle dissolution apparatus USP type II was used to determine the drug release studies. About 10 mg of drug containing proliposomes were accurately weighed and filled into a hard gelatin capsule. The capsule was placed in a non- reacting muslin cloth. The mesh was then tied with nylon thread.

The diffusion studies were carried out in USP type II apparatus using phosphate buffer (pH 7.4) as a medium. The test was carried out for about 10 hours. For every hour 5 ml of sample was withdrawn and same amount of fresh medium was replaced. The absorbance was determined in UV spectrophotometer at 272.5 nm.

$$\% \text{ drug release} = \frac{\text{amount of sample (mg)}}{\text{dose (mg)}} \times 100$$

$$\% \text{ cumulative drug release} = \frac{\text{volume of sample withdrawn (ml)}}{\text{bath volume (ml)}} \text{ x } P(t-1) \text{x } Pt$$

**Drug release kinetics**

To understand the mechanism and kinetics of drug release from proliposomal formulations the results of the in vitro drug release study were fitted with various kinetic equations like zero order, first order, and Higuchi model. The drug release data was further analyzed by Korsmeyer- Peppas models.

**Stability Studies**

The stability studies were carried out at an accelerated condition as per ICH guidelines. The stability study was carried out for a period of 3 months at 40$^{o}$C and at 75% RH. The sample was later analyzed for physical appearance, percent drug content and entrapment efficiency.

**RESULTS AND DISCUSSION**

**Formulation of proliposomes of Clopidogrel bisulfate**

Proliposomes were prepared using Film-deposition on carrier method (Figure 1).

FTIR was conducted on the selected formulation f4 the pure drug shows characteristic peaks at 1727.51 cm$^{-1}$, 1082.34 cm$^{-1}$, 2827.51 cm$^{-1}$. The principle peaks of the Clopidogrel bisulfate were found to be unaltered with peaks of physical mixture. This indicates that the Clopidogrel bisulfate and excipients were compatible with each other (Figure 2, 3).

The results of flow properties of Clopidogrel bisulfate proliposomes are shown in table 2. The results have shown acceptable range of flow properties [13].

**Scanning Electron Microscopy (SEM)**

Scanning electron microscope photos of Clopidogrel bisulfate proliposomes are shown in Figure 4. The SEM photographs were taken at different magnifications. The particle size of the proliposomes was from 20 -3 μm respectively. The size represents the proliposomes which are prepared are according to the specifications [14].

**Hydration study**

Hydration of Clopidogrel bisulfate proliposomes have been performed which have shown complete hydration of proliposomes in a minute. This shows that that the proliposomes can be easily converted into liposome's when come in contact with body fluids when they are taken orally.

**Percentage yield, drug content and drug entrapment efficiency of Clopidogrel bisulfate proliposomes**

The results are shown in the table 3. The percentage yield was found to be in the range of 69.8% - 89.4%. The result show an

increase in percentage yield with increase in the lecithin concentration [15]. Percent drug content has been determined and it was found between 67.9% - 82.6%. The result has shown an increase in drug content with increase in lecithin concentration [12]. The percentage drug content of F4 batch was found to be higher. The entrapment efficiency of Clopidogrel bisulphate may be due to its poor aqueous solubility. The entrapment efficiency was found in the range of 54.9% - 75.6%. The result has shown an increase in drug entrapment efficiency with increase in lecithin concentration. The percentage drug entrapment efficiency of F4 batch was found to be higher.

***In vitro* drug release studies**

The dissolution studies were carried out in USP type II apparatus using phosphate buffer (pH 7.4) as a medium, for about 10 hours. The results are given in table 4.

The drug release was found to be varied with different ratios of drug to phospholipids ratios. The cumulative drug release was found to be dependent on the concentration of phospholipids. The drug release was increased with increase in lecithin concentration to an extent, later the drug release was found to be decreased with increase in concentration of lecithin [12]. F4 formulation has showed highest drug release at 10 hours (Figure 5).

**Drug release kinetics**

The result of drug release kinetics is shown in table 5.

Results from table 6 reveals that the release of Clopidogrel bisulfate proliposomes best fitted to the zero order release model, as the $r^2$ value is 0.9875. The graphical representation of % cumulative drug release $v_s$ square root of time in hours follows higuchi release model, as the value of coefficient of correlation $r^2$ is 0.990. This confirms that the drug release is proportional to square root of time which indicates the release of Clopidogrel from proliposomes was diffusion controlled. The n value from the Korsmeyer-peppas model for Clopidogrel proliposomal formulation is 0.788, which is less than 0.89, which shows Anomalous transport of diffusion [16].

**Stability Studies**

The stability study was carried for the optimized proliposomal formulation for a period of 3 months at 40$^{o}$C and at 75% RH. They were analyzed for the following parameters: hydration study, percent drug content and percent drug entrapment efficiency. The results are shown in the table 7.

After three months of storage of Clopidogrel proliposomes, they were found to be free flowing and they formed immediately into liposomes on hydration. The percent drug content and percent drug entrapment efficiency changed from85.84 ± 0.54 and 75.6± 0.59 to 83.57 ± 0.90and 73.38 ± 0.45 within 3 months. Proliposomes exhibited better stability when compared with liposomes [17].

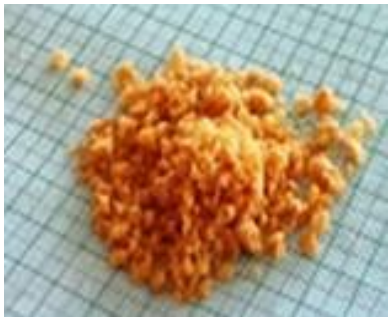


**Fig 1: Prepared proliposomes of Clopidogrel bisulfate**

## Compatibility study of Clopidogrel bisulfate with excipients

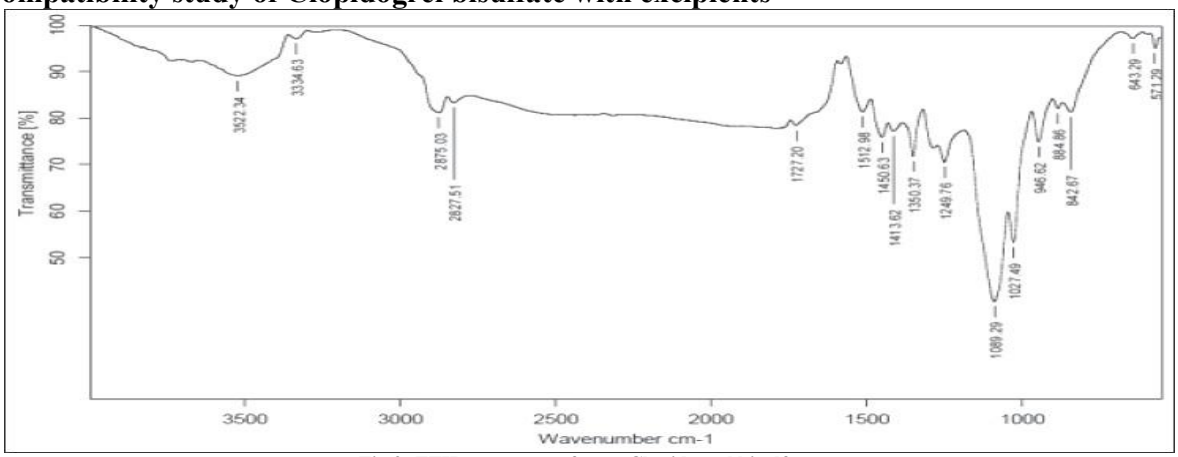



**Fig 2: FTIR spectrum of pure Clopidogrel bisulfate**

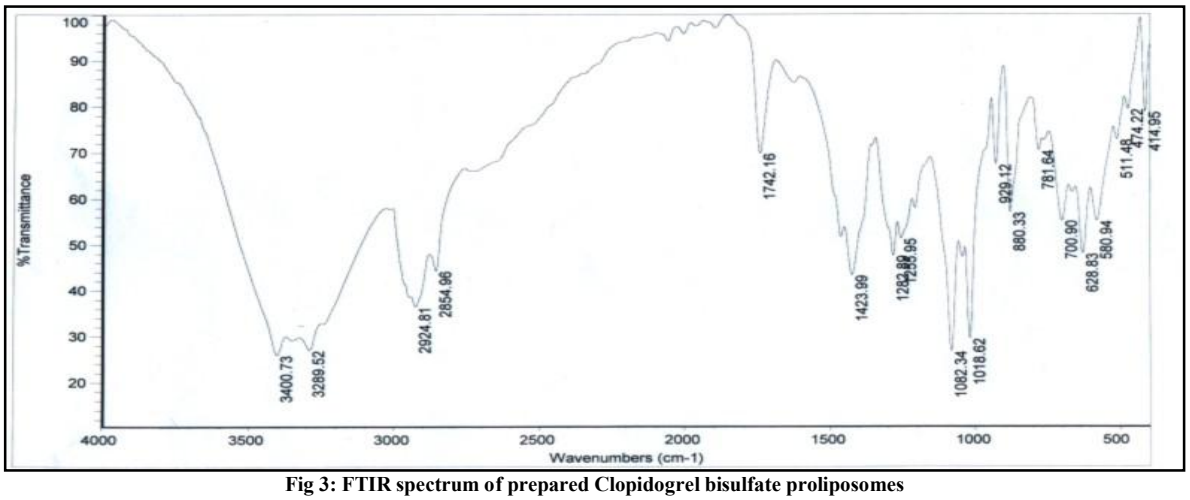



**Fig 3: FTIR spectrum of prepared Clopidogrel bisulfate proliposomes**

## Characterization of proliposomes of Clopidogrel bisulfate Flow properties

**Table 2: Flow properties of Clopidogrel bisulfate proliposomes**

| Batches | Bulk Density (g/ml) ± SD | Tapped Density (g/ml) ± SD | Carrs'index (%) ± SD | Hausener's Index ± SD | Angle of repose. ($o^o$) ± SD |
|---|---|---|---|---|---|
| F1 | 0.57 ± 0.01 | 0.67 ± 0.005 | 13.8 ± 2.2 | 1.16 ± 0.03 | 38.3± 2.57 |
| F2 | 0.59 ± 0.005 | 0.73 ± 0.02 | 18.3 ± 2.3 | 1.22 ± 0.02 | 33.03 ± 1.92 |
| F3 | 0.57 ± 0.01 | 0.68 ± 0.01 | 15.3± 0.5 | 1.17 ± 0.005 | 37.5 ± 1.71 |
| F4 | 0.59 ± 0.005 | 0.71 ± 0.02 | 15.6 ± 1.1 | 1.19 ± 0.02 | 38.05 ± 2.85 |
| F5 | 0.56 ± 0.01 | 0.66 ± 0.03 | 13.3 ± 0.5 | 1.15 ± 0.005 | 34.3 ± 1.05 |
| F6 | 0.58 ± 0.02 | 0.69 ± 0.03 | 16.8 ± 0.02 | 1.18 ± 0.02 | 37.3 ± 1.05 |

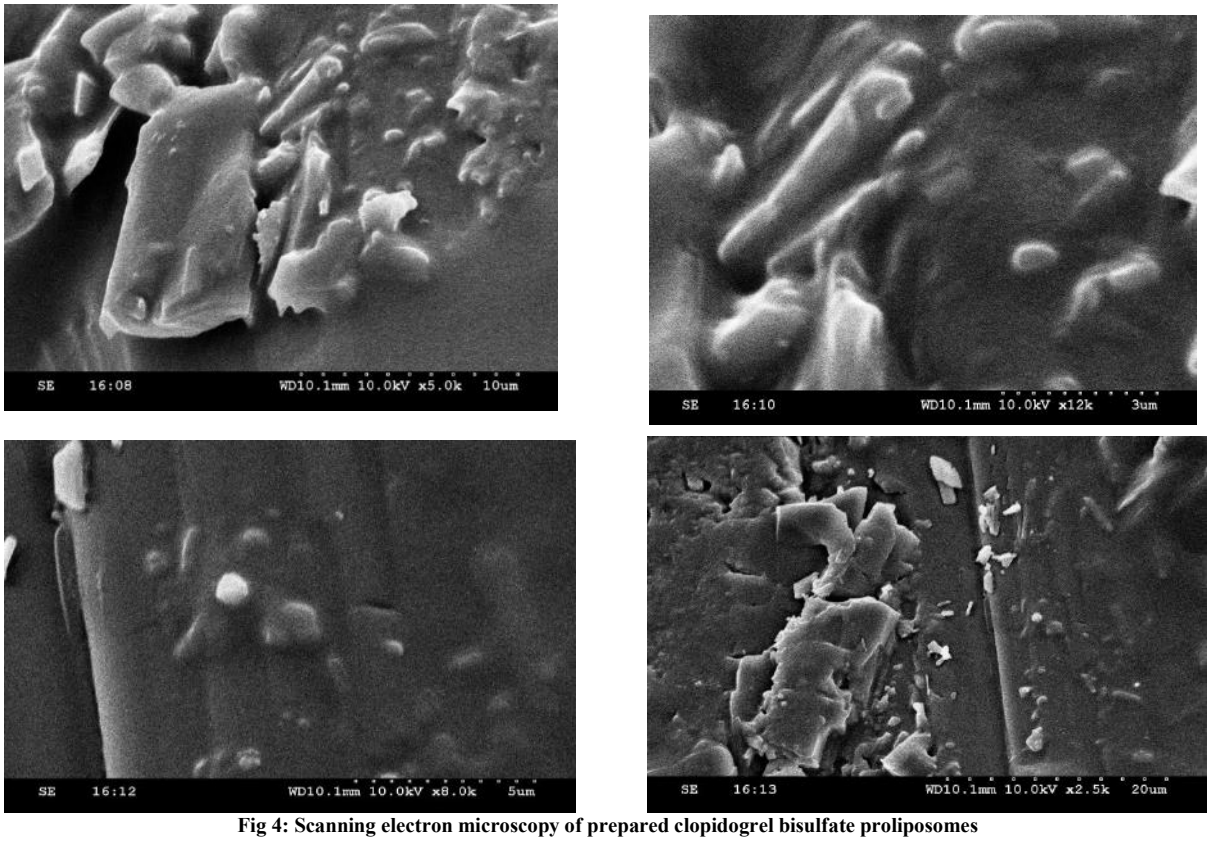



**Fig 4: Scanning electron microscopy of prepared clopidogrel bisulfate proliposomes**

**Table 3: Percentage Yield of Clopidogrel bisulfate proliposomes**

| Batches | % Yield ± SD | % Drug Content ± SD | % Drug entrapment efficiency ± SD |
|---|---|---|---|
| F1 | 79.89 ± 0.65 | 72.66 ± 0.71 | 54.9 ± 0.52 |
| F2 | 74.62 ± 0.54 | 75.96 ± 0.37 | 61.7± 0.63 |
| F3 | 81.3 ± 0.84 | 78.87 ± 0.58 | 64.4± 0.37 |
| F4 | 86.1 ± 0.61 | 85.84 ± 0.54 | 75.6± 0.59 |
| F5 | 89.4 ± 0.51 | 83.2 ± 0.32 | 71.7± 0.26 |
| F6 | 84.1 ± 0.41 | 80.64 ± 0.56 | 67.16± 0.46 |

**Table 4: Percent cumulative drug release of Clopidogrel bisulfate proliposomes**

| Time (h) | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 9.9 ± 0.66 | 9 ± 2.5 | 10.8 ± 1.9 | 15.3 ± 1.5 | 13.5 ± 2.15 | 12.6 ± 0.67 |
| 2 | 16.25 ± 0.99 | 17.15 ± 2.13 | 20.13 ± 2.1 | 23.48 ± 1.7 | 22.57 ± 1.5 | 21.67 ± 1.34 |
| 3 | 26.19 ± 1.3 | 28.89 ± 1.4 | 29.81 ± 1.8 | 36.13 ± 0.56 | 30.72 ± 1.5 | 32.52 ± 0.73 |
| 4 | 32.54 ± 1.9 | 39.76 ± 1.6 | 41.56 ± 2.9 | 42.5 ± 0.72 | 38.87 ± 0.96 | 40.68 ± 1.65 |
| 5 | 41.58 ± 2.1 | 46.12 ± 1.5 | 48.83 ± 2.1 | 49.73 ± 1.4 | 47.01 ± 1.83 | 44.32 ± 0.98 |
| 6 | 44.33 ± 1.37 | 50.65 ± 1.4 | 53.37 ± 0.72 | 61.47 ± 2.1 | 54.26 ± 2.35 | 51.54 ± 1.11 |
| 7 | 52.44 ± 2.7 | 56.08 ± 1.8 | 56.99 ± 2.7 | 69.64 ± 2.3 | 59.7 ± 1.37 | 55.18 ± 2.31 |
| 8 | 2.19 ± 2.2 | 65.11 ± 1.3 | 67.81 ± 1.2 | 77.7 ± 1.9 | 70.53 ± 1.54 | 66.00 ± 1.6 |
| 9 | 73.2 ± 1.6 | 75.06 ± 0.92 | 80.47 ± 1.0 | 81.43 ± 0.98 | 79.59 ± 1.38 | 77.76 ± 2.2 |
| 10 | 77.80 ± 2.3 | 81.41 ± 0.75 | 84.14 ± 0.88 | 88.65 ± 1.45 | 86.84 ± 1.9 | 85.03 ± 1.8 |

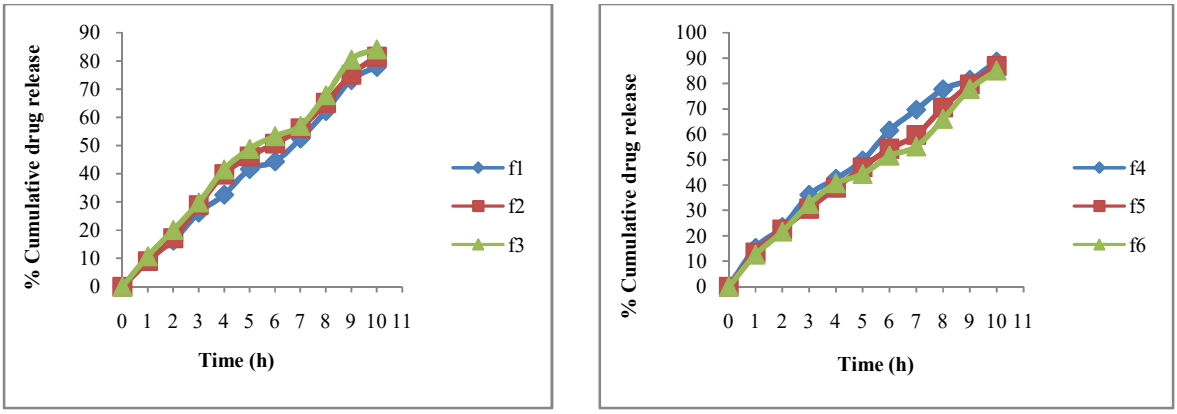



Fig 5: Graphical presentation of %CDR of formulations $f_1$ to $f_6$

Table 5: Release kinetics of Clopidogrel bisulfate Proliposomes

| Zero order | | Higuchi's | | Peppas's | | First Order | |
|---|---|---|---|---|---|---|---|
| Time (h) | Cumulative % Drug Release | Sq.Root of Time | Cumulative % Drug Release | Log Time | Log Cumulative% Drug Release | Time (h) | Log % of Drug Remaining |
| 1 | 15.3 | 1.00 | 15.3 | 0.00 | 1.18 | 1 | 1.98 |
| 2 | 23.48 | 1.414 | 23.48 | 0.30 | 1.37 | 2 | 1.88 |
| 3 | 36.13 | 1.73 | 36.13 | 0.47 | 1.55 | 3 | 1.8 |
| 4 | 42.5 | 2.00 | 42.5 | 0.60 | 1.62 | 4 | 1.75 |
| 5 | 49.73 | 2.23 | 49.73 | 0.69 | 1.69 | 5 | 1.65 |
| 6 | 61.47 | 2.44 | 61.47 | 0.77 | 1.78 | 6 | 1.54 |
| 7 | 69.64 | 2.64 | 69.64 | 0.84 | 1.84 | 7 | 1.43 |
| 8 | 77.7 | 2.82 | 77.7 | 0.90 | 1.89 | 8 | 1.34 |
| 9 | 81.43 | 3.00 | 81.43 | 0.95 | 1.91 | 9 | 1.26 |
| 10 | 88.65 | 3.16 | 88.65 | 1.00 | 1.94 | 10 | 1.15 |

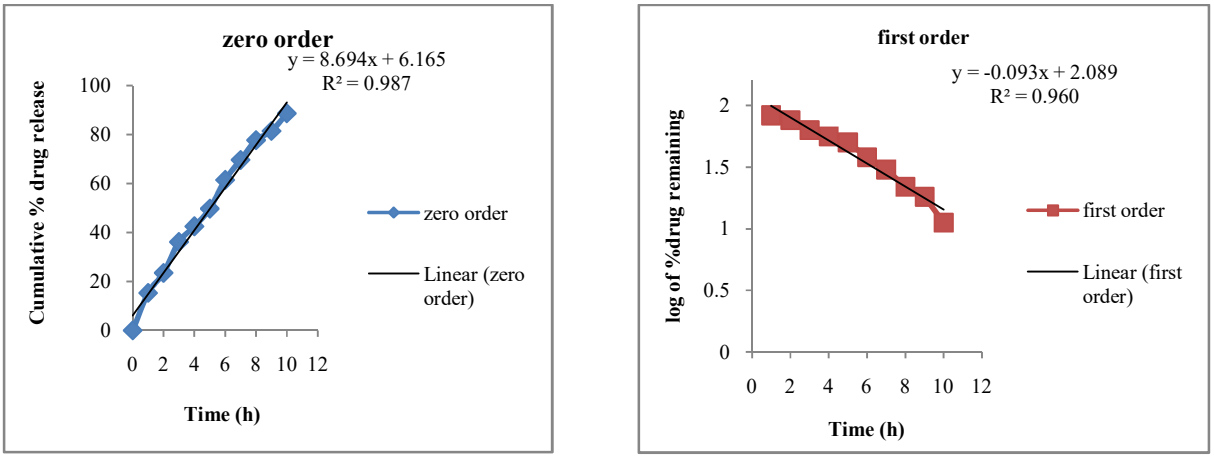
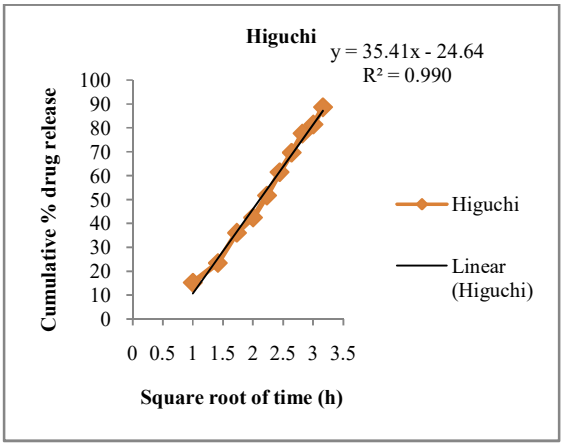



Fig 6: Graphical presentation of zero and first order release kinetics



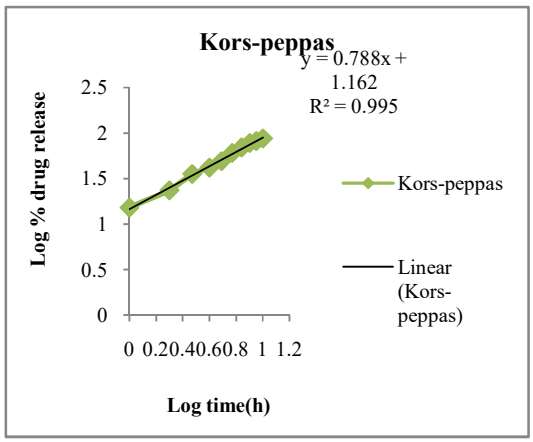




**Fig 7: Graphical presentation of Higuchi and Korsmeyer-peppas kinetic analysis data**

**Table 6: Determination of Order of Release of Clopidogrel bisulfate Proliposomes**

| Model name | $R^2$ value | Slope |
|---|---|---|
| Zero order model | 0.987 | 8.694 |
| First order model | 0.967 | -0.092 |
| Higuchi's model | 0.990 | 35.41 |
| Korsmeyer-peppas model | 0.995 | 0.788 |

**Table 7: Stability study of Clopidogrel bisulfate Proliposomes**

| Formulation code | Test after time In days | Percent drug content (%) | Percent drug entrapment (%) |
|---|---|---|---|
| F4 | 0 | 85.84 ± 0.54 | 75.6± 0.59 |
| F4 | 30 | 85.27 ±0.61 | 74.51 ± 0.62 |
| F4 | 60 | 84.14 ± 0.55 | 73.94 ± 0.39 |
| F4 | 90 | 83.57 ± 0.90 | 73.38 ± 0.45 |

## CONCLUSION

Clopidogrel bisulfate proliposomes with good flowability and sustained released characteristics can be obtained by controlling drug and lecithin concentration. Proliposomes prove to be efficient drug carriers for sustained drug delivery of Clopidogrel bisulfate.

## AKNOWLEDGEMENT

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## CONFLICT OF INTEREST

Authors declare no conflict of interest.

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