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Formulation and in-vivo evaluation of modified dosage form of Clopidogrel bisulfate

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ABSTRACT

The present study was carried out with an objective to formulate modified dosage form of Clopidogrel bisulfate. Clopidogrel bisulfate is available in the form of oral immediate release tablets. It is modified into both pharmaceutical aqueous injection and oral solid floating tablet formulations. *In-vitro* and *in- vivo* evaluation of both the formulations were evaluated. The aqueous injection comprises solubilizer and aqueous solvent. Floating tablet were formulated in different ratio's of natural based swelling polymer Swelstar MX-I. The floating tablets were based on effervescent approach using sodium bicarbonate as gas generating agent. The effect of polymers concentration on drug release profile was evaluated. The formulations containing sodium bicarbonate 34 mg per tablet provided desired buoyancy (floating lag time of about 2 minutes and total floating time of >24 hours). The *in –vivo* study on rabbits to see the effect of bleeding time, clotting time, platelet count and partial thromboplastin time were investigated. The results indicates that the bleeding time of clopidogrel floating tablets exhibited effect upto 12 hrs and the effect was maintained for 24 hours compared to reference product. The IV bolus solution showed maximum bleeding time in 1 hr and than decreased significantly as compared to normal control. No significant change in mean platelt count was observed. The clotting time of treated groups significantly increased in test formulation group as compared to normal group upto12 hours but regains to baseline in 24 hours.

Keywords: Aqueous injection, Floating tablet, Swelstar MX-I, Bleeding time

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INTRODUCTION

The focus of pharmaceutical research in the area of cardiovascular disorder has being continuously carried out and formulated for conventional oral dosage form for the management of cardiovascular disorder. The underperformance of antihypertensive therapy in some major interventions trials in reducing the occurrence of coronary heart disease has paved the way of Novel dosage form of cardiovascular drugs.[1]

Thrombosis superimposed on arteriosclerosis is the principal cause of mortality and morbidity in patients with arteriosclerosis.[2] The use of antiplatelet agents and anticoagulants in the treatment of arteriosclerosis is well established, based on many large randomized trials. For high-risk patients such as those with acute coronary syndromes (ACS; unstable angina, myocardial infarction), antiplatelet therapy with clopidogrel is indicated, based on results of the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial.[3]

Clopidogrel bisulfate is an oral antiplatelet agent (thienopyridine class) to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Platelet glycoprotein IIb/IIIa agents are powerful inhibitors of platelet function and are also effective in ACS, but the benefit is confined to high-risk patients. These agents are also indicated for patients undergoing percutaneous coronary intervention. Prolonged dual antiplatelet therapy (at least 6 months) is recommended for patients receiving drug-eluting stents.[4]

Clopidogrel Immediate release dosage provides a peak concentration of active metabolite in 1-3 hr post –dosing. This would provide high concentration of active metabolite immediately after dosing and lower concentration 24 hr post dosing. In summary the patient is exposed to bleeding risk after peak level. A Modified release formulation would extent the level of active metabolite beyond first hour post dosing. Therefore the aim of the study is to prepare modified release drug delivery of clopidogrel bisulfate with prolonged residence time in the stomach as the dug has higher solubility at lower pH values thus also reduces the resistivity among the prolonged antiplatelet therapy patients.

Clopidogrel bisulfate floating drug delivery system has been formulated with low density system. The bulk density of the tablet is less than gastric fluids and hence it remains buoyant in the stomach, prolonging the retention time at lower pH, and facilitating better bioavailability as clopidogrel bisulfate has more solubility at lower pH.[5] The drug releases slowly at the desired rate from the tablet and in this way there is a better control of drug in the plasma drug concentration.

Clopidogrel is available in the form of immediate release tablets equivalent to 75 mg base. The oral formulation takes 1 to 2 hours to exhibit its pharmacological activity. Intravenous injections are recognized to present the activity instantaneously. The parenteral route of administration has benefit over oral administration for quick action, as there is no delay in time to the drug reaching sufficient concentration in the systemic circulation to start surgery in cases of coronary intervention. Coronary intervention is a medical emergency, during which the rapid achievement of therapeutic drug concentrations in the blood and rapid onset of action is a main concern and to achieve the same, intravenous administration of clopidogrel is the best preferred route. Moreover there is need of injection dosage form for patients who are in urgency for medication and for whom oral administration is difficult. Intravenous injection should be aqueous based so that biocompatibility is achieved. Clopidogrel aqueous solution can be administered parenterally.

Y.Chandra Sekhar *et a*l. have worked on floating and sticking tablets of clopidogrel bisulphate using polymers of HPMC (100K-LV) and HPMC 4K as the polymer and sodium bicarbonate as gas generating agent. HPMC is a synthetic based polymer. Natural polymers remain striking area of interest for research since they are inexpensive, easily abundantly available, and compatible due to their origin. Swelstar MX-I, a natural based excipient is used in the present study for modulating the dug release. It is pregelatinized starch, alpha - amylase resistant, able to form gel –matrix, and is pH –independent under high ionic strength conditions.[8] It is a matrix material that possesses both swelling and controlled release properties.

Additionally, in this current work, the parenteral drug delivery of clopidogrel bisulfate was prepared by dissolving clopidogrel bisulfate in ethanol in presence of Solutol HS -15. Both the solution were mixed and stirred properly.

MATERIALS AND METHODS

Materials

Clopidogrel bisulfate was used as the active ingredient. Swelstar MX-I was used as the rate controlling polymers. Sodium bicarbonate was used an effervescent agent. The other ingredients used were Avicel pH 102 and Hydrogenated castor oil. PEG 8000 was used as solubilizer. All the materials used in experimental works were obtained from Dr. Reddy's Research Laboratories, Hyderabad, India. All reagents used were of analytical grade.

Preparation of Modified release floating tablets of Clopidogrel bisulfate.

The qualitative and quantitative composition of different formulation of Clopidogrel bisulfate is shown in [Table 1]. Clopidogrel bisulfate (97.87 mg equivalent to 75 mg of Clopidogrel) and all

other ingredients were weighed separately and passed through sieve no. 40. The active ingredient, Swelstar MX-1, Avicel 102 (Microcrystalline cellulose), Sodium bicarbonate and were mixed together. The mixture was then aqueous granulated to form granules. The granules were then milled and passed through sieve no. 20. The final blend was lubricated with magnesium stearate and finally compressed into tablets using single punch tablet rotary press (Cadmach).

Preparation of Aqueous Injection formulations of Clopidogrel bisulfate.

Clopidogrel bisulfate is added to Cremophor EL and mixed till dispersion is formed. Other ingredients like Sodium Acetate, EDTA Sodium are dissolved in small amount of water and added to drug mixture with continuous stirring. Preservatives like methyl paraben, propyl paraben are dissolved in hot water and after cooling to room temperature, added to drug mixture. Finally, the volume is made up with water for inj.

Table 1: New Dosage form formulations of Clopidogrel bisulfate

S.No	Ingredients	FC-I*	FC-2*	FC-3*	FC-4*	FI-1*
1	Clopidogrel bisulfate [@, $]	125	125	125	125	2% w/w
2	Swelstar MX-I	30	45	60	60	-
3	Sodium bicarbonate	34	34	34	36	-
4	Avicel pH 102	66	51	36	34	-
5	Hydrogenated castor oil	5	5	5	5	-
6	Cremophor EL	-	-	-	-	20 % w/v
7	Sodium Acetate	-	-	-	-	q.s.
8	EDTA Sodium	-	-	-	-	0.05 % w/v
9	Methyl Paraben	-	-	-	-	0.12% w/v
10	Propyl Paraben	-	-	-	-	0.06% w/v
11	Water for Injection	-	-	-	-	q.s.

Note:

@ - Total dose for Sustained release upto 24 Hrs – Initial Loading dose (1 Hr) + Maintenance dose (23 Hrs) i.e. 24 mg + 72 mg = 96 mg of Clopidogrel bisulfate
(Calculated from the Pharmacokinetic data of Clopidogrel bisulfate)
$ - denotes for 96 mg of Clopidogrel bisulfate which is molar equivalent of 125 mg Clopidogrel base.
* FC-1, 2, 3 and 4 are oral solid floating tablets
** FI-1 is an aqueous based injection of Clopidogrel with a dose of 15 mg / ml

Fourier transforms infrared spectroscopy (FT-IR)

Fourier transform infrared spectroscopy was analyzed by Shimadzu, Japan. The pure drug was weighed and mixed with previously dried potassium bromide KBr. The mixture was carefully grinded and the homogenous mixture was placed in an IR pellet die subjected to a pressure of 900 Mpa ($9t.cm^{-2}$) for about 2 minutes to form a disc.[9] The spectral scanning was done in the range of 4000 to 400 cm -1. The spectra of Clopidogrel bisulfate as per se and binary mixture of

Clopidogrel bisulfate and Swelstar MX-I were done.

Differential scanning calorimetry (DSC)

The differential scanning calorimeter was done to evaluate the compatibility study of drug with polymer. The binary mixture of Clopidogrel bisulfate and Swelstar MX-I in the ratio of 1:2 were subjected to DSC. The sample was heated from 50 to 300 ^{0}C at a heating rate of 10 ^{0}C/ minute under argon atmosphere using a micro calorimeter (DuPont, USA).

p- XRD:

To confirm the physical state of the clopidogrel bisulfate form I, X-ray diffracton was done in an X-ray diffractometer (X'pert, Philips, the Netherlands). The result was observed with a Cu anode used at a voltage of 40 KV at 30Ma. The scan type was continous and the scan start position was 2.012 to 38.27. The scan step time was 3.0/40 seconds. The scattered intensities were measured at ambient temperature and the results were recorded. The wavelength to compute d-spacing was 1.54059 A (cu/k-alpha1)

***In- vitro* buoyancy studies**

The in vitro buoyancy was observed by the floating lag time and total floating time. The floating lag time was determined as the time to rise to the surface and float. The duration of time the dosage form constantly remained on the surface was determined as the total floating time

***In-vitro* dissolution studies**

The drug release of clopidogrel bisulfate from the floating tablets (n = 6) was determined using USP XXXII dissolution testing apparatus type II (paddle method). The dissolution was performed using 1000 ml of 0.01 N HCl, at 37 ± 0.5°C for 50 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at predetermined time intervals (1, 2, 3, 6, 9, 12, 15, 18 and 24 hours) and the samples were replaced with fresh dissolution medium. The samples were filtered through 0.45 µ membrane (nylon) filter and diluted to suitable concentration with 0.01 N HCl. The samples were analyzed for drug release against 0.01 N HCl as a blank at a wavelength of 220 ηm using a Shimadzu UV-1700UV/Vis double beam spectrometer.

***In-vivo* studies**

The experiments were carried out on New Zealand white rabbits of 4 months, of both sexes, weighing between 2.0 to 2.5 kg. They were provided from Sapience Bio-analytical Research Lab, Bhopal, (M.P.). The animals were acclimatized to the standard laboratory conditions in cross ventilated animal house at temperature 25±2°C relative humidity 45% vegetables and water ad libitum during experiment. The experiment was approved by the institutional ethics

committee and as per CPCSEA guidelines (approval no. 1413/PO/a/11/CPCSEA). Animals were divided into four groups and each group contains three animals. Group I- Served as normal control (Untreated). Group II- administered Clopidogrel tablet (Reference- Immediate release tablet of 75 mg from Torrent Pharmaceuticals limited, India). Group III- administered Clopidogrel intravenously (0.1mg/kg bolus). Group IV- administered Clopidogrel tablet (Sustained release floating tablet). After the treatment, the needle was pricked in rabbit marginal vein and the pricking time was noted. The blood was collected in appendorff tube containing EDTA and bleeding time was noted. The Platelet count and Partial Thromboplastin Time (PTT) were estimated. For X-Ray Imaging test, tablets were prepared using X-ray opaque material barium sulfate to ensure visibility by X-ray. The X-ray imaging was performed in Group IV rabbit. The tablet was administered by natural swallowing to rabbit followed by 50 ml of water. During the experiment the rabbit was not allowed to eat, but water was available. After 1hr, X-ray of the abdomen was taken using an X-ray machine (Regius unitea, Manufacturer-Konica Minolta) from rabbit in a standing position. The distance between the source of X-rays and the object was the same for all imaging. This allowed us to see the tablet in the body of stomach, antrum and or pyloric part of the stomach so that observations of the tablet movements could be made.[10]

RESULTS AND DISCUSSION

Fourier transforms infrared spectroscopy (FT-IR)

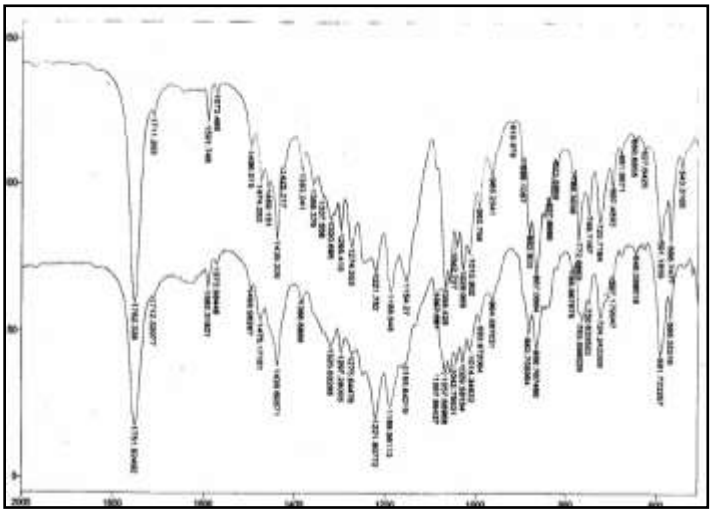


Figure 1: Overlay FTIR spectra of drug and excipient

The characteristic wave number peak of clopidogrel bisulfate was found to be 1753.03 cm^{-1}, 1651.18 cm^{-1}, 1174.39 cm^{-1} and 2924 cm^{-1} corresponding to C=0, C=C, C-O and C-H stretching respectively. Binary mixture of clopidogrel bisulfate with Swelstar showed no significant change

in the peak of the active ingredient indicating compatibility. Figure 1 presents an overlay graph of the active ingredient with the excipient.

Differential scanning calorimetry (DSC)

Clopidogrel bisulfate showed a sharp exothermic peak at 174.19 ^{0}C. Compatibility of Clopidogrel bisulfate along with the polymer Swelstar exhibited a sharp exothermic peak at 179.25 ^{0}C. It was observed that the onset temperature remains at ± 5 ^{0}C with the polymer Swelstar in the ratio of 1:2 indicating that the polymer is compatible.

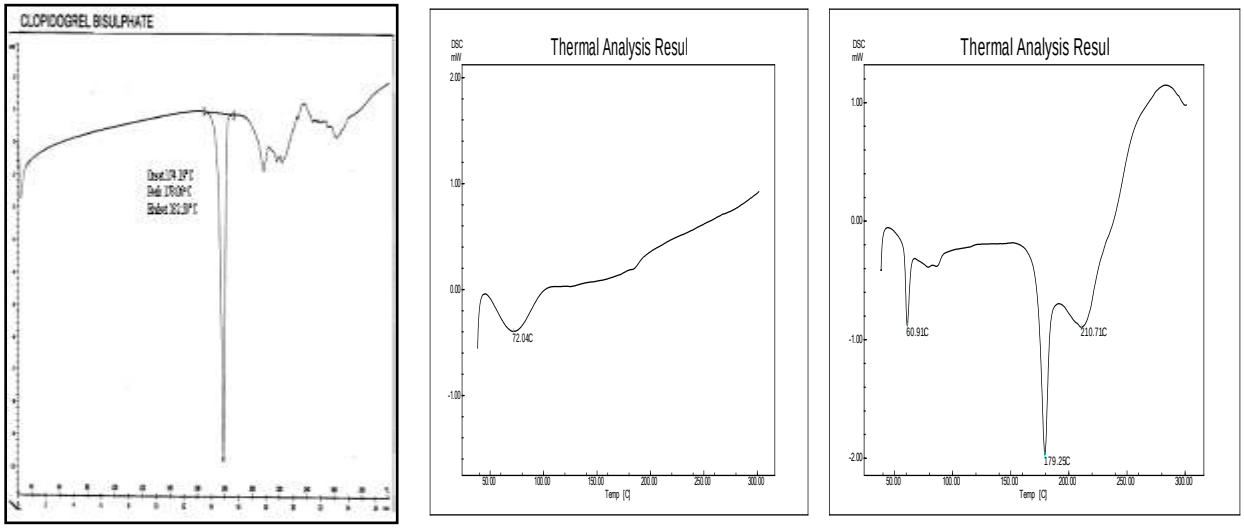
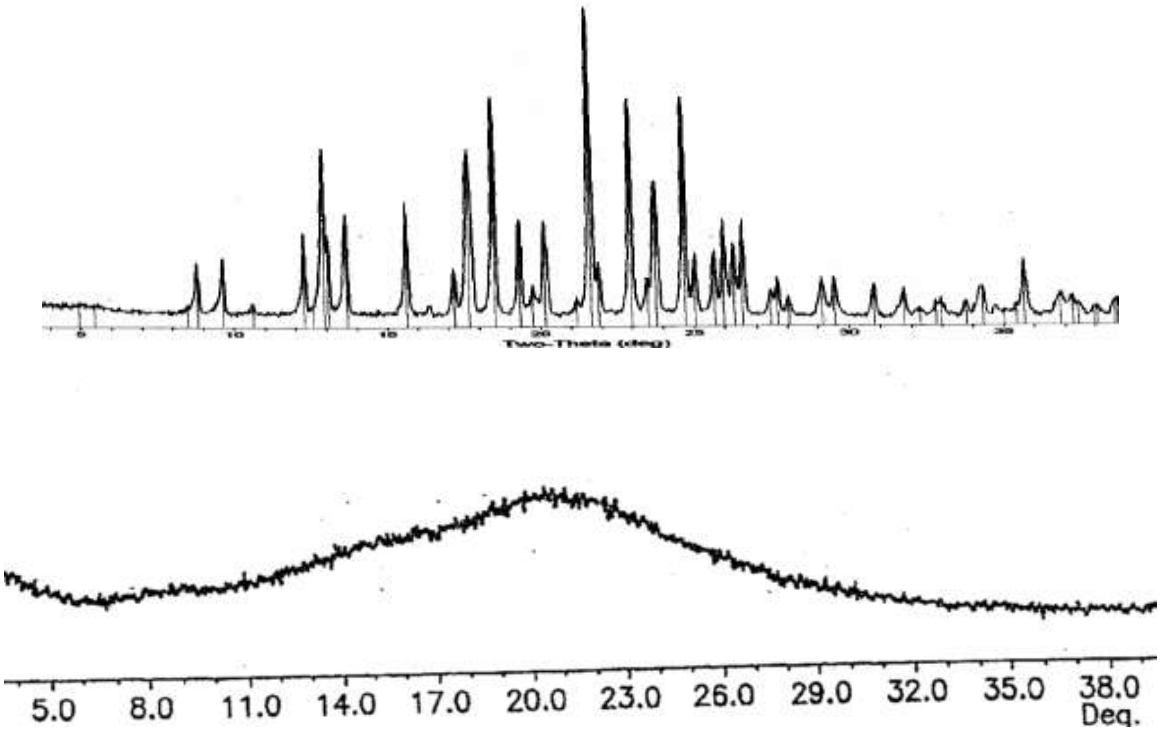


Figure 2: (a) **DSC of Clopidogrel bisulfate (b) DSC of Swelstar MX-1 (c) DSC of physical mixture clopidogrel bisulfate and Swelstar MX-I**



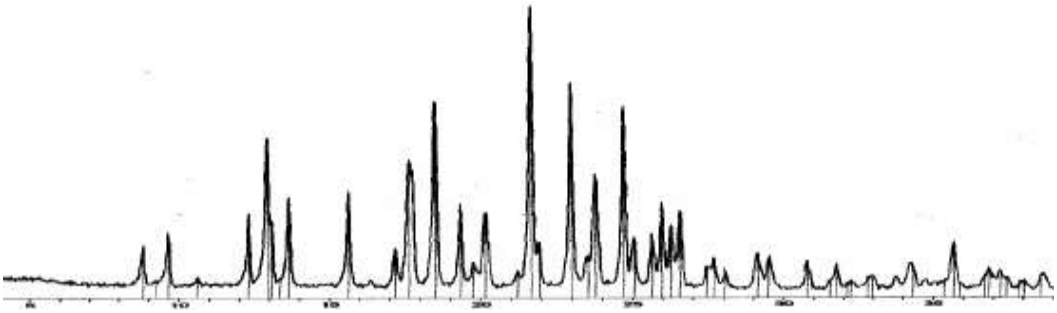


Figure 3: (a) Clopidogrel bisulfate (b) Swelstar MX-I (c) Swelstar and Clopidogrel bisulfate

p- XRD:

The 2- theta of clopidogrel bisulfate was found to be 21.5 and at d (A^O) 4.02 at height 4920. The mixture of clopidogrel and Swelstar shows no significant change in the 2-theta. The 2-theta value of clopidogrel sulfate confirms it's to be form II and the polymorphic form remains intact with rate controlling polymer Swelstar MX-I

In- *Vitro* Buoyancy

The effervescent technique uses sodium bicarbonate as a gas generating agent. The carbon dioxide gas which is formed remains ensnared in the gel, formed by the hydration of Swelstar, which finally results in decreasing the density of the tablet. The tablet starts to swell and float since the density of the tablets falls below 1 g/ml, hence the tablet floats. The effect of sodium bicarbonate on the floating behavior was evaluated at two levels of 34 and 36 mg per tablet. The in-vitro buoyancy results are shown in Table 2. Both the formulations maintained the matrix integrity for more than 24 hours and floating time of the formulations was more than 24 hours. It was observed that the quantity of carbon dioxide produced is proportional to the quantity of sodium bicarbonate used in the tablet. Hence the increase in the floating time was found in the formulation with 36 mg / tablet which can be attributed to the increased amount of carbon dioxide which gets entrapped in the gel and provides buoyancy. Hence, sodium bicarbonate at a quantity of 34 mg per tablet was essential to achieve optimum in vitro buoyancy.

Figure 4: Clopidogrel Bisulfate Floating tablet

Table 2. Buoyancy of formulated clopidogrel bisulfate tablets:

Sr. No.	Batch No	Floating lag time	Total floating time	Matrix integrity
1.	FC-1	120-150	>24 Hrs	Ok
2.	FC-2	90-110 sec	>24 Hrs	Ok
3.	FC-3	50-90 sec	>24 Hrs	Ok
4.	FC-4	20-45 sec	>24 Hrs	Ok

In *vitro* dissolution studies The result of in- vitro dissolution study has been listed in [Table 3]

Table 3. Dissolution results of Clopidogrel bisulfate tablets:

S.N.	Batch No	Cumulative % drug release ± SD							
		1 Hr	2 Hr	4 Hr	6 Hr	8 Hr	12 Hr	16 Hr	24 Hr
1	FC-1	25±2.1	48±1.8	62±3.4	79±1.7	92±2.8	102±1.7	102±1.4	102±1.2
2	FC-2	21±1.5	43±1.1	58±2.4	67±3.1	85±1.1	92±0.9	101±3.1	101±0.8
3	FC-3	20±1.7	39±1.4	49±1.5	56±2.5	65±2.6	76±1.4	85±1.9	98±1.1
4	FC-4	26±2.9	31±1.6	40±2.8	45±1.8	56±2.1	65±1.3	78±1.6	100±0.9

Effect of polymer concentrations over drug release

The influence of Swelstar on the release mechanism was studied at different levels. The dug release profile has been shown in Figure 1. The formulation containing higher quantity of the polymer showed dug release at the end of 24 hrs. The results indicate that the drug release decreases with increase in the concentration of polymers. At higher polymer, the matrix gel strength increases which results in the diffusion coefficient of the drug and finally retard the drug release mechanism. The drug polymer matrix changes from initial dry stage to gel like glossy rubbery state while the media is permeating though the tablet surface. The dug release is inversely proportional to the gel strength and the quantity of polymer used. The higher proportion of Swelstar in the clopidogrel tablet enables the formation of a thicker hydrogel layer and subsequently increases the time required for drug release.

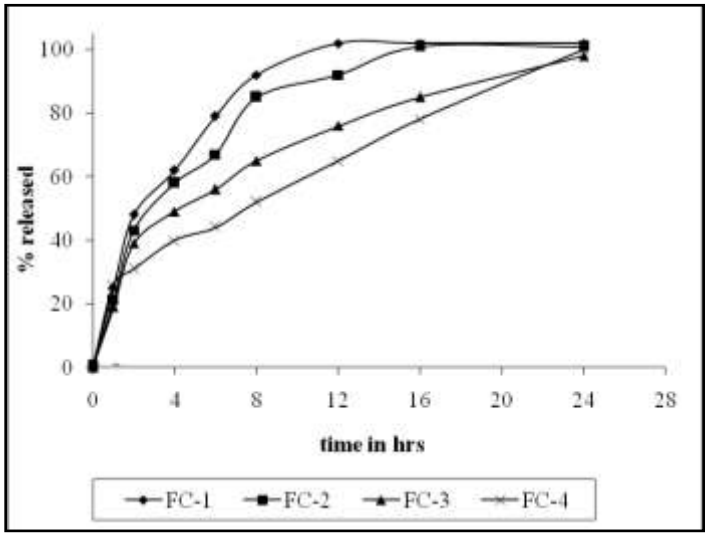


Figure 4 : Cumulative % drug released Vs time in Clopidogrel formulations

In-Vivo results

All the values are expressed as mean standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc tukey-Kramer Multiple Comparisons Test by employing statistical software, Graphpad InStat 3. Differences between groups were considered significant at $P < 0.05$ levels.

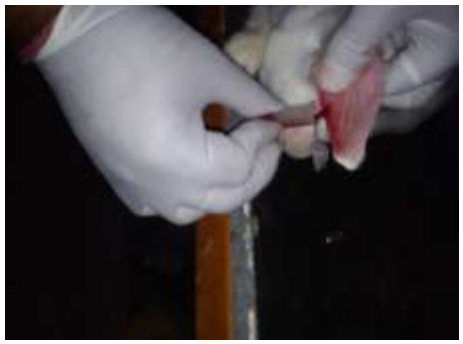


Figure 5: The blood collected in appendorff tube containing EDTA and bleeding time noted.

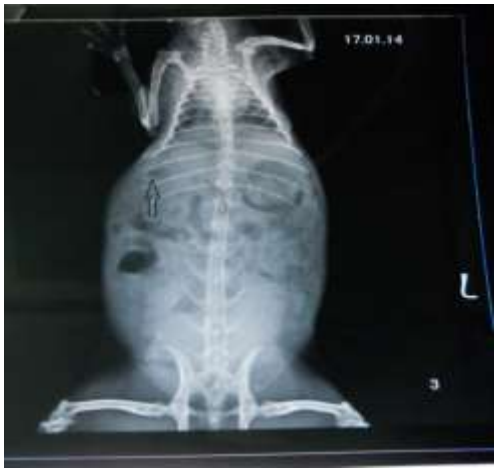


Figure 6: X-ray of the abdomen taken using an X-ray machine from rabbit in a standing position

Investigation on the effect of bleeding time, clotting time, Platelet count and Partial Thromboplastin Time of clopidogrel formulations in rabbits were done. The duration of bleeding time of reference and test formulations (solution & floating tablet) is summarized in Table 4 , in the test formulation (floating tablet) group It was found that bleeding time of clopidogrel floating tablets showed effect upto 12 hours and than decreased gradually in 24 hours. The results indicate that the Clopidogrel showed sustained effect compared to reference tablets. The test formulation (solution i.v.) showed maximum bleeding time after 1hour and than decreased significantly as compared to normal control. The clotting time of treated groups is summarized in Table 5, clotting time significantly increased in test formulation (floating tablet) group as

compared to normal control group up to 12 hours but clotting time regained to baseline in 24 hours. Results of Partial Thromboplastin Time of clopidogrel formulations are summarized in Table 6, result of the test formulation showed almost similar as clotting time in treated groups. No significant change in mean Platelet count, summarized in Table-7. On the basis of above results, it may be concluded that maximum concentration of the formulation (floating tablet) was found after 8 hours and then gradually decreased after 12 & 24 hours

Table 6: Effect of clopidogrel bisulfate formulations on partial thromboplastin time.

Group	Partial Thromboplastin Time (PTT) Mean ±S.E.M.	
	1 hr	8 hr
Group I	33.0±1.52	31.33±0.88
Group II	39.66±2.02	36.33±0.88a*
Group III	43.0±1.73a**	41.0±1.52a**
Group IV	34.66±0.88c*	38.33±0.88a**

All values are mean ± SEM, n = 3, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$,

a- significant difference as compared to normal control (Group-I)

b- Significant difference as compared to Group-II

c- Significant difference as compared to Group-III

Table 7: Effect of clopidogrel bisulfate formulations on platelet count.

Group	Platelet count, Mean ±S.E.M. ($X10^3/mm^3$)	
	1 hr	8 hr
Group I	336.33±4.66	339.66±7.17
Group II	341.0±8.96	343.33±13.28
Group III	352.66±8.87	352.0±6.11
Group IV	345.66±11.4	346.33±7.88

Table 4: Effect of clopidogrel bisulfate formulations on bleeding time.

Group	Bleeding time (Sec)					
	1 hr	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**
I	73.33±2.02	77.0±1.73	76.66±2.9	74.66±2.4	71.66±1.66	73.33±1.66
II	118.33±7.26a**	161.66±4.41a***	180.0±2.88a***	173.33±1.66a***	145.0±2.88 a***	123.33±4.41a***
III	253.33±4.41a***,b***	243.33±4.41a***, b***	233.33±3.33a***, b***	223.33±8.81a***, b***	190.0±5.77 a***,b***	138.33±4.41 a***
IV	90.0±5.77c***	143.33±7.26a***, c****	165.0±7.63a***,c ***	176.66±6.0a***,c ***	171.66±4.41a***,b **,c*	156.66±4.41a***,b **,c*

All values are mean ± SEM, n = 3, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, a- significant difference as compared to normal control (Group-I) b- Significant difference as compared to Group-II c- Significant difference as compared to Group-III

Table 5: Effect of clopidogrel bisulfate formulations on clotting time.

Group	Clotting time (Sec) Mean ± S.E.M.					
	1 hr	**2 hr**	**4 hr**	**8 hr**	**12 hr**	**24 hr**
I	37.0±1.73	35.66±1.45	33.66±1.2	32.66±1.45	32.33±2.18	30.33±1.45
II	42.33±2.33	43.0±0.57a*	45.33±0.88a***	43.33±0.66a***	39.0±0.57a*	31.33±0.88
III	43.66±1.85	43.33±1.76a*	45.0±1.0a***	45.0±1.15a***	40.0±0.57a*	31.33±1.85
IV	38.33±1.85	41.66±0.88a*	44.0±1.15a***	45.33±0.88a***	41.0±1.52a**	31.0±2.08

All values are mean ± SEM, n = 3, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, a- significant difference as compared to normal control (Group-I). b- Significant difference as compared to Group-II c- Significant difference as compared to Group-III

CONCLUSION

The effervescent based approach was used for preparation of floating tablets of clopidogrel bisulfate. Natural based polymer Swelstar MX-1 was used along with sodium bicarbonate as gas generating agent. It was observed that increasing the concentration of polymer retarded the drug release. Increasing the quantity of sodium bicarbonate decreased the floating lag time. The tablet matrix remained integrated up to 24 hours indicating that there is no influence of sodium bicarbonate on the matrix integrity. The clopidogrel parenteral was prepared by solubilizer Cremophor EL in aqeous based injection and the antiplatelet effect of clopidogrel was studied in-vivo on rabbits.

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