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ENHANCEMENT OF DISSOLUTION OF CLOPIDOGREL MINI-TABLETS IN INTESTINAL FLUIDS WITH THE AID OF IN-SITU ACIDIFYING AGENT

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ABSTRACT: The main objective of the present study is to enhance the dissolution of clopidogrel bisulphate in intestinal fluids by formulating into mini-tablets with in-situ acidifying agent mini-tablets. The solubility data reveals, Clopidogrel bisulphate is freely soluble in pH 2.0 and the solubility decreases with increase in pH. Whereas, with weak acid, the solubility of clopidogrel is increased at pH 5.0 blank fed state simulated intestinal fluid and & pH 6.5 blank fasted state simulated intestinal fluid. Hence, tartaric acid is selected in the core tablet to enhance the dissolution of clopidogrel tablets at intestinal fluid pH 5.0 & pH 6.5. Formulation was evaluated with different levels of tartaric acid in core tablet. Formulation with 50mg/unit of tartaric acid showed the extent of dissolution of 100% in pH 5.0 blank fed state simulated intestinal fluid and 96% in pH 6.5 blank fasted state simulated intestinal fluid. To control the impact of humidity on degradation, a moisture protective layer is coated on core tablet with non-aqueous solvent, using conventional coating pan. The coated mini tablets are encapsulated in a hard gelatin capsule shell. The filled capsules are evaluated for description, assay, dissolution, water by KF.

INTRODUCTION: Clopidogrel, an inhibitor of platelet aggregation, 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 [1]. Clopidogrel bisulfate is a white to off-white powder. It is practically insoluble in water at neutral pH but freely soluble at pH 1.0.

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Absorption is at least 50%, based on urinary excretion of clopidogrel metabolites [2]. The solubility of clopidogrel decreases as the pH increase above 3.0. It is well documented that the influence of the changes in pH within the gastrointestinal tract upon the bioavailability of pharmaceuticals.

The absorption of drug is largely dependent upon diffusion, which varies with pH of the individual regions within the gastrointestinal tract [3]. In the stomach the pH is around 1 to 2 and in the intestine the pH is between 5.0 -6.5. The intestinal fluids simulating to fasting and fed conditions exists in the pH range of 5.0 to 7.5 [4]. The absorption of drug is largely dependent upon diffusion, which varies with pH of the individual regions within the

gastrointestinal tract, the pKa of the drug and permeability, which are not only moderated by the surface area of the region in which it is released, but also the regional pH effects upon drug ionization.

By applying a pH change, poorly water soluble drugs with parts of the molecule that can be protonated (base) or deprotonated (acid) may potentially be dissolved in water. While the importance of critical parameters like salt selection and pH adjustment has been stressed on pre-formulation, the use of pH-altering excipients within drug delivery systems is also of significant utility. pH adjustment can in principle be used for both oral and parenteral administration [5].

MATERIALS AND METHODS:

Materials: The following chemicals were obtained from commercial suppliers and used as received: Clopidogrel bisulphate (Orchid chemicals and pharmaceuticals ltd., Chennai) Tartaric acid (Merck, USA), Opadry AMB white 80W68912 (Colorcon, India), Silicon dioxide (syloid 244 FP) (Grace division, USA), Microcrystalline cellulose (Avicel PH 112) (FMC, USA), Mannitol (pearlitol SD 200) (Roquette pharma, france), Hydrogenated castor oil (Kolwax HCO)(BASF, India), (Talc (Luzenac, Italy), pregealtinised starch (Starch 1500, Dow, USA), Isopropyl alcohol and methylene chloride was procured from RFCL Limited., New Delhi, India. All chemicals were reagent grade or higher.

Digital weighing balance (C-220) (make: Saritorious), Mechanical sifter with the screens of ASTM 40# & ASTM 60#, Octagonal blender 4L (Sams tech, India), 16 station rotary compression machine (Cadmach, India), conventional coating pan(a Remi mechanical propellant stirrer (RA124) (make: Remi), Manual capsule filling machine (MAC 300) (make: Pam machineries), Tray drier (make : Ganson engg), double beam UV Visible spectrophotometer (make: Schimadzu), Dissolution test apparatus (Electrolab),

Methods:

a. **Solubility of Clopidogrel Bisulphate at different pH:** Solubility evaluation for the biopharmaceutical classification of a substance is based on a determination of the equilibrium under conditions of physiological pH. The FDA suggests that the test be carried out at a temperature of 37 ± 1°C in aqueous media, varying the pH from 1 to 7.5 [6]. Buffer solutions are prepared as per EP 5.0[7]. In addition to that blank FaSSIF pH 6.5 buffer & blank FeSSIF pH 5.0 buffer was evaluated, as per the formula presented in **table 1**.

TABLE 1: FORMULA FOR PREPARATION OF FASTED AND FED STATE SIMULATED INTESTINAL FLUID.

Blank Fasted State Simulated Intestinal Fluid pH 6.5		Blank Fed State Simulated Intestinal Fluid pH 5.0	
Sodium dihydrogen phosphate monohydrate	3.438g	Glacial acetic acid	8.650g
Sodium chloride	6.186g	Sodium chloride	11.874g
Sodium hydroxide	0.348g	Sodium hydroxide	4.04g
Deionised water qs to	1 litre	Deionised water qs to	1 litre

Clopidogrel bisulphate was evaluated for saturated solubility in the following media (0.1N Hcl(pH 1.2), pH 4.5 Acetate buffer, pH 6.8 Phosphate buffer, pH 7.5 phosphate buffer, Purified water, blank FaSSIF, blank FeSSIF), by shake flask method, and the resulting solution was filtered through 0.45micron filter, measured for the absorbance using UV-Visible spectrophotometer.

b. **Preparation of Mini-Tablets [8]:** Formulation of clopidogrel mini tablets involves 3 stages

   a) Stage – I: Granulation and blending

   b) Stage - II: Compression

   c) Stage - III: Film coating

Stage I- Granulation & Blending:

- Clopidogrel, tartaric acid, Mannitol, microcrystalline cellulose is co -sifted through ASTM 40#.
- Hypromellose E5 LV is dissolved in methylene chloride and isopropyl alcohol. The above sifted materials are loaded in fluid bed processor GPCG 1.1, and granulated using Hypromellose E5LV solution; the granules are passed through ASTM 30#.

- Talc and silicon dioxide are sifted through ASTM 60#, loaded in to blender and mixed for 15 minutes, at 15 RPM.
- Hydrogenated castor oil is sifted through ASTM 60#, loaded into the blender and mixed for 5 minutes. Each formula was having the batch size of 4000 units.

Stage II- Compression:

- The lubricated blend was compressed using 2.5mm multi-tip punch with the target weight of 20mg/unit and 8 units/unit dose.
- The compressed mini-tablets were evaluated for hardness, thickness and disintegration time.
- The lot size for coating was 4000 units.

Stage III- Film coating:

- Opadry AMB white 80W50612 suspended in Isopropyl alcohol and methylene chloride admixture under stirring.
- Stirring for continued for 30 minutes.
- The resultant suspension was coated on compressed mini-tablets with different percentage weight gain by using conventional coating pan.
- During the preparation of coating solution the 10% of excess was prepared to recover the loss during practical work. And the coating solution was sprayed over barrier coated pellets using Fluid bed coater until weight gain was achieved and % yield was calculated.
- The solid content of film coating suspension was 7% w/w

Encapsulation:

- The coated mini-tablets were filled in to size "0" hard gelatin capsules, and evaluated for assay and dissolution.

TABLE 2: COMPOSITION OF CLOPIDOGREL FILM COATED MINI-TABLETS

S. No.	Ingredients	F21	F22	F23	F24	F25
1	Clopidogrel Bisulphate	97.875	97.875	97.875	97.875	97.875
2	Tartaric acid	-	15	20	25	30
3	Hypromellose E5LV	5	5	5	5	5
3	Mannitol	50.125	35.125	30.125	25.125	20.125
5	Microcrystalline cellulose (Avicel PH112)	75	75	75	75	75
6	Silicon dioxide (Syloid 244FP)	3	3	3	3	3
7	Talc	3	3	3	3	3
8	Hydrogenated castor oil	6	6	6	6	6
	Sub total	**240**	**240**	**240**	**240**	**240**
	Film coating					
1	Opadry AMB white	14.4	14.4	14.4	14.4	14.4
2	Methylene chloride	qs	qs	qs	qs	qs
3	Isopropyl alcohol	qs	qs	qs	qs	qs
	Total	**254.4**	**254.4**	**254.4**	**254.4**	**254.4**

TABLE 3: IN-PROCESS PARAMETERS AT VARIOUS STEPS:

Parameters	Compression	Coating
Description	White to off-white circular biconvex mini tablets	White to off-white circular biconvex mini tablets
Uniformity of tablet weight	20mg ± 2mg	Target weight ± 10%
Hardness (N)	10-20N	20-40N
Thickness (mm)	3.1- 3.5mm	3.1- 3.5mm
Disintegration time	NMT 15 min	NMT 15 min

Stability study [9]: Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. FDA and ICH specify the guidelines for stability testing of new drug products, as a technical requirement for the registration of pharmaceuticals for human use.

The ICH Guidelines have established different temperatures and period of stability testing. The film coated mini tablets of formulation F24 was filled in size'0'capsules, packed in HDPE bottle, and loaded on stability chamber as per ICH guidelines, as mentioned in **table 4**.

TABLE 4: ICH GUIDELINES FOR STABILITY STUDY

Study Storage	Condition	Time
Long term	25°C±2°C / 60% RH±5% RH	12 month
Intermediate	30°C±2°C /65% RH±5% RH	12 months
Accelerated	40°C±2°C /75% RH±5% RH	6 months

To evaluate the impact of barrier coating in short period, the product is evaluated at accelerated stability condition.

Evaluation of clopidogrel film coated mini tablets

1. **Uniformity of Tablet Weight Test [8]:** Ten capsules from the batch were randomly selected, individual weight of the selected representative was determined using a digital electronic balance. The average tablet weight and the standard deviation from the mean were calculated.

2. **Capsule disintegration Test [8]:** Six capsules randomly selected were introduced into the six baskets of the disintegration testing apparatus (Electrolab, India). The disintegrating medium was de ionized water maintained at 37°c + 1.0°c. The time taken for each capsule to disintegrate to break up into a smaller units and passes through the screen mesh orifices at the bottom of the basket was recorded.

Dissolution [10]:

Medium : pH 2.0 HCl

Apparatus : apparatus 1 (basket)

Speed : 50 rpm

Temperature : 37±0.5°C

Run time : 60 mins

Procedure: 6 capsules were placed in each of 6 dissolution flasks containing 900 ml of pH 2.0 HCl, previously maintained at 37±0.5°C. The apparatus was run for 60 minutes. A suitable volume of sample was withdrawn at regular intervals of time and filtered through 0.45 µm membrane filter.

The absorbance of the sample preparations were measured at 249 nm, using pH 2.0 HCl as blank.

Assay [11]: Accurately weighed the contents 10 capsules of clopidogrel mini-tablets filled in capsules and fine powdered.

The powder equivalent to 100mg of Clopidogrel bisulfate was transferred to 100ml volumetric flask and 20ml distilled water pH 1 solution is added and sonicated for 15 minutes to dissolve the Clopidogrel bisulfate in it and made the volume to mark with same.

The solution was filtered through Whatmann filter paper No.40. 10 ml of this was diluted with distilled water pH 1 solution diluted with same as blank.

The concentration of Clopidogrel bisulfate present in marketed tablet formulation was determined.

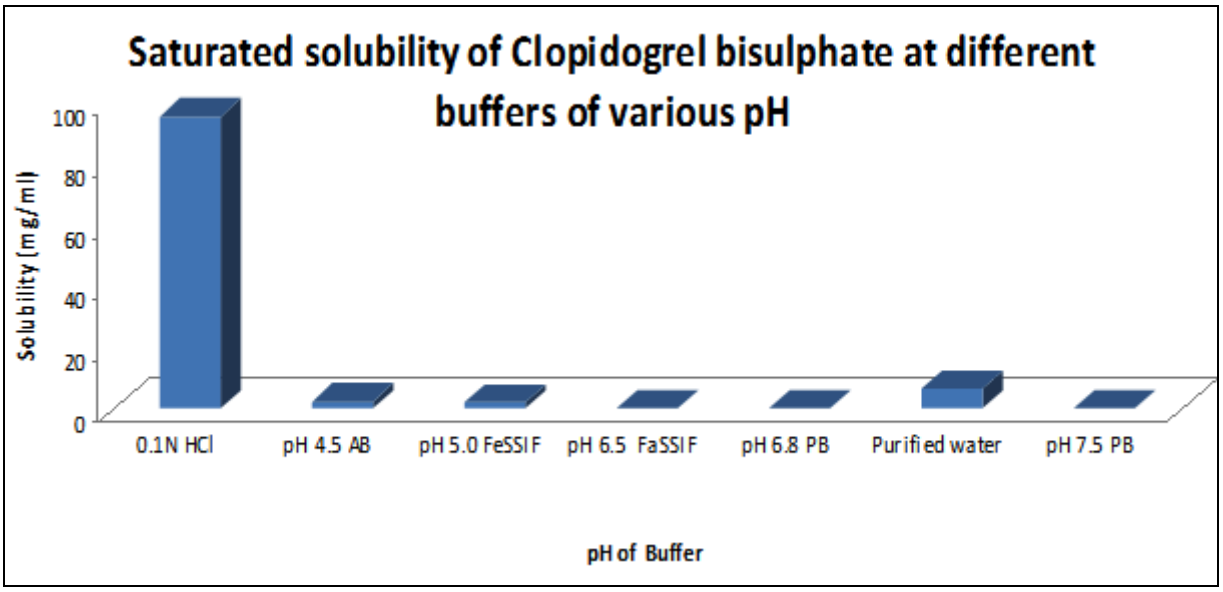
Water content [12]**:** Water content of capsule was evaluated by direct titrimetric method, as per USP 34–NF 29, physical tests/⟨921⟩ water determination and the values are presented in **table 7.**

RESULTS & DISCUSSIONS:

a) **Solubility study:** The saturated solubility of clopidogrel bisulphate is evaluated in various buffers, the results are tabulated below:

TABLE 5: SOLUBILITY OF CLOPIDOGREL BISULPHATE IN VARIOUS BUFFERS:

S. No.	Buffers	Solubility of Clopidogrel bisulphate (mg/ml)	Solubility of Clopidogrel bisulphate (mg/250ml)
1	0.1N HCl	95.13	23781.70
2	pH 4.5 Acetate buffer	2.30	574.53
3	pH 5.0 blank FeSSIF	1.97	492.00
4	pH 6.5 blank FaSSIF	0.017	4.13
5	pH 6.8 phosphate buffer	0.010	2.45
6	Purified water	6.50	1625.00
7	pH 7.5 phosphate buffer	0.015	3.70



GRAPH 1: SATURATED SOLUBILITY OF CLOPIDOGREL BISULPHATE AT DIFFERENT BUFFERS OF VARIOUS pH

The results of solubility study reveal that the drug exhibits poor solubility from pH 4.5 onwards. Hence, a solubility enhancer is required to improve the dissolution in pH 4.5 Acetate buffer, pH 5.0 FeSSIF, pH 6.5 FaSSIF, pH 6.8 phosphate buffers, purified water & pH 7.5 phosphate buffers.

Physical Characterization of Clopidogrel mini-tablets filled in capsules:

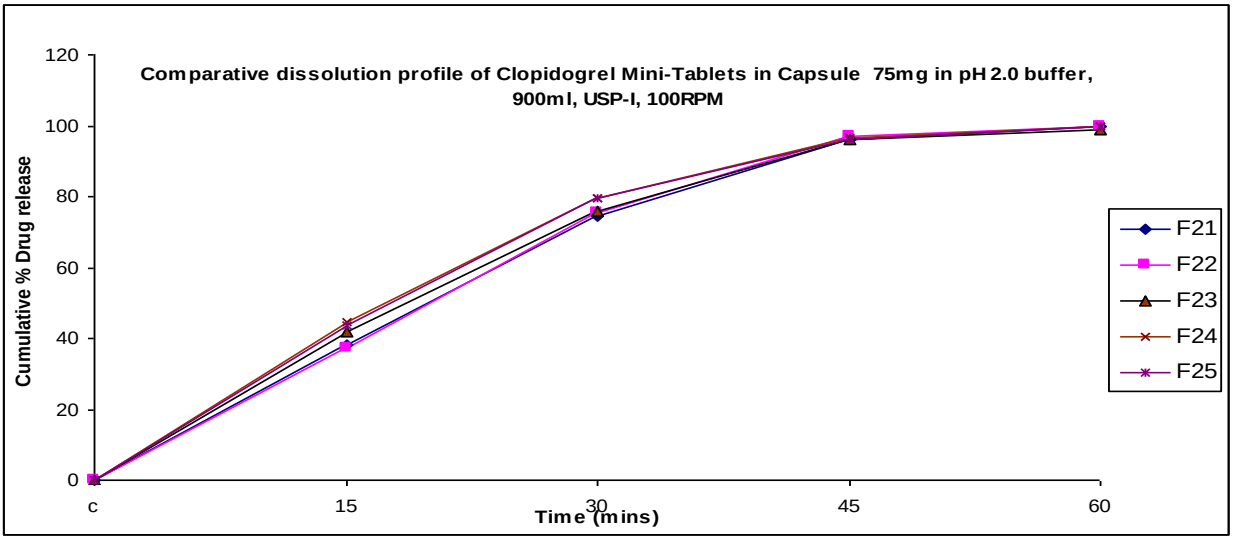
TABLE 6: PHYSICAL CHARACTERIZATION OF CLOPIDOGREL MINI-TABLETS FILLED IN CAPSULES

Parameters	Specification	Batch Number				
		F21	F22	F23	F24	F25
Description	white to off-white colored mini tablets filled in hard gelatin capsule	white to off-white colored mini tablets filled in hard gelatin capsule	white to off-white colored mini tablets filled in hard gelatin capsule	white to off-white colored mini tablets filled in hard gelatin capsule	white to off-white colored mini tablets filled in hard gelatin capsule	white to off-white colored mini tablets filled in hard gelatin capsule
Number of mini-tablets per capsule	**12**	12	12	12	12	12
Uniformity of tablet weight	**Mean ± SD**	21.3 ± 0.10	21.2 ± 0.08	21.2 ± 0.12	21.3 ± 0.16	21.2 ± 0.08
Disintegration time	**NMT 15 min**	5 min 20 sec	4 min 15 sec	3 min 45 sec	2 min 40 secs	2 min 45 sec

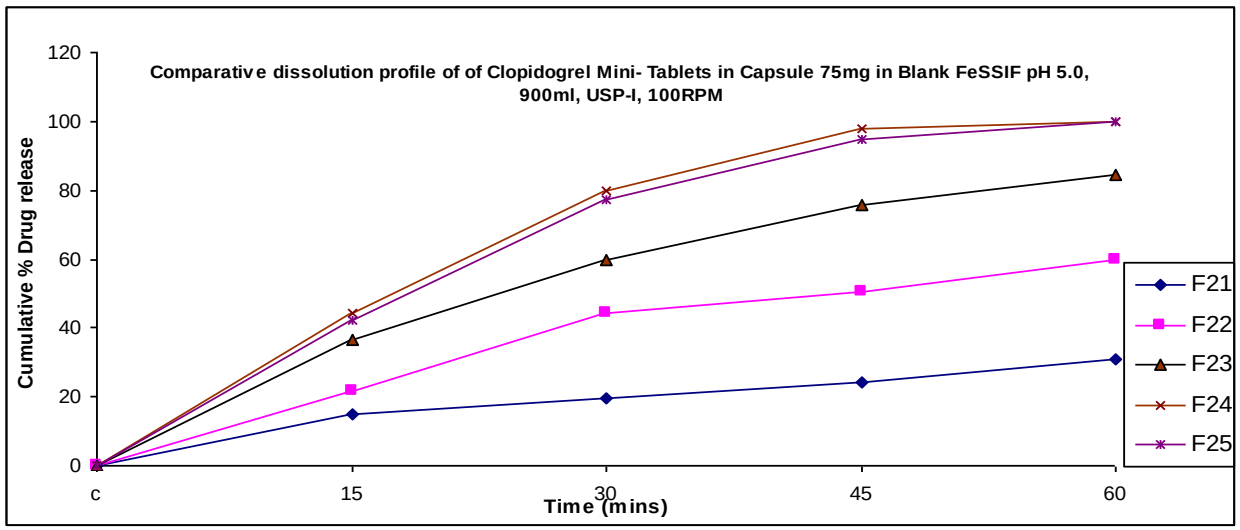
Chemical Characterization of Clopidogrel mini tablets filled in capsules:

TABLE 7: CHEMICAL CHARACTERIZATION OF CLOPIDOGREL MINI-TABLETS FILLED IN CAPSULES

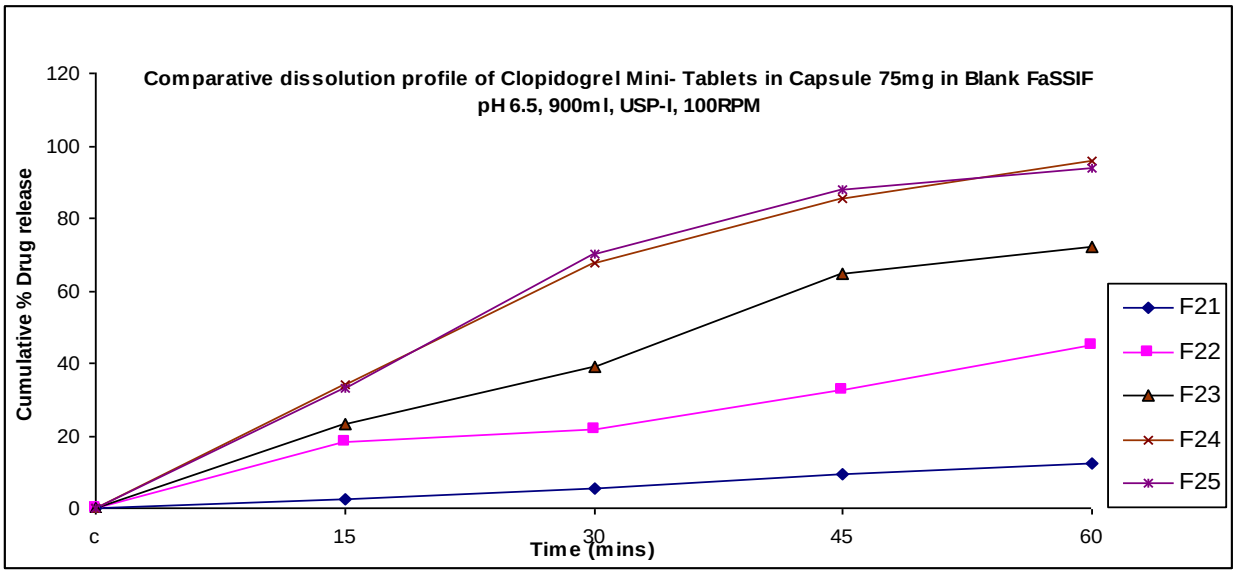
Parameters	Specification		Batch Number				
			F21	F22	F23	F24	F25
Assay	90-110 % of label claim		100.7 ± 0.81	99.9 ± 0.35	100.5 ± 0.06	100.3 ± 0.42	100.3 ± 0.8
Water (by KF) (% w/w)	NMT 4		1.25	1.42	1.5	1.52	1.54
Dissolution in pH 2.0 (HCl, 900ml, USP-I, 100RPM)	NLT 75%(Q) in 45 min	15 min	38.3 ± 0.6	37.3 ± 1.2	41.7 ± 0.6	44.7 ± 0.6	43.7 ± 0.6
		30 min	74.7 ± 0.6	75.3 ± 1.5	76 ± 2.0	79.7 ± 1.5	79.7 ± 1.5
		45 min	96 ± 1.7	97.0 ± 1.0	96.3 ± 0.6	96.7 ± 0.6	96.3 ± 0.6
		60 min	100 ± 1.0	100.0 ± 1.0	99 ± 1.0	100 ± 1.0	99.7 ± 1.2
Dissolution in pH 5.0 FeSSIF (900ml, USP-I, 100RPM)	NLT 75%(Q) in 45 min	15 min	14.7 ± 0.6	21.7 ± 0.6	36.7 ± 0.6	44.3 ± 0.6	42.3 ± 1.5
		30 min	19.7 ± 0.6	44.3 ± 2.1	60 .0 ± 1.0	79.7 ± 1.5	77.0 ± 1.0
		45 min	24.3 ± 0.6	50.3 ± 2.1	75.7 ± 0.6	97.7 ± 0.6	94.7 ± 0.6
		60 min	30.7 ± 1.5	60.0 ± 1.0	84.7 ± 0.6	99.9 ± 0.1	100.0 ± 1.0
Dissolution in pH 6.5 FaSSIF (900ml, USP-I, 100RPM)	NLT 75%(Q) in 45 min	15 min	2.3 ± 1.1	18.3 ± 0.6	23.3 ± 1.2	34.0 ± 1.7	33.3 ± 1.2
		30 min	5.6 ± 1.2	21.7 ± 0.6	39.0 ± 1.0	67.7 ± 0.6	70.3 ± 1.2
		45 min	9.5 ± 0.2	32.7 ± 1.2	64.7 ± 0.6	85.3 ± 0.6	87.7 ± 0.6
		60 min	12.4 ± 0.5	45.0 ± 1.0	72.0 ± 1.0	95.7 ± 0.6	94.0 ± 1.0



GRAPH 2: COMPARATIVE DISSOLUTION PROFILE OF CLOPIDOGREL MINI-TABLETS FILED CAPSULES IN pH 2.0 HYDROCHLORIDE BUFFER



GRAPH 3: COMPARATIVE DISSOLUTION PROFILE OF CLOPIDOGREL MINI-TABLETS FILED CAPSULES 75MG IN BLANK FED STATE SIMULATED INTESTINAL FLUID pH 5.0



GRAPH 4: COMPARATIVE DISSOLUTION PROFILE OF CLOPIDOGREL MINI-TABLETS FILED CAPSULES 75MG IN BLANK FASTED STATE SIMULATED INTESTINAL FLUID pH 6.5

Results indicated that batch number F21, F22 & F23 are showing poor extent of dissolution in blank FeSSIF pH 5.0 buffer and blank FaSSIF pH 6.5 buffer. Whereas, the batch number F24 & F25 shown the better extent of dissolution in blank FeSSIF pH 5.0 buffer and blank FaSSIF pH 6.5 buffer. From the results, it was concluded that 25mg of tartaric acid per unit is required to solublize 75mg of clopidogrel in simulated intestinal fluids.

Accelerated stability study of Clopidogrel min-tablets filled in capsules: The filled capsules are packed in HDPE container with 30's count, loaded in accelerated stability condition. Initial and 3M accelerated stability samples of the filled capsules were evaluated for Description, Assay, water content and dissolution. The results are tabulated in **Table 8**.

TABLE 8: ASSAY, WATER BY KF AND DISSOLUTION: (INITIAL VS 3M ACCELERATED CONDITION)

Accelerated Stability data of Clopidogrel Mini-tablets filled in capsules			
Batch Number : ASC-C-F24		**Pack: HDPE bottle with 30's count**	
Parameters	**Specification**	**Initial**	**3M 40/75**
Description	white to off-white colored mini tablets filled in white opaque hard gelatin capsule	white to off-white colored mini tablets filled in white opaque hard gelatin capsule	white to off-white colored mini tablets filled in white opaque hard gelatin capsule
Assay	NLT 90% and NMT 110% of label claim	100.3 ± 0.42	99.7 ± 0.32
Water (by KF) (% w/w)	NMT 4	1.52	1.98
Dissolution in pH 2.0 (0.01N HCl, 900ml, USP-I, 100RPM)	NLT 75 (Q) % in 45 min	97 ± 0.6	93 ± 1.5

*Listed value indicates mean value of results and Standard deviation (Where n=3)

The above data reveals, that batch number ASC-C-F24 is not having significant difference from initial to 3M accelerated condition in Assay, dissolution, water content. The description remains unchanged from initial to accelerated condition.

CONCLUSION: The saturated solubility result concludes that Clopidogrel bisulphate exhibits poor solubility above pH 4.5.

A weak acid was evaluated from 15mg/unit to 30mg/unit. With the aid of weak acid 25mg per unit, the product's dissolution was improved in blank fasted state simulated fluid pH 6.5 buffer and blank fed state simulated fluid pH 5.0 buffer. The formulation was evaluated at accelerated condition for 3 months, and no significant change was observed, while comparing to initial.

Hence, the formulation with 25mg of tartaric acid per unit was finalized as stable formulation.

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