



Research Article

DISSOLUTION METHOD DEVELOPMENT AND ENHANCEMENT OF SOLUBILITY OF CLOPIDOGREL BISULFATE

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ABSTRACT

Dissolution of drug is the rate determining step for oral absorption of the poorly water soluble drugs and solubility is the basic requirement for the absorption of the drug from GIT. The development of a suitable dissolution procedure for drug products with lower water solubility has been a provocation to both the pharmaceutical industry and the agencies that regulate them. This study demonstrates the systematic development of a discriminatory dissolution method for clopidogrel bisulfate, a BCS class II drug exhibiting highly pH-dependent solubility. The method was developed by testing solubility of clopidogrel bisulfate in different concentrations of sodium lauryl sulfate. The results showed 900 ml of 0.1 N HCl and phosphate buffer pH 6.8 containing 1% SLS with a paddle speed of 50 rpm was selected as discriminative dissolution test conditions for clopidogrel bisulfate tablets. In conclusion surfactants and pH changes are very effective ways to increase solubility. The *in vitro* dissolution must serve as both a quality control tool and a potential surrogate marker of drug bioavailability and bioequivalence.

Keywords: Clopidogrel bisulfate, Dissolution, BCS Class II, phosphate buffer

INTRODUCTION

Development of the dissolution method for poorly soluble or insoluble drug has been a challenge for scientists. Drugs with limited water solubility are usually lipophilic, and drug release is usually the rate-limiting process for oral drug absorption of these substances¹.

To release the drug from the dosage form it may involve mainly two steps: the disintegration which ensure liberation of the drug from the formulation matrix, then followed by the dissolution of the drug in the liquid medium².

According to the biopharmaceutical classification system (BCS) of drugs, the class II category has low solubility and high permeability drugs and identified as potential drug candidates for investigation. Bioequivalence problems arise in class II and class IV categories of the biopharmaceutical classification of drugs. The low solubility aspects can be handled in order to develop a biorelevant and discriminating method for dissolution.

A dissolution medium containing surfactant can better simulate the environment of the gastrointestinal tract than a medium containing organic solvents or other non-physiological substances, making the dissolution test conditions more useful in evaluating drug quality³⁻⁴.

Generally, when developing a dissolution procedure, one goal is to have sink conditions, which defined as the volume of medium at least three times that required in order to form a saturated solution of drug substance as outlined in USP⁵.

It has also been recommended that the drug concentration in the dissolution medium should not exceed 15% to 20% of saturation solubility of the drug in order to provide sink conditions. Non

sink conditions may give unpredictable release kinetics and suppression of release profiles⁶.

Clopidogrel bisulfate is a thienopyridine antiplatelet drug known chemically as methyl (+)-(*S*)- α -(2-chlorophenyl)-6,7-dihydrothieno[3,2-*c*] pyridine-5(4*H*)-acetatesulfate (1:1). It has a chemical formula (C₁₆H₁₆ClNO₂S.H₂SO₄) and a molecular weight of 419.9 g/mol. It has a dissociation constant (pK_a) value of 4.55⁷⁻⁸. The chemical structure of clopidogrel bisulfate is shown in (Figure 1).

Clopidogrel bisulfate appears as a white to off-white powder. It has a melting point of about 184°C. It is practically insoluble in water at neutral pH; freely soluble in aqueous buffer at pH 1, and in methanol, sparingly soluble in methylene chloride, and practically insoluble in ethyl ether⁸⁻³.

The dissolution medium available in the monographs for clopidogrel bisulfate consisted of 1000 mL of a pH 2.0 hydrochloric acid buffer; the objective of this study was to determine the saturation solubility of the drug in different buffer and the effect of surfactant concentration to obtain sink condition suitable for the dissolution of clopidogrel bisulfate solid oral dosage forms by using phosphate buffer pH 6.8 to support product development and quality control efforts.

MATERIALS AND METHODS

Clopidogrel bisulfate sample was supplied by Zhejiang Menovo Pharmaceutical Co., LTD, China. Citric acid monohydrate was supplied from Mumbai, India. Di-sodium hydrogen-o-phosphate Na₂HPO₄ from Qualikems Fine Chem Pvt.Ltd. India. Sodium lauryl sulfate from Sd Fine-Chem limited Mumbai, India. All reagents used were of analytical grade.

Calibration Curves of Clopidogrel Bisulfate

Standard solutions of clopidogrel bisulfate in 0.1 N HCl and phosphate buffer (pH 6.8) solutions were prepared from stock solutions. Clopidogrel bisulfate stock solution of concentration (0.12 mg/ml) was prepared; samples were analyzed spectrophotometrically at the wave length of maximum absorbance in 0.1N HCl and in phosphate buffer (pH 6.8) with 1% sodium lauryl sulfate. Absorbance was recorded and plotted versus concentration⁹.

Determination of Clopidogrel Bisulfate Saturation Solubility

The solubility of clopidogrel bisulfate in various solutions was determined by saturation solubility technique. An excess amount of clopidogrel bisulfate was added separately to 20 ml of 0.1 N HCl (pH 1.2), acetate buffer solutions at pH (3.5, 4, 4.5 and 5.12), and phosphate buffer solution at pH (6.6, 6.8).

The saturation solubility of clopidogrel bisulfate in phosphate buffer (pH 6.8) solution containing 0.25%, 0.5% and 1% w/v of sodium lauryl sulfate as a surfactant was also determined. The mixtures were shaken for 24 hours at 37°C then filtered using filter paper 0.45µm and the filtrate was diluted suitably so that can be analyzed spectrophotometrically (at wave length specific for each media) for the amount dissolved^{10,11}.

Preparation of Phosphate Buffer pH 6.8

The composition of phosphate buffer pH 6.8 was shown in (Table 1), equation (1) was used to prepare 100 ml of buffer solution in which the value of x is 23.5.

$$X \text{ ml (A)} + (100-x) \text{ ml (B)} \text{ ----- equation (1)}^{12}$$

In Vitro Dissolution Profile of Clopidogrel Bisulfate Tablets

In vitro dissolution study was performed using USP dissolution test apparatus-II (paddle assembly). The dissolution was performed using 900 ml of 0.1 N HCl solution (pH 1.2) and phosphate buffer solution (pH 6.8) (containing 1% SLS) as dissolution media maintained at $37 \pm 0.5^\circ\text{C}$ and 50 rpm.

Samples (5ml) were withdrawn at regular intervals of 10 minutes for 60 minutes in 0.1N HCl (pH 1.2) and 120 minutes in phosphate buffer solution and replaced with fresh dissolution medium to maintain sink condition. Samples were filtered through filter paper and assayed spectrophotometrically on UV-visible spectrophotometer at 270 nm wavelength for 0.1 N HCl (pH 1.2) and at 269 nm for phosphate buffer solution (pH 6.8). For each formulation, the release was repeated in triplicate, results are expressed as a mean \pm S.D.

Table 1: Composition of Phosphate Buffer Solution (pH 6.8)¹²

Category	Material	Concentration (g/L)
A	Citric acid (monohydrate) C ₆ H ₈ O ₇ · H ₂ O	21.01
B	Disodium hydrogen phosphate (dihydrate) Na ₂ HPO ₄ · 2H ₂ O	35.6

Table 2: Saturation Solubility of Clopidogrel Bisulfate

Buffer Solutions	Solubility (mg/ml)
pH 1.2	40
pH 3.5	1.79
pH 4	0.279
pH 4.5	0.171
pH 5.12	0.083
pH 6.6	0.082
pH 6.8	0.079
pH 6.8 + 0.25% (w/v) SLS	0.1
pH 6.8 + 0.5% (w/v) SLS	0.3
pH 6.8 + 1% (w/v) SLS	2

Table 3: Saturation Solubility and Relative Sink Conditions of Clopidogrel Bisulfate in Phosphate Buffer (pH 6.8) at Different Surfactant Concentrations

Buffer Solution	Solubility (mg/ml)	Sink condition (Cs/Cd)
pH 6.8 + 0.25% (w/v) SLS	0.1	0.92
pH 6.8 + 0.5% (w/v) SLS	0.3	2.8
pH 6.8 + 1% (w/v) SLS	2	18.4

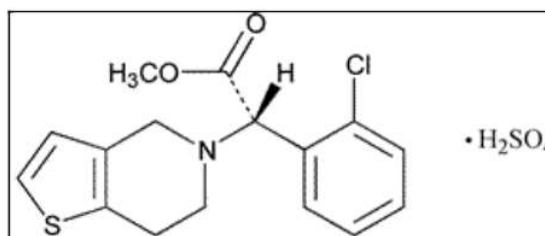


Figure 1: Chemical structure of clopidogrel bisulfate⁸

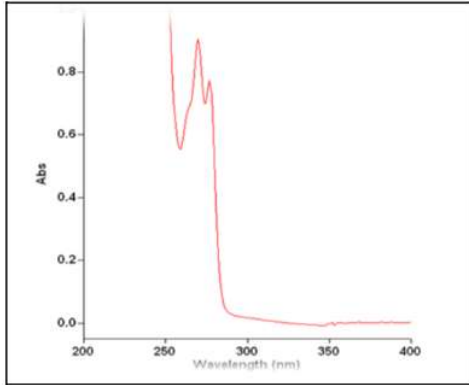


Figure 2: UV scan of clopidogrel bisulfate in 0.1 N HCl (pH 1.2)

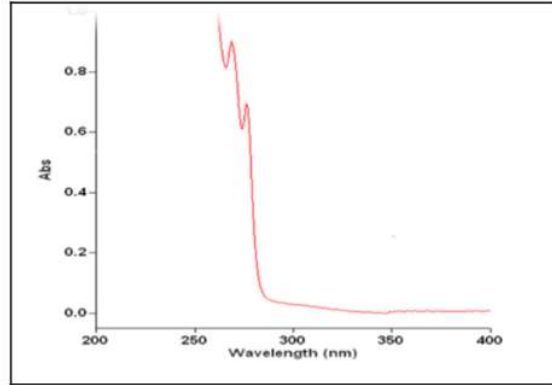


Figure 3: UV scan of clopidogrel bisulfate in phosphate buffer pH 6.8 with 1% w/v sodium lauryl sulfate

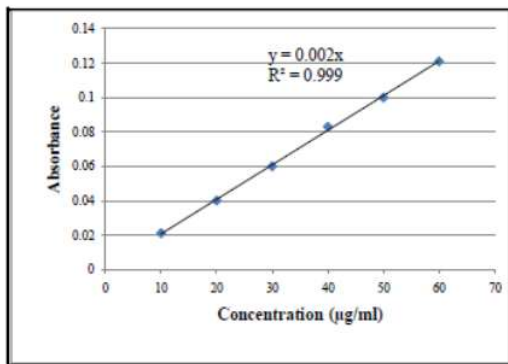


Figure 4: Calibration curve of clopidogrel bisulfate in 0.1N HCl (pH 1.2)

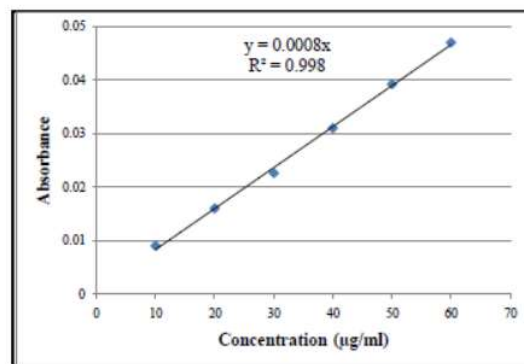


Figure 5: Calibration curve of clopidogrel bisulfate in phosphate buffer solution (pH 6.8) containing 1% SLS

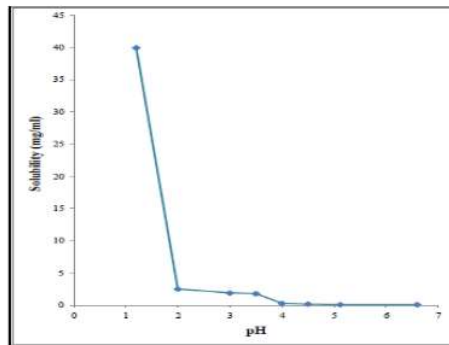


Figure 6: pH-solubility profile of clopidogrel bisulfate

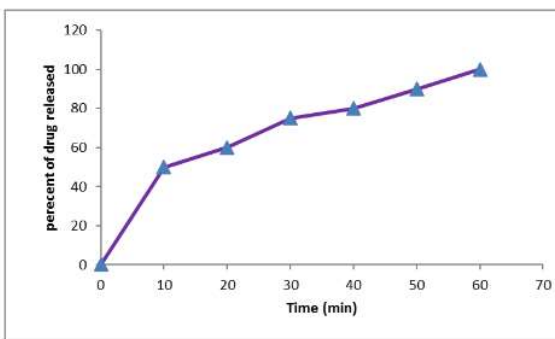


Figure 7: Dissolution profile of clopidogrel bisulfate tablet in 0.1N HCl

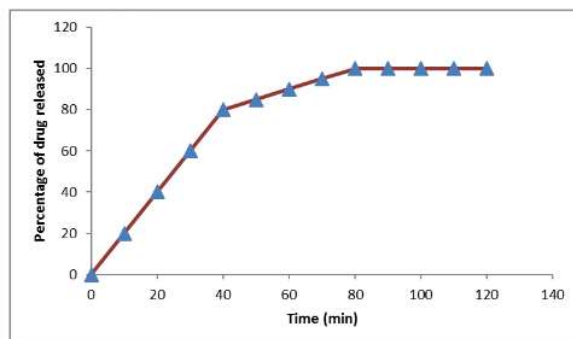


Figure 8: Dissolution profile of clopidogrel bisulfate tablet in phosphate buffer pH 6.8 with 1% w/v SLS

RESULTS AND DISCUSSION

The UV scan of standard solutions between 200–400 nm showed the absorption maxima at 270 nm, 277 nm in 0.1N HCl and at 269 nm, 276 nm in phosphate buffer solution pH 6.8 as shown in (Figure 2 and 3). This means that a change in the pH of buffer causes shifting in the λ_{max} .

The Beer's law was verified from the calibration curve by plotting a graph of concentration versus absorbance, the plot shown in (Figure 4 and 5). Regression analysis showed very good correlation. The calibration plot revealed zero intercept which is clear by the regression analysis equation $Y = mX + C$ (where Y is absorbance, m is the slope and X is the concentration) the results obtained which indicate that these curves obey Beer- Lambert's law within the concentrations used¹³.

Determination of Clopidogrel Bisulfate Saturation Solubility

According to the Biopharmaceutics Classification System (BCS), clopidogrel bisulfate is categorized as a class II agent (poorly water soluble and highly permeable)¹⁴. The results of saturation solubility of clopidogrel bisulfate are showed in (Table 2). It showed wide variation in solubility in physiological pH range. Clopidogrel bisulfate exhibited higher solubility in pH 1.2 of 0.1N HCl. In case of pH 6.8 phosphate buffer, solubility was found to be less as compared to solubility in acidic conditions. These findings are highly expected because clopidogrel is weak base with pKa value of 4.5¹⁰. Therefore, clopidogrel solubility is strongly pH dependent and it is very soluble at pH value < 3 ¹⁵.

Sodium lauryl sulfate (SLS) is an anionic surfactant which lowers the surface tension of the medium in which it is dissolved, and/or the interfacial tension with other phases. It has high hydrophilic-lipophilic balance (HLB) value of 40, but the solubilization process depends on the concentration of SLS added^{16, 17}. SLS is the most popular surfactant suggested by FDA to use in dissolution media, SLS was selected as suitable surfactant for solubilization of clopidogrel bisulfate in phosphate buffer solution (pH 6.8) and to maintain sink condition in the dissolution study. A marked increase in solubility was found when SLS was added in media. Maximum effect on solubility was observed when the concentration of SLS was 1% w/v in phosphate buffer solution (pH 6.8). To determine the concentration of SLS that will maintain sink condition can be obtained from Cs/Cd ratio.

The ratio of saturation solubility to drug concentration (dose), expressed as Cs/Cd, represents the closeness to sink conditions; values greater than 3 being considered sink. Sink conditions is the volume of medium at least three times greater than that required to form a saturated solution of drug substance^{18, 19}. The concentration of 1% w/v of SLS gave Cs/Cd greater than 3 as shown in (Table 3). Hence 1% SLS was selected as satisfactory concentration for the dissolution media to maintain sink condition.

Cs indicates saturation solubility of clopidogrel bisulfate in 900 mL of dissolution medium, Cd dose of clopidogrel bisulfate in tablet formulation. Being a weak base, clopidogrel bisulfate presents a pH-dependent solubility profile, solubility decreasing with increasing pH as in (Figure 6) and being predictive of intestinal-dissolution and/or absorption limitations.

Understanding the physicochemical characteristics of the active pharmaceutical ingredient API is a key starting point for any

dissolution method development. In particular, information on equilibrium solubility provides quick direction on the selection of a suitable dissolution medium. The results of the equilibrium solubility studies for clopidogrel bisulfate demonstrate that its solubility is highly pH-dependent, which is consistent with earlier reports.

The choice of a medium, like any other experimental conditions for dissolution testing should be linked to appropriate physiological characteristics which are similar to those of the gastrointestinal tract²⁰. The most common dissolution medium is dilute hydrochloric acid, however other media commonly used include buffers at physiological pH and stimulated gastric or intestinal.

In this case, we examined the dissolution of clopidogrel bisulfate in two media (hydrochloric acid and phosphate buffer pH 6.8) in order to see which one was suitable for dissolving it. The solubility results of the two media demonstrate that 0.1N HCl was the best media for dissolving clopidogrel bisulfate and the phosphate buffer was the least suitable.

Dissolution Release Profiles Comparison

The dissolution profile results showed a significant difference in the dissolution profile of the drug, the percent released was almost 100% after 55 minutes, in 0.1N HCl while in phosphate buffer pH 6.8 with 1% w/v SLS the percent released was 100% after 75 minutes as shown in (Figures 7 and 8) respectively.

From the study of percentage drug release profiles it was observed that it is possible to establish dissolution test parameters, which could be used as an alternative to food and drug administration FDA dissolution test for clopidogrel tablets. The use of 900 ml of 0.1 N HCl and phosphate buffer pH 6.8 solutions at 37°, USP-II dissolution apparatus at the paddle speed of 50 rpm provided discriminative results for clopidogrel tablet.

Based on the results obtained above, 900 ml of 0.1 N HCl and phosphate buffer pH 6.8 solutions with a paddle speed of 50 rpm were selected as discriminative dissolution test conditions for clopidogrel bisulfate tablets.

CONCLUSION

Understanding the physicochemical properties of a drug is crucial for determining the most effective strategy for enhancing dissolution. Typically, the greatest enhancement in the dissolution of poorly soluble compounds is made by changing the dissolution medium to increase compound solubility. Surfactants and pH changes are very effective ways to increase solubility. The *in vitro* dissolution must serve as both a quality control tool and a potential surrogate marker of drug bioavailability and bioequivalence. The proposed method could be applied for routine analysis in quality control laboratories and to help researchers in their own projects.

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