

# Preparation and Characterization of Clopidogrel Bisulfate Solid Dispersion using *Vigna radiata* Extract as a Natural Drug Carrier

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## Abstract

**Context:** Clopidogrel is a potent inhibitor of platelet aggregation, active *in vivo* against a large spectrum of inducers. The drug has low solubility in biological fluid, lead to the poor bioavailability after oral administration. **Aim:** This study aims at enhancement of dissolution profile of clopidogrel bisulfate using green gram (*Vigna radiata*) as a natural carrier by solid dispersion (SD) technique. **Materials and Methods:** Natural carrier was prepared by hot water extraction method. The natural carrier was characterized for swelling index, viscosity, hydration capacity, and angle of repose. The natural carrier green gram (*V. radiata*) was also characterized by differential scanning calorimetry and X-ray diffraction technique for physical interaction and crystallinity, respectively. SD was prepared by solvent evaporation method using the natural carrier in the ratio 1:1, 1:2, and 1:3. **Results:** The extracted water-soluble carrier from *V. radiata* shown requires properties for the preparation of SD. SD 1:3 formulation shows the higher *in vitro* drug release within 120 min. **Conclusions:** From this study, it concludes that green gram could be a novel approach as a carrier with an enhancing effect on solubility of poorly water-soluble drug in SD.

**Key words:** Clopidogrel bisulfate, dissolution enhancement, solid dispersion, *Vigna radiata* (green gram)

## INTRODUCTION

*Vigna radiata*, alternatively known as moong bean, green gram, and lentil, is plant species in the legume family. The mung bean is mainly cultivated today in India, China, and Southeast Asia. The green gram forms a very nutritious article of diet. It is consumed in the form of whole dried seeds and in the form of dal prepared by splitting the seeds in a mill. Many natural polymers are obtained from seeds and have prominent pharmaceutical application.<sup>[1,2]</sup>

Now a days, continuous search on new polymers is going on that will be helpful for making dosage of more optimize. The dissolution rate of drugs from the formulations containing polymer which is viscous in nature is generally low due to the formation of gel layer on the hydrated surfaces, which prevents the drug release during dissolution.

The most important parameter which may greatly affect the performance of drug is solubility.

Aqueous solubility is an important physicochemical property of drug in therapeutic activity.<sup>[3]</sup> The mechanisms involved in solubility and dissolution rate enhancement include the transformation of unstable modifications into more stable and amorphous state by reduction of particle size possibly to the molecular level as well as enhancement of wettability and solubility of drug using the carrier material.<sup>[4]</sup>

Many carriers used in solid dispersions (SDs) are hygroscopic in nature and have high viscosity. Better alternative for this type of polymer is natural polymers with low viscosity and high swelling capacity. Natural polymer is more beneficial because of their easy availability, low cost, biocompatibility, and biodegradability. Furthermore, aqueous extraction gives hydrophilic polymers, and on absorption, they swell and form

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**Received:** 04-11-2015

**Revised:** 17-01-2016

**Accepted:** 28-01-2016

a viscous gel layer around the drug and increases wetting property.<sup>[5,6]</sup>

Clopidogrel bisulfate is a potent inhibitor of platelet aggregation, active *in vivo* against a large spectrum of inducers. Clopidogrel is rapidly absorbed after oral administration. Clopidogrel bisulfate is poorly water-soluble drug with high permeation rate.

Sekiguchi and Obi developed a practical method whereby many of the limitations with bioavailability enhancement of poorly water-soluble drugs. This method which was later termed SD involves the formation of eutectic mixture of drug with water-soluble carriers.<sup>[7-10]</sup>

The main aim of this study was to improve the solubility of poorly water-soluble clopidogrel bisulfate. The objective was to invent the new natural polymer from a natural source and its use as a carrier in SD by solvent evaporation technique.

## MATERIALS AND METHODS

Clopidogrel bisulfate was obtained as a gift sample from Cipla Pharmaceuticals Ltd., Mumbai, and a *V. radiata* seed was carrier procured from local market. The other chemicals were used of analytical grade.

### Extraction of water-soluble contents from *V. radiata*

*V. radiata* (green gram) seeds were procured from local market and weighed accurately 100 g crushed fragmented particles. The material was soaked petroleum ether and ethanol overnight to remove fatty materials and any coloring substances, respectively. The dried residue of green gram seeds was soaked into distilled water at 65°C in water bath for 5 h. The content was filtered, centrifuged at 2000 rpm to get clear supernatant liquid. Five times more acetone was added into filtrate to obtained precipitate. The precipitate was dried, triturate/pulverized into fine powder. The powder was purified by isopropyl alcohol.

### Characterization of extracted materials of *V. radiata* as a natural carrier

#### Swelling index

Swelling index of natural carrier, *V. radiata* was determined as per method described by Patel *et al.*<sup>[8]</sup> Swelling index was expressed as a percentage and calculated according to the following equation:

$$SI = \frac{(X_t - X_0)}{X_0} \times 100 \quad (1)$$

Where,  $X_0$  is the initial height of the powder in a graduated cylinder, and  $X_t$  denotes the height occupied by the swollen powder of natural carrier.

#### Viscosity measurement

The viscosity of 1% (w/v) natural carrier solution was measured according to the US Pharmacopoeia (USP XXII at 37°C) specification, using Brookfield Viscometer.

#### Angle of repose

The angle of repose was determined by the funnel method. The diameter of the funnel heap was measured, and angle of repose was calculated using Equation 2.

$$\theta = \tan^{-1}(H / R) \quad (2)$$

Where,  $H$  - Height of powder heap,  $R$  - Radius of powder heap.

#### Hydration capacity

The hydration capacity of natural carrier *V. radiata* was determined as per method by Aggarwal *et al.*<sup>[11]</sup> The difference between the original volume of the mucilage and the volume drained was taken as water retained by the sample referred as hydration capacity.

$$\text{Hydration capacity} = \text{Weight of hydrated sample} / \text{weight of dry sample} \quad (3)$$

#### Fourier transform infrared spectroscopy (FTIR)

The FTIR (Shimadzu IR Spectrophotometer) study was carried out for the natural carrier. The sample preparation involves mixing of sample (1 mg) with potassium bromide (K-Br), triturating in glass mortar and finally placing in a sample holder. The spectrum was scanned over the frequency between 4000 and 400/cm and at 1 cm resolution.

#### Differential scanning calorimetry (DSC)

The DSC study of the natural carrier was carried out at scan rate 10°C/min at temperature 30-300°C (Make SII Nanotechnology-SEIKO model/series/DSC 6220) for purity of drug sample.

#### X-ray diffraction study

One of desire property for preparation of SD is crystalline and amorphous nature of drug. The X-ray diffraction study of the natural carrier was carried out using measurement program-wide range, owner jagtar, and Cu-K $\alpha$  radiation. The ratio of K alpha 2/K alpha 1 = 0.5, diversion slite 1.52, scan axis = Gonio, and scan range was 4.997-50.00045.

## Preparation of SDs and physical mixture (PM)

PM by cogrinding drug and carrier in different ratios were prepared and denoted as PM 1:1, PM 1:2, and PM 1:3. Meanwhile, SD of clopidogrel bisulfate was also prepared by solvent evaporation method using green gram (*V. radiata*) as the natural carrier in the ratio of SD1:1, SD1:2, and SD1:3.

## Characterization of SD

### Drug content

Pure drug and SD of each formulation equivalent to 75 mg of clopidogrel bisulfate was weighed and dissolved in sufficient quantity of methanol and make up the volume up to 100 ml by water and sonicated for 10 min. 1 ml sample withdrawn and diluted up to 100 ml by distilled water again. The solution was filter through Whatman filter paper, and absorbance was measured at 273.8 nm using a double beam ultraviolet (UV) spectrophotometer.

### DSC

The DSC study of an optimized batch of SD was carried out at scan rate 10°C/min at a temperature from 30°C to 300°C in nitrogen atmosphere.

### X-ray diffraction study

One of desire property for preparation of SD is crystalline and amorphous nature of the drug. The X-ray diffraction study of SD containing drug and natural carrier were studied using diffractometer Philips PW 3071, Cu-K $\alpha$  radiation. The ratio of K alpha 2/K alpha 1 = 0.5, Diversion slite 1.52, Scan axis = Gonio, and scan range was 4.997-50.00045.

### Phase solubility study and solubility study

An excess amount of clopidogrel bisulfate was added to the aqueous solution containing increasing concentration of individual carrier (i.e. 0.25%, 0.50%, 1%, and 2% w/v). The volumetric flasks were sealed and shaken 37°C for 48 h. Then, the samples were filtered. The filtrate was suitably diluted, and absorbance was measured at 273.8 nm using the UV-visible spectrophotometer. The absorbance was taken in triplicate. Meanwhile, solubility of the pure drug was also determined by the same method.

### In vitro dissolution study

The USP dissolution testing apparatus II (Paddle type) was used for the *in vitro* dissolution studies of SD formulations and PMs. The dissolution test was performed using 900 ml of 6.8 phosphate buffer at 37  $\pm$  0.5°C and 75 rpm. 1 ml sample solution was withdrawn by using calibrated pipette at suitable time interval (5, 10, 20, 30, 40, 60, 80, 100, and 120 min) and filter through Whatman filter paper. The sink condition

was maintained throughout the study. The samples were then analyzed at 273.8 nm by UV-visible spectrophotometer (Shimadzu 1601, Japan). The study was carried out in triplicate.

## RESULT AND DISCUSSION

Extracted product as the natural carrier was completely solubilized in given amount of distilled water. After shaking test tube containing solution for 10 min, the solution was found clear. Hence, the product is water soluble. It might be due to the hot water extraction process. Swelling index study of green gram seeds extracted product was found to be 266.66%. It indicates the natural carrier has good swelling nature. Due to the swelling nature of carrier, the extensive surface of carrier is increased during the dissolution of product in distilled water. This indicated that the dissolution rate of poorly water-soluble drug will enhance by increasing wetting property of drug. The viscosity of product powder sample was found 50 cp which is very less as compared to the synthetic polymer as drug carrier. It was prove that the viscosity of carrier increased, the dissolution rate was decreased.<sup>[12]</sup> The lower viscosity of extracted product is one of the good indicators for increasing solubility of poorly water-soluble drug. The result of primary characterization of the natural carrier is given in Table 1.

### FTIR spectroscopy

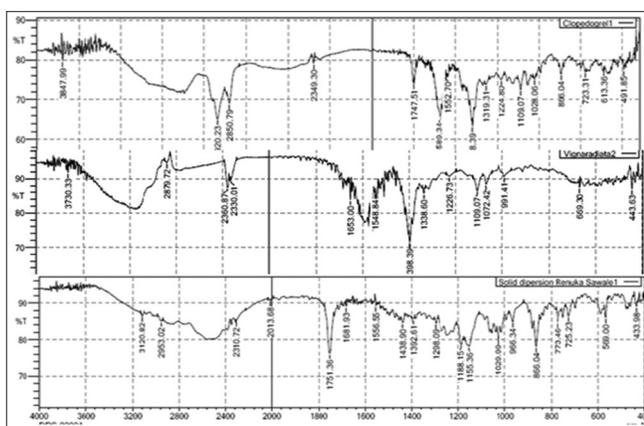
FTIR spectroscopy shows various vibrations between the functional groups at the different bond. The corresponding Figure 1 shows clear stretching vibration due to varying functional groups and indicating no overlapping found over the peaks. Hence, the carrier and drug are compatible with each other.

**Table 1: Evaluation parameters of *Vigna radiata***

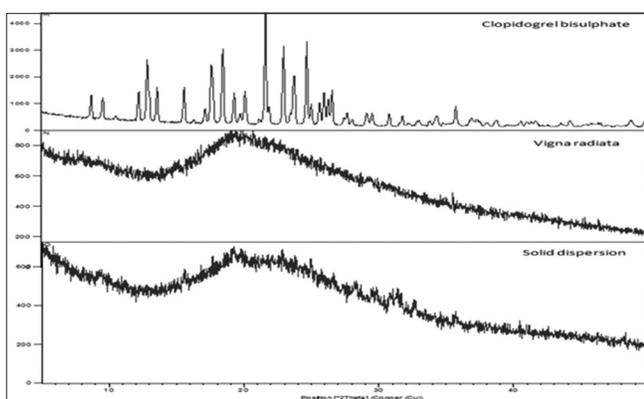
Parameter	
Swelling index	266.66% $\pm$ 2.31
Viscosity	50.33cp $\pm$ 6.23
Angle of repose	32.33 $\pm$ 0.942
Hydration capacity	7.094 $\pm$ 0.58

**Table 2: Phase solubility and solubility study**

Carrier concentration (%)	Solubility mg/ml
0.25	1.89 $\pm$ 0.024
0.50	4.75 $\pm$ 0.041
1	23.09 $\pm$ 1.01
2	33.27 $\pm$ 0.32
Pure drug	0.10 $\pm$ 0.01



**Figure 1:** Fourier transform infrared spectra of clodigregel bisulfate, *Vigna radiata*, and solid dispersion



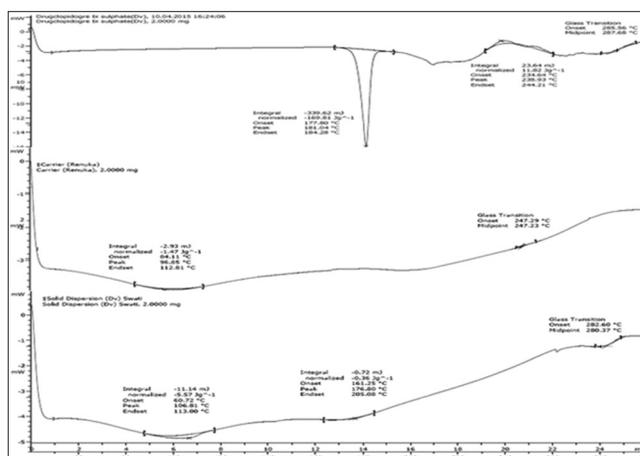
**Figure 2:** Powder X-ray diffraction of clodigregel bisulfate, *Vigna radiata*, and solid dispersion

### X-ray diffraction study

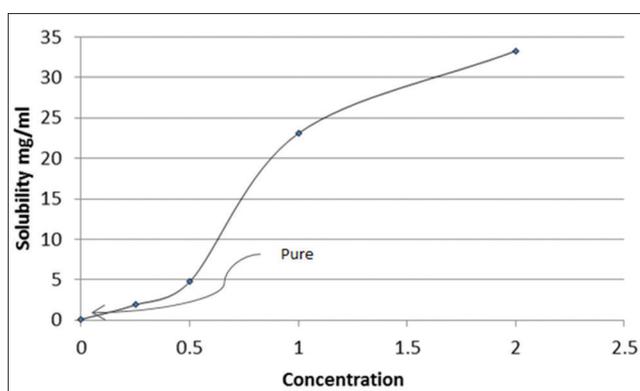
In powder X-ray diffraction (PXRD) study, sharper diffraction peaks indicate more crystalline materials. X-ray diffraction spectrum of pure clodigregel bisulfate showed that the drug was crystalline in nature as demonstrated by distinctive peaks [Figure 2] pure *V. radiata* do not show any intensive peak, which indicated that the extract as the natural carrier is amorphous in nature. The SD of clodigregel bisulfate and *V. radiata* exhibited the absence of characteristics peaks of clodigregel bisulfate, suggesting that the drug is completely soluble in the liquid phase with *V. radiata* carrier. The spectrum of drug, carrier, and SD was shown in Figure 2.

### DSC

The DSC spectrum of *V. radiata* is shown in Figure 3. The spectrum does not show any intensive peak. Hence, it reveals that the extract of *V. radiata* seeds was found amorphous in nature. The spectrum shows slight peaks at 96.85°C with glass transition temperature is 247.29°C actually during melting point study the extract of *V. radiata* was charred.



**Figure 3:** Differential scanning calorimetry thermogram of *Vigna radiata*



**Figure 4:** Phase solubility study of clodigregel bisulfate

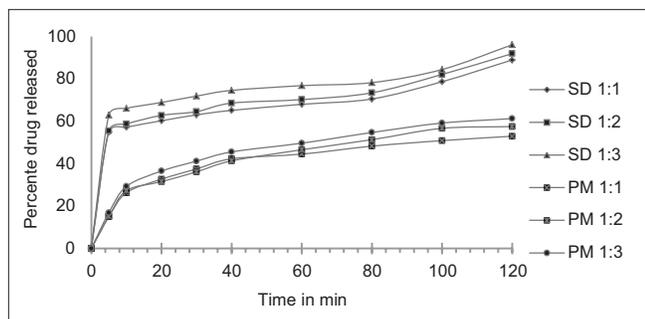
### Phase solubility study

The solubility of clodigregel bisulfate in distilled water was found 0.10 mg/ml at 25°C; therefore, clodigregel bisulfate can be considered to be a poorly water-soluble drug. The phase solubility curve of clodigregel bisulfate in the presence of *V. radiata* seeds extract at different concentrations is shown in Figure 4 a systematic increased solubility of clodigregel bisulfate was observe with an increasing concentration of *V. radiata* seed extract as the natural carrier (Table 2).

### In vitro dissolution study

The use of water-soluble carrier in SD improves the solubility of the poorly water-soluble drug. SD formulated with natural carrier exhibited significant improvement in dissolution parameter. There was a significant increase in % drug dissolved in case of SD 1:3 ratios with the function of time. The maximum drug release was found in formulation 1:3 (96.12%) while 61.28% of drug was released in PM with 1:3 ratio [Figure 5].

Dissolution of SD was significantly improved compared to that of PM. Significantly more drug released from SD was



**Figure 5:** Dissolution profile of solid dispersion and physical mixture

observed, which may be due to the higher dispersion of the drug, the reduction of the particle size, wettability, and the disappearance of the drug crystalline verified by PXRD.

## CONCLUSION

SD containing natural carrier, which is non-toxic, biocompatible, and easy available, is an alternative and best choice for improving solubility of poorly water-soluble drug (BCS-II). From this study, it can be conclude that *V. radiata* could be used as potential natural carrier in the solubility and dissolution rate enhancement of poorly water-soluble drug. It proves that natural carrier is the best alternative for the synthetic one in preparation of SD.

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**Source of Support:** Nil. **Conflict of Interest:** None declared.