

Preparation and *In-Vitro* Evaluation of Clopidogrel Bisulfate Liquisolid Compact

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Abstract

Liquisolid (LS) compact is the most promising technique for increasing dissolution rate and consequently the bioavailability of poorly soluble drugs. Clopidogrel is an oral antiplatelet used for treatment and prophylaxis of cardiovascular and peripheral vascular diseases related to platelets aggregation. Clopidogrel has low solubility at high pH media of the intestine and low oral bioavailability (about 50%). The purpose of this work was to enhance the dissolution pattern of the clopidogrel through its preparation as liquisolid compact. A mathematical model was used to calculate the optimum quantities of Tween 80 as non-volatile liquid vehicle, Avicel PH 102 as carrier material and Aerosil 200 as coating material needed to prepare acceptably flowing and compactible powder mixtures. The liquisolid compacts were evaluated for hardness, friability, weight variation, content uniformity, and disintegration time and *in-vitro* drug release rate. In addition, Differential Scanning Calorimetry (DSC), Fourier Transforms Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) were used for the assessment of the physicochemical properties of the drug and compatibility with excipients in liquisolid compacts. Twelve formula were prepared and the selected formula (LS-2) containing (50% w/w) clopidogrel in Tween 80 at the excipient ratio (R) of (20:1). Compact of (LS-2) released (92.2%) of drug during the first 10 minutes compared to (13.6%) of the directly compressible tablet and (24.2%) of marketed tablet (Plavix®). In conclusion, the dissolution rate of clopidogrel can be enhanced to a great extent by liquisolid technique in comparison to conventional tablets.

Keywords: Liquisolid compact, Clopidogrel bisulfate, Tween 80, Dissolution rate.

تحضير وتقييم خارج الجسم لمضغوط الكلوبيدوكريل بايسلفيت السائل المصلب

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الخلاصة

مضغوط السائل المصلب هي أغلب تقنية واعدة لزيادة معدل الذوبان والتوافر الحيوي للأدوية قليلة الذوبان. يعتبر الكلوبيدوكريل بايسلفيت من مثبطات تجمع الصفائح الدموية المعطاة عبر الفم يستخدم للعلاج والوقاية من أمراض القلب الوعائية المحيطية والأمراض المتعلقة بتجمع الصفائح الدموية. الكلوبيدوكريل عقار قليل الذوبانية في الوسط القاعدي العالي للأمعاء وله توافر حيوي قليل والذي يبلغ (حوالي 50%) بعد الجرعات الفموية. الغرض من هذه الدراسة هو تحسين معدل الذوبان لعقار الكلوبيدوكريل من خلال تحضيره بشكل مضغوط سائل مصلب. تم استخدام نموذج رياضي لحساب الكميات المثلى من توين 80 (سائل غير متطاير كواسطة نقل)، أفيسيل بي أج ١٠٢ (كمادة حاملة) والاروسيل ٢٠٠ (كمادة مغلفة) لاعداد خليط مسحوق يتدفق بشكل مقبول وقابل للكبس. تم تقييم مضغوطات السائل المصلب من حيث الصلابة، الهشاشة، اختلاف الوزن، توحيد المحتوى، وقت التفكك ومعدل تحرر الدواء خارج الجسم. بالإضافة الى ذلك، تم استخدام المسح الحراري المقارن (دي أس سي)، طيف الأشعة تحت الحمراء (أف تي أي آر)، حيود أشعة أكس (أكس آر دي) والمسح المجهر الإلكتروني (أس أي أم) لتقييم الخصائص الفيزيوكيميائية للدواء والتوافق مع السواغات في مضغوطات السائل المصلب. تم تحضير ١٢ تركيبة والتركيبية المختارة ٢ محتوية على ٥٠% وزن/وزن كلوبيدوكريل في توين ٨٠ ونسبة سواغات (٢٠:١). حرر مضغوط (الال أس ٢) ٩٢,٢% من محتواه الدوائي خلال ال ١٠ دقائق الأولى مقارنة مع الأقراص المكبوسة مباشرة ١٣,٦% والأقراص المتداولة تجارياً (بلافكس) ٢٤,٢%. وفي النهاية، فإن تقنية المضغوط السائل المصلب قد أدت الى تحسين معدل اذابة العقار مما قد يؤدي الى زيادة توافره الحيوي.

الكلمات المفتاحية: مضغوط سائل مصلب، كلوبيدوكريل بايسلفيت، توين ٨٠، معدل الذوبان.

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Introduction

Oral delivery of 50 % of the drug compounds is hampered because of the high lipophilicity of the drug itself ⁽¹⁾. Nearly 40 % of new drug candidates exhibit low solubility in water, which leads to ineffective absorption, low bioavailability, and therapeutic failure ⁽²⁾.

There are various techniques available to improve the solubility of poorly soluble drugs. These include micronization, solid dispersions, complexation with β -Cyclodextrins, solubilization by surfactants, and liquisolid technique which is the most promising and novel technique developed by Spireas *et al.* to improve the dissolution rates of the poorly water soluble drugs ⁽³⁾.

Liquisolid systems are acceptably flowing and compressible powdered forms of liquid medications. The 'liquid medication' involves oily liquid drugs and solutions or suspensions of water insoluble solid drugs carried in suitable nonvolatile solvent systems termed liquid vehicles. Liquid medication converted into a dry-looking, non-adherent, free flowing and readily compressible powder by a simple blending with selected powder excipients referred to as carrier and coating materials. Various grades of cellulose, starch and lactose may be used as the carriers, whereas very fine particle size silica powders may be used as the coating materials ⁽⁴⁾.

Liquisolid compacts of poorly soluble drugs containing a drug solution or drug suspension in a solubilizing vehicle show enhanced drug release due to an increased surface area of drug available for release, an increased aqueous solubility of the drug, and an improved wettability of the drug particles. Accordingly, this may result in a higher drug absorption and improved oral bioavailability ⁽⁵⁾.

This technique was successfully used to improve the solubility and dissolution rate of several poorly water soluble drugs as Naproxen, Famotidine, Carbamazepine, Piroxicam, Indomethacin, Refecoxib, Hydrocortisone and Prednisolone ⁽⁶⁾.

Clopidogrel bisulfate is methyl (+)-(S) - α -(2chlorophenyl) - 6,7 - dihydrothieno [3, 2 - c] pyridine -5 (4H) -acetate sulfate (1:1). It is white to off-white powder. It is practically insoluble in water at neutral pH; freely soluble in buffer (pH 1) ⁽⁷⁾. It belongs to class II according to Biopharmaceutics Classification System (BCS). It has low bioavailability (about 50%) ⁽⁸⁾. The pka value of clopidogrel is (4.56) ⁽⁹⁾. The absorption of drug is largely dependent upon diffusion, which varies with pH of the individual regions within the

gastrointestinal tract ⁽¹⁰⁾. The active metabolite of clopidogrel selectively inhibits platelet aggregation. It is used in the prevention of ischemic stroke, myocardial infarction, unstable angina and peripheral arterial diseases ⁽¹¹⁾.

The aim of this work was to improve the solubility and dissolution rate of clopidogrel bisulfate in phosphate buffer at pH 6.8 which was similar to intestinal media via liquisolid technique. This may subsequently, enhance its oral bioavailability.

Materials and Methods

Materials

Clopidogrel bisulfate standard powder was supplied from Pioneer Pharmaceutical Company, Iraq, sulaimaniah) as a gift, clopidogrel 75mg tablets (Plavix®, Sanofi Aventis, France), Microcrystalline cellulose (Avicel PH 102) (Thomas Baker, India), Colloidal silicon dioxide (Aerosil 200) (Evonik Degussa Corp, Germany), Sodium starch glycolate (SSG) (ASG, India), Tween 80 and polyethylene glycol (PEG 400) (BDH chemical LTD, UK), propylene glycol (PG) (Thomas baker, India), glycerin (Romil, UK) and sodium lauryl sulfate was supplied from SDI, Samarra, Iraq) as a gift. All other reagents and chemicals were of analytical grade.

Methods

Solubility studies

Solubility studies of clopidogrel bisulfate were carried out in 0.1N HCl (pH 1.2), phosphate buffer (pH 6.8) alone and with 1% sodium lauryl sulfate (SLS), Tween 80, propylene glycol (PG), polyethylene glycol (PEG 400), glycerin and distilled water. Saturated solutions were prepared by adding an excess amount of drug to the vehicles and shaking in a shaker water bath for 48 hr at 25 ± 0.5 °C under constant vibration. After this period the solutions were filtered, diluted and analyzed by UV spectrophotometer (Shimadzu, Japan) at λ_{\max} 220 nm.

Mathematical model for design of liquisolid systems

The formulation design of liquisolid systems was done in accordance with new mathematical model described by Spireas *et al.* clopidogrel bisulfate was dispersed in Tween80 (Tween80 was used as liquid vehicle to prepare liquid medication). Avicel PH 102 and Aerosil 200 were used as the carrier and coating materials, respectively. The concentration of the drug in the liquid vehicle were (40, 50, 60 and 70% w/w) and the carrier: coating ratio were (R = 20:1, 15:1 and 10:1). Flowable liquid retention potential (Φ value) of powder excipients was used to calculate

the required ingredient quantities. In Tween 80, the Φ -value was (0.16) for the carrier Avicel PH 102 and (3.33) for the coating material Aerosil 200⁽¹²⁾. The liquid load factor (Lf) was computed from the Φ -value of the carrier and coating materials with different ratio (R) in accordance to equation (1):

$$Lf = \Phi Cr + \Phi Co (1/R) \text{ ----- (1)}$$

Where, Φ Cr and Φ Co are the flowable liquid retention potentials (Φ -values) of carrier (Avicel PH 102) and coating (Aerosil 200) powder materials, respectively.

However, liquid load factor (Lf) is defined as the ratio of the weight of liquid medication (W) to the weight of the carrier powder (Q) in the system, which should be possessed by an acceptably flowing and compressible liquisolid system. The most suitable quantities of carrier (Q) were calculated using equation (2):

$$Lf = W / Q \text{ ----- (2)}$$

The optimum quantities of coating material (q) were obtained from equation (3):

$$R = Q / q \text{ ----- (3)}$$

Where, R is the ratio by weight of carrier (Q) and coating (q) materials present in the formulation^(13, 14).

Preparation of clopidogrel bisulfate liquisolid compact

Liquisolid compacts (LS) represented by formula 1 – 12, each containing 98 mg of

clopidogrel bisulfate (equivalent to 75mg clopidogrel). Different drug concentrations in Tween 80 (40, 50, 60 and 70% w/w) were prepared by dispersing the drug in the non-volatile vehicle (Tween 80). Also, a bindery mixture of the carrier (Avicel PH 102) and coating material (Aerosil 200) was prepared at a ratio R of (20:1, 15:1 and 10:1). Then after, it was mixed with the liquid medication. The mixing process was carried out in three stages. In the first stage, the binary powder mixture was blended with liquid medication using a porcelain mortar with the aid of a pestle at a mixing rate of one rotation per second for approximately one minute in order to evenly distribute the liquid medication into the powder. In the second mixing stage, the liquid/powder admixture was evenly spread as a uniform layer on the surfaces of the mortar and was left standing for approximately ten minutes to allow the liquid medication to be absorbed in the interior of the powder particles, and then saturation adsorption occurred on the surface of these particles. In the third stage, sodium starch glycolate (SSG) as a super-disintegrant was added at 5% w/w and mixed for 10 minutes. The final mixture was compacted using a single punch-tablet machine (Korsch EKO, Germany)^(15,16). The composition and characteristics of liquisolid compact were demonstrated in the table 1.

Table 1. Composition and characteristics of clopidogrel compact (LS) and DCT.

| Formula number | Drug (mg) | Drug conc. in Tween 80 (% w/w) | Carrier: Coating ratio (R) | Liquid loading factor (Lf) | Liquid Vehicle (Tween 80) (mg) | Carrier (Avicel PH 102) (mg) | Coating (Aerosil 200) (mg) | Super-disintegrant (SSG) (mg) | Compact weight (mg) |
|----------------|-----------|--------------------------------|----------------------------|----------------------------|--------------------------------|------------------------------|----------------------------|-------------------------------|---------------------|
| LS-1 | 98 | 40 | 20 | 0.326 | 147 | 751.5 | 37.5 | 51.7 | 1085 |
| LS-2 | 98 | 50 | 20 | 0.326 | 98 | 601.2 | 30 | 41.3 | 868.5 |
| LS-3 | 98 | 60 | 20 | 0.326 | 65.3 | 500.9 | 25 | 34.4 | 723.6 |
| LS-4 | 98 | 70 | 20 | 0.326 | 42 | 429.4 | 21.4 | 29.5 | 620.3 |
| LS-5 | 98 | 40 | 15 | 0.379 | 147 | 646.4 | 43 | 46.7 | 981.2 |
| LS-6 | 98 | 50 | 15 | 0.379 | 98 | 517.1 | 34.4 | 37.3 | 784.8 |
| LS-7 | 98 | 60 | 15 | 0.379 | 65.3 | 430.8 | 28.7 | 31.1 | 653.9 |
| LS-8 | 98 | 70 | 15 | 0.379 | 42 | 369.3 | 24.6 | 26.6 | 560.5 |
| LS-9 | 98 | 40 | 10 | 0.493 | 147 | 496.9 | 49.6 | 39.5 | 831.1 |
| LS-10 | 98 | 50 | 10 | 0.493 | 98 | 397.5 | 39.7 | 31.6 | 664.9 |
| LS-11 | 98 | 60 | 10 | 0.493 | 65.3 | 331.2 | 33.1 | 26.3 | 553.9 |
| LS-12 | 98 | 70 | 10 | 0.493 | 42 | 283.9 | 28.3 | 22.6 | 474.8 |
| DCT | 98 | - | 20 | - | - | 601.2 | 30 | 41.3 | 770.5 |

Preparation of directly compressed tablets (DCT)

Compressed tablet containing 98 mg of clopidogrel bisulfate was prepared with direct compression method without the addition of any non-volatile liquid vehicle (the same composition of the selected LS compact but without Tween 80). The drug powder was mixed with suitable amounts of Avicel PH102 and Aerosil 200. Afterwards, 5% of SSG was added and mixed for 10 minutes. The final blend was compressed using (Korsch, Germany) tablet machine ⁽¹⁷⁾.

Pre-compression studies of the prepared liquisolid Powder system**Flow properties of liquisolid system****Angle of repose (θ)**

The angle of repose was determined by fixed funnel method. The height of the funnel was adjusted in such a way that the tip of the funnel just touches the apex of the heap of the powder. Accurately, weighed blend is allowed to pass through the funnel freely on the surface. The angle of repose (θ) was calculated using the following equation ⁽¹⁸⁾.

$$\tan \theta = h / r \text{ ----- (4)}$$

Where, θ is the angle of repose, h is the height of pile in cm and r is the radius of pile in cm. Depending on the value of angle of repose, the flowability of liquisolid system were evaluated ⁽¹⁹⁾.

Carr's index and Hausner's ratio

The bulk and tapped densities were used to calculate the Carr's index and the Hausner's ratio of powder according to the equations ⁽²⁰⁾:

$$\text{Carr's Index} = (\text{tapped density} - \text{bulk density}) \times 100 / \text{tapped density} \text{ ----- (5)}$$

$$\text{Hausner ratio} = \text{tapped density} / \text{bulk density} \text{ --- ----- (6)}$$

$$\text{Bulk density} = W_t / V_o \text{ ----- (7)}$$

$$\text{Tapped Density} = W_t / V_f \text{ ----- (8)}$$

Where, W_t is the weight of powder, V_o is the bulk volume of powder and V_f is the tapped volume of powder.

System with compressibility value (Carr's Index) greater than 20-21 % has been found to exhibit poor flow properties. In addition, the system has good flowability when Hausner's ratio is lower than 1.2 while, if the ratio is more than 1.2 this indicates that the flowability is bad ⁽²¹⁾.

Differential scanning calorimetry analysis

Thermal behavior of pure Clopidogrel, Avicel PH 102, powder mixture of conventional (DCT) and liquisolid (LS) compact were attained by DSC. Samples (5 mg) were placed in an aluminum pan and heated in the DSC-60 (Shimadzu, Japan) at a constant rate of 10 °C/min,

in an atmosphere of nitrogen over a temperature range of 25-300 °C ⁽²²⁾.

Fourier transforms infrared spectroscopy (FTIR)

Each of pure clopidogrel bisulfate, Avicel PH 102, powder mixture for conventional and liquisolid samples of 2-3 mg were mixed with about 100 mg of dry potassium bromide powder and compressed into transparent discs then scanned over a range of 4000-400 cm^{-1} using the Infrared spectrophotometer (Lambda 7600, Australia) ⁽²³⁾.

X-ray diffraction (XRD)

The (XRD) patterns were determined for pure drug, excipients used in formulation (Avicel PH 102), physical mixture of drug and excipients (DCT) and finally, for the prepared liquisolid system (LS compact). The operating conditions were; voltage 40 kV, current 30 mA and scanning speed 8.000 (deg /min.) ⁽²⁴⁾.

Scanning electron microscopy (SEM) study

Scanning electron microscopy (SEM) is utilized to assess the morphological characteristics of the pure drug and the liquisolid system. Samples were first loaded on sample stub using double side carbon tape then coated with gold and examined in the Zeiss Supra 55 VP Scanning electron microscope ⁽²⁵⁾.

Post-compression studies**Hardness**

The hardness of formulated liquisolid compacts was assessed using a pharma test hardness tester and the mean hardness of three tablets \pm standard deviation (SD) was determined. The hardness was expressed as a force in kg/cm^2 required to crush the compact ⁽²⁶⁾.

Friability

Pharma test friabilator was used in this study by taking 20 liquisolid compacts from each formula. These compacts were weighed accurately (W_t initial) then after, rotated in the friabilator for 4 min at 25 rpm. The compacts were re-weighed (W_t final). The friability was calculated as a percentage according to equation ⁽²⁷⁾.

$$\text{Friability \%} = [(W_t \text{ initial} - W_t \text{ final}) / W_t \text{ initial}] \times 100 \text{ ----- (9)}$$

The acceptable friability value is up to 1%.

Weight variation test

Twenty compacts were taken and their weight was determined individually and collectively on a digital weighting balance (Precisa instruments Ltd, Switzerland). The average weight of the compact was determined from the collective weight and comparing the

individual tablet weight to average weight variation tolerance according to British pharmacopoeia⁽²⁸⁾.

Content uniformity

This test was carried out by applying USP method. Ten compacts were individually assayed for their content. Each compact was grinded and the powder placed in 50ml of 0.1N HCl, sonicated for 5min. and cooled. Then, transfer 5ml of this solution to volumetric flask, dilute with 0.1N HCl to 50 ml. Then after, filter and discard the first 5ml of filtrate. After that, the amount of clopidogrel was determined spectrophotometrically by measuring the absorbance at appropriate λ_{\max} . The percent of content uniformity for each compact was calculated and compared with the mean for each formula according to the USP specification⁽³⁹⁾.

Disintegration time study

The *in-vitro* disintegration studies were carried out using tablet disintegration test apparatus (Pharma test, Germany). One compact was placed in each of the six tubes of the basket assembly and disk was added to each tube. This assembly was then suspended in one-liter beaker containing 0.1N HCl pH (1.2) maintained at $37 \pm 2^\circ\text{C}$. The basket was then moved up and down through a distance of 5 to 6 cm at a frequency of 32 cycles per min. The time required for complete disintegration of the compact was recorded⁽³⁰⁾.

In-vitro dissolution test

The test was studied in USP Type-II dissolution apparatus (Pharma test, Germany) employing a paddle stirrer at speed of 50 rpm. In this study 900 ml of simulated intestinal fluid (SIF) (phosphate buffer pH 6.8 with 1% SLS) or simulated gastric fluid (SGF) (0.1N HCl pH 1.2) was used as a dissolution media. The temperature of dissolution media was maintained at $37 \pm 0.5^\circ\text{C}$ throughout the experiment. Samples of (5 ml) were withdrawn at the predetermined intervals of the time (5, 10, 20, 30, 60 and 90 min.). Then after, filtered through a 0.45 μm filter and analyzed for drug release by measuring the absorbance at λ_{\max} of drug using UV-visible spectrophotometer (Shimadzu, Japan). The volume withdrawn at each time-interval was replaced with the equal volume of fresh dissolution media to maintain sink condition and constant volume. Each preparation was tested in triplicate and the mean value of reading was calculated⁽³¹⁾.

Dissolution parameters like mean dissolution time (MDT) and percent dissolution efficiency (% DE)

were applied for comparison of dissolution profiles to select the best formulation⁽³²⁾.

Statistical analysis

All the results were expressed as the mean \pm SD. One way analysis of variance (ANOVA) was used to test for significance at a 5% significance level. So, that, statistical difference dealing with ($P < 0.05$) was considered significant.

Results and Discussions

Saturation solubility

The solubility of clopidogrel in different solvents was given in table 2. Clopidogrel exhibited the highest solubility in Tween 80 ($55.92 \pm 2.33\text{mg/ml}$). Since, the aim of this study was to increase the dissolution rate of clopidogrel, Tween 80 was exploited as a nonvolatile solvent in preparation of liquisolid systems.

Table 2. Solubility of clopidogrel bisulfate in various solvents.

| Solvents | Solubility (mg/ml) Mean \pm S.D |
|------------------------------------|--------------------------------------|
| 0.1N HCl (pH 1.2) | 37.36 ± 1.64 |
| Phosphate buffer (pH 6.8) | 0.84 ± 0.03 |
| Phosphate buffer (pH .8) + 1% SLS. | 3.92 ± 0.49 |
| Tween 80 | 55.92 ± 2.33 |
| PG | 2.39 ± 0.20 |
| PEG 400 | 2.99 ± 0.12 |
| Glycerin | 2.76 ± 0.08 |
| Water (pH 5.5) | 1.47 ± 0.23 |

n=3

Pre-compression evaluation of the prepared liquisolid system

Flow properties of liquisolid system

The flowability of a powder is of critical importance in the production of pharmaceutical dosage forms to reduce high dose variations. The angle of repose (θ) is a characteristic of the internal friction or cohesion of the particles. In addition, Carr's Index is a measure of the propensity of a powder to be compressed. Also, the flowability of a system is represented by its Hausner's ratio. The results were demonstrated in table 3. It was shown that most LS formulas with high excipient ratio ($R = 20$) exhibited good flowability. These results related to the presence of high amount of Avicel (carrier) and lower amount of Aerosil (coating). However, Avicel has excellent flow properties while, Aerosil is a fluffy powder due to its low density (0.05g/ml)⁽³³⁾.

Table 3. Angle of repose, carr's compressibility index and Hausner's ratio for clopidogrel liquisolid systems

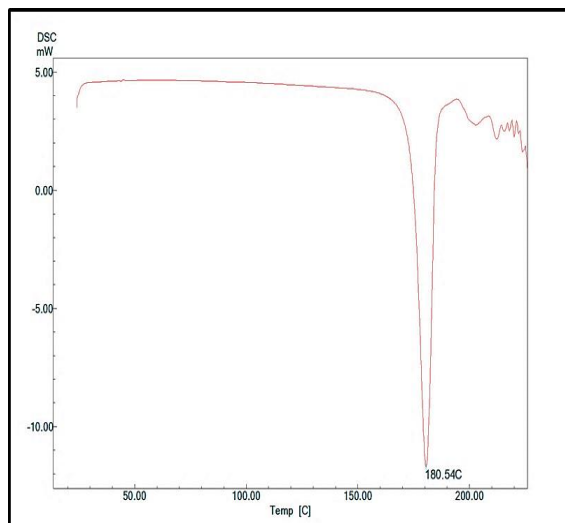
| Formula Number | Angle of Repose (Θ) | Carr's Index (Compressibility) | Hausner's Ratio | Type of Flow |
|----------------|------------------------------|--------------------------------|-------------------|--------------|
| LS-1 | 36.02 ± 0.254 | 18.16 ± 0.687 | 1.215 ± 0.021 | Fair |
| LS-2 | 33.09 ± 0.535 | 15.87 ± 0.09 | 1.178 ± 0.087 | Good |
| LS-3 | 31.11 ± 0.265 | 14.61 ± 0.15 | 1.161 ± 0.018 | Good |
| LS-4 | 30.73 ± 0.733 | 14.18 ± 0.43 | 1.157 ± 0.023 | Good |
| LS-5 | 36.94 ± 0.403 | 19.05 ± 0.640 | 1.2 ± 0.062 | Fair |
| LS-6 | 34.01 ± 0.854 | 17.25 ± 0.702 | 1.191 ± 0.044 | Good |
| LS-7 | 32.76 ± 0.565 | 15.79 ± 0.47 | 1.185 ± 0.028 | Good |
| LS-8 | 31.48 ± 0.705 | 15.32 ± 0.276 | 1.172 ± 0.049 | Good |
| LS-9 | 37.71 ± 0.2 | 19.61 ± 0.459 | 1.242 ± 0.031 | Fair |
| LS-10 | 35.89 ± 0.415 | 17.43 ± 0.346 | 1.197 ± 0.056 | Fair |
| LS-11 | 33.97 ± 0.867 | 15.98 ± 0.194 | 1.184 ± 0.020 | Good |
| LS-12 | 32.07 ± 0.815 | 15.5 ± 0.271 | 1.180 ± 0.020 | Good |
| DCT | 37.83 ± 0.830 | 23.47 ± 0.365 | 1.342 ± 0.034 | Passable |

Results as mean \pm S.D, n=3.

Differential scanning calorimetry (DSC)

Differential Scanning Colorimetry analysis was applied to determine thermotropic properties of the system and any physicochemical interaction between drug and excipients. The DSC thermogram of pure clopidogrel bisulfate was shown in figure 1. The clopidogrel bisulfate peak demonstrated as a clear sharp characteristic endothermic peak at 180°C corresponding to its melting point. Such a sharp endothermic peak showed that clopidogrel bisulfate used was in a pure crystalline state. The DSC of Avicel PH 102 was shown in figure 2. While, figure 3 showed the DSC of physical mixture of directly compressed tablet (DCT) which exhibited endothermic peak at 178°C, which was the peak of the drug. The low intensity of peak may be related to low quantity of drug in test sample. Also there was another peak at 75.02°C which related to Avicel.

On the other hand, the DSC thermogram of liquisolid compact (LS-2) showed disappearance of the characteristic peak of clopidogrel melting point as shown in figure 4 giving a strong indication that the drug lost its crystallinity state and converted to an amorphous form⁽³⁴⁾.

**Figure 1. DSC thermogram of pure clopidogrel.**

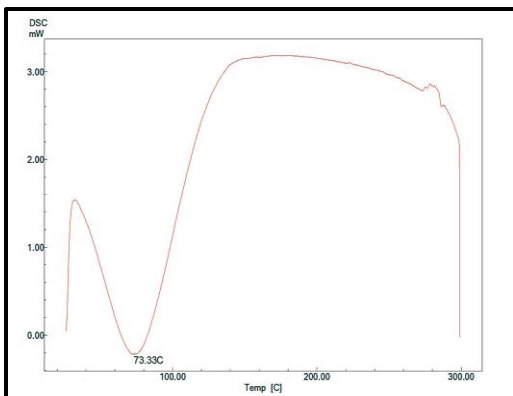


Figure 2. DSC thermogram of Avicel PH 102.

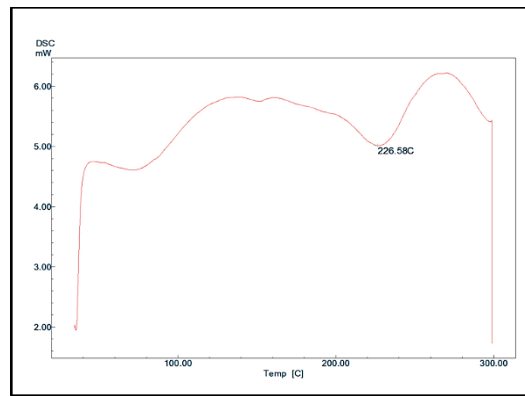


Figure 4. DSC thermogram of liquisolid compact LS-2.

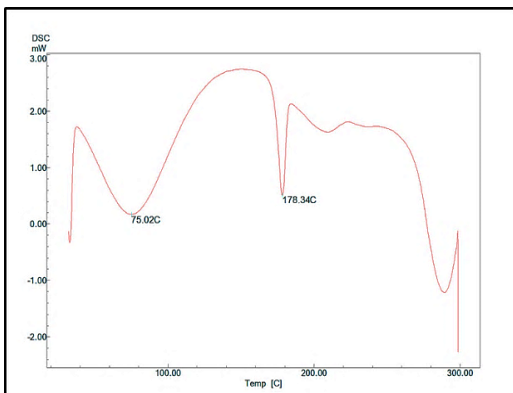


Figure 3. DSC thermogram of directly compressed tablet (DCT).

Fourier transforms infrared spectroscopy (FTIR).

This study was performed to know the compatibility between the drug and excipients (Figures 5 – 8). The FTIR spectrum of pure clopidogrel bisulfate figure 5 showed a strong absorbance band due to C=O stretching vibrations at 1752 cm^{-1} and due to O–H stretching of the hydrogen sulfate moiety around 3012 cm^{-1} . The band due to aromatic C–H stretching vibrations represented at 3121 cm^{-1} . The FTIR spectrum included also, broad absorbance band at 2505 cm^{-1} which can be attributed to the stretching vibrations of bonded N–H resulting from salt formation between the quaternary nitrogen of clopidogrel and –OH of hydrogen sulfate. The band associated with C–O stretching appeared at 1062 , 1155 and 1186 cm^{-1} . These results were confirmed by the appearance of the same characteristic absorption peaks in the spectrum of the physical mixture of DCT and LS – 2 without any changes in their position figures (7 and 8) which, indicated absence of chemical interactions between the drug and excipients⁽³⁵⁾.

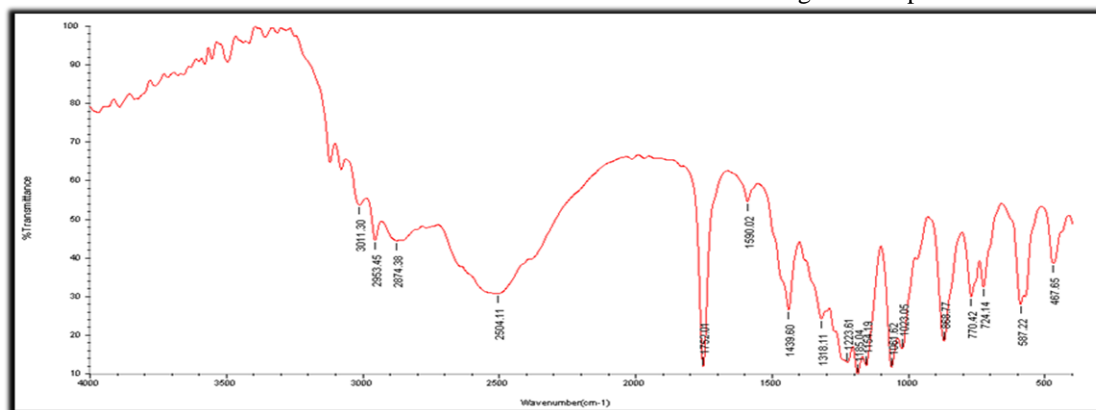


Figure 5. FTIR spectrum of pure clopidogrel.

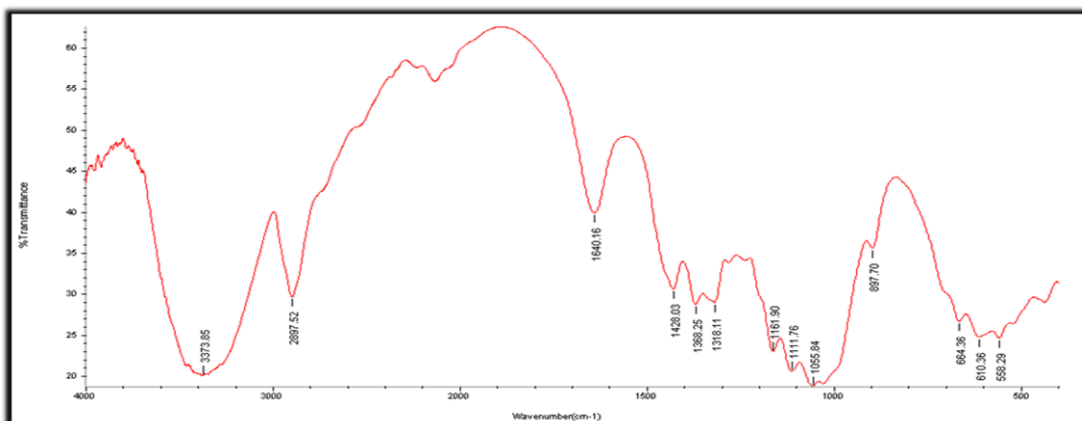


Figure6. FTIR spectrum of Avicel PH 102.

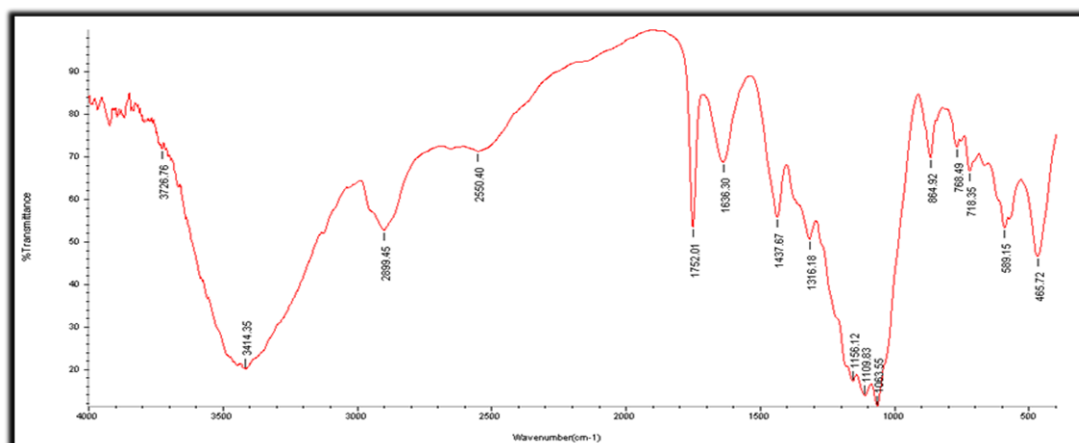


Figure 7. FTIR spectrum of directly compressed tablet (DCT)

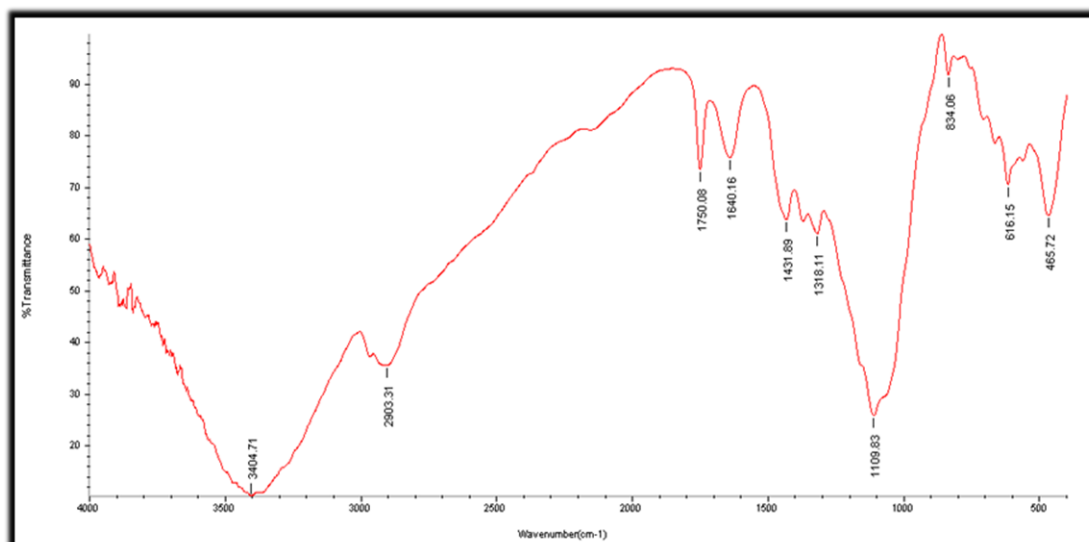


Figure 8. FTIR spectrum of liquisolid compact formula LS-2.

X-ray diffraction (XRD).

For characterization of the crystalline state, the x-ray diffractogram of pure clopidogrel bisulfate exhibited several sharp peaks in the region of 5° to 50° 2θ as shown in figure 9. Two high intensity peaks at 21.69° and 23.0° 2θ . At the lower 2θ angle, unique peaks were present at 8.91° and 12.44° 2θ suggested that, the drug existed as crystalline state. Figure 10 showed one sharp peak at 2θ angle of 22.5 of Avicel PH 102.

Clopidogrel bisulfate characteristic peaks were observed in the physical mixture of conventional formulation (DCT) as shown in figure 11, demonstrating that its crystalline structure remained unchanged during the physical blend. The liquisolid powder (LS-2) diffraction pattern figure 12 showed only one sharp diffraction peak at 2θ angle of 22.5 belonging to Avicel PH 102 indicating that only Avicel PH 102 maintained its crystalline state⁽³⁶⁾.

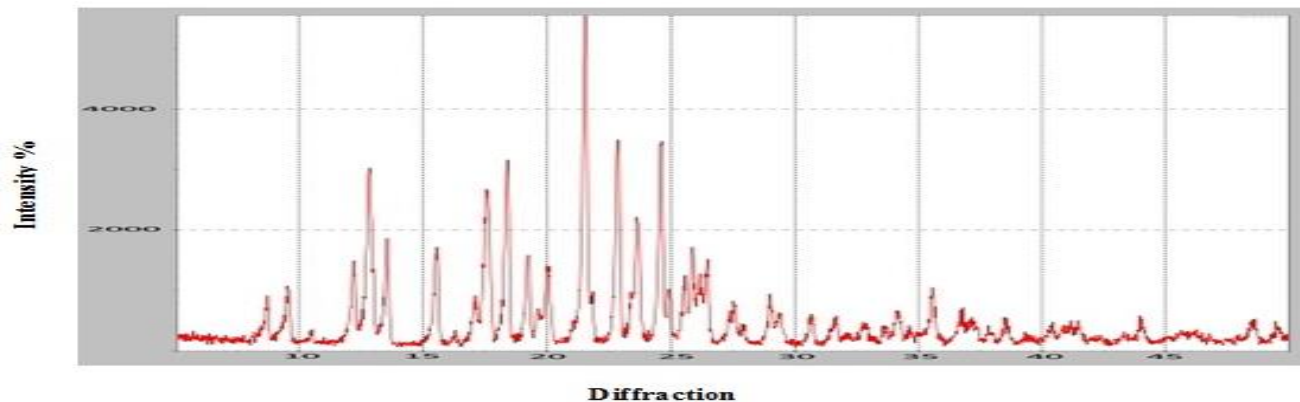


Figure 9. X-Ray diffraction of pure clopidogrel

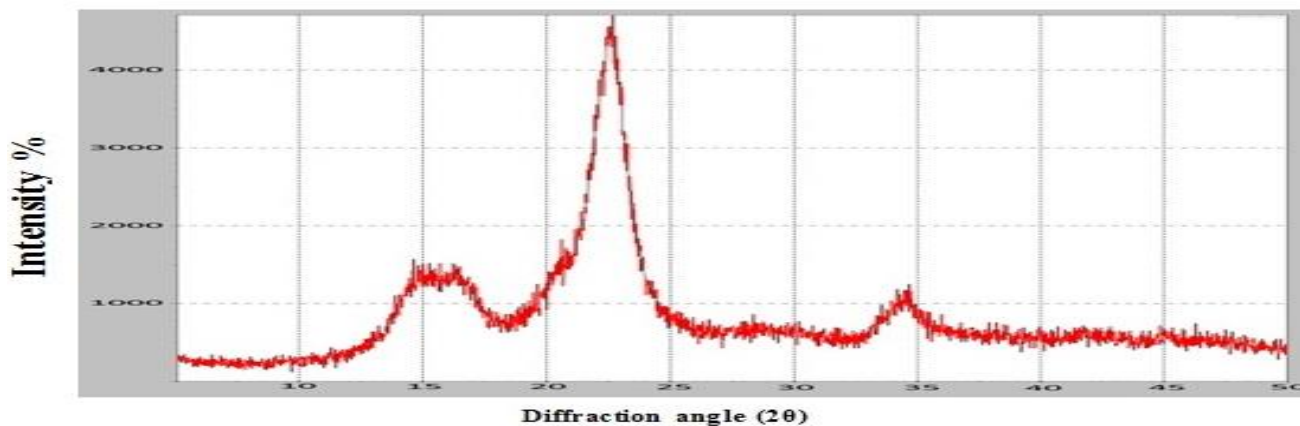


Figure 10. X-Ray diffraction of Avicel PH 102.

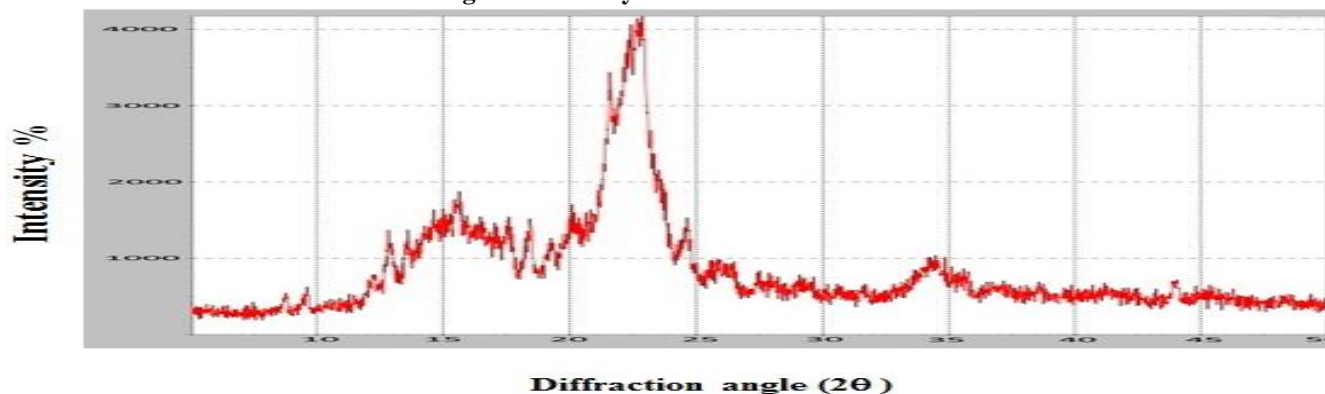


Figure 11. X-Ray diffraction of directly compressed tablet (DCT)

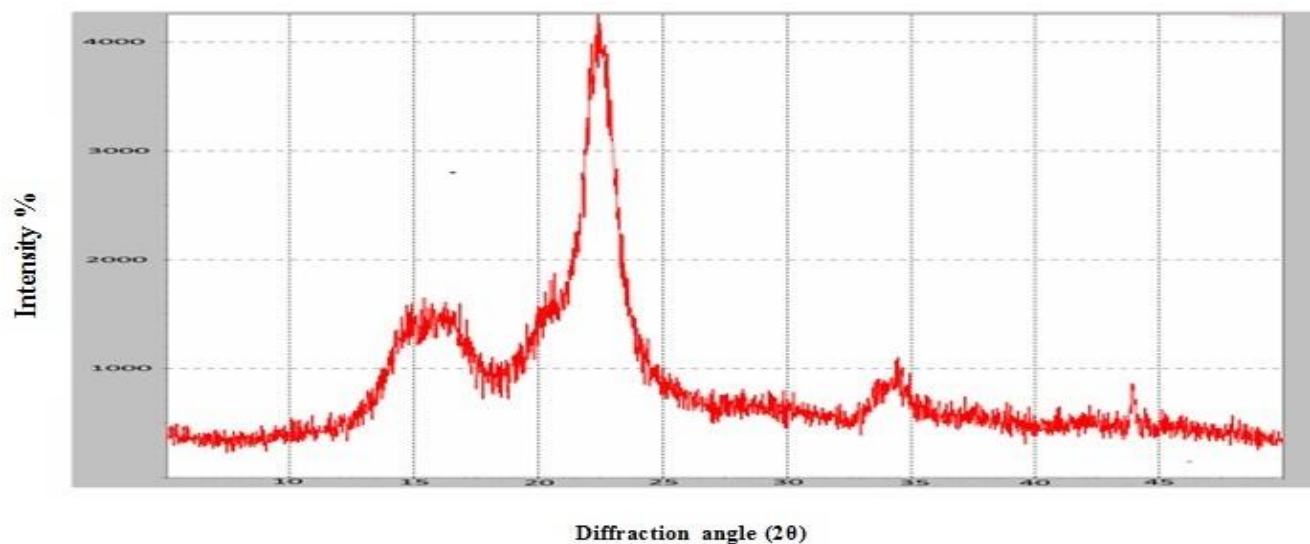


Figure 12.X-Ray diffraction of liquisolid system LS-2

Scanning electron microscopy (SEM)

Figure 13 illustrated the SEM of the pure clopidogrel bisulfate. It appeared that, the drug had crystalline nature, as proved previously by DSC and XRD. Figure 14 represented the photomicrograph of optimized liquisolid system (LS – 2). The drug particles in LS system entrapped within excipients, confirming FTIR and DSC data analysis. This surface modification ensured the decrease in crystallinity of the drug particles. These images indicated the change in surface morphology of drug particle due to entrapment into the respective carrier and coating materials⁽³⁷⁾.

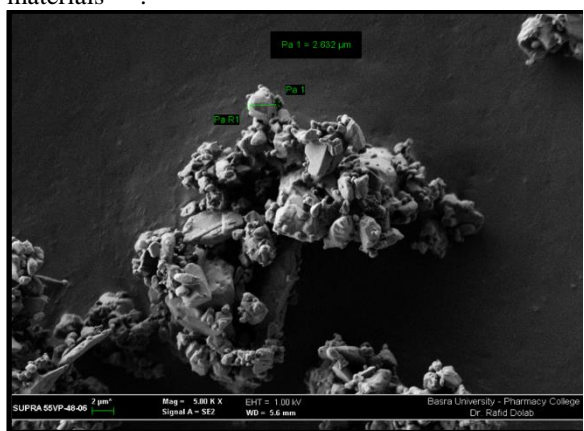


Figure 13. SEM of pure clopidogrel bisulfate.

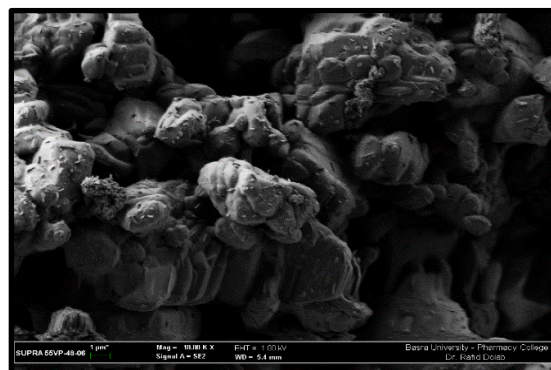


Figure 14.SEM of liquisolid compact (LS-2)

Post-compression evaluation

Hardness test

The average hardness of a liquisolid compacts ranged from (4.3 ± 0.173) to (6.46 ± 0.251) kg/cm² while, the hardness of DCT was (5.65 ± 0.45) kg/cm² as shows in table 4. As excipients ratio increased the hardness of the tablets increased. This was due to the hydrogen bonds between groups on adjacent cellulose molecules in Avicel PH 102⁽³⁸⁾.

Friability

All clopidogrel LS compacts had acceptable friability as none of the tested formulas had percentage loss in compacts weight that exceeded 1%. Also, no compact cracked, split or broken in either formula⁽³⁸⁾, as shown in table 4.

Weight variation

Compacts of each formula were subjected to weight variation test, the difference in weight

and percent deviation was calculated for each tablet. The results of the test as demonstrated in table 4 showed that, the compact weights were within the pharmacopeial limit ⁽³⁹⁾.

Content uniformity

Percentages of content uniformity for all clopidogrel formulas ranged from (95 – 100.8%) as shown in table 4. This complied with USP content uniformity specification that is 85%-115% of content in each individual compact indicating that, the processing method were convenience ⁽³⁹⁾.

Disintegration time

The disintegration time for the prepared clopidogrel LS compacts was shown in table 4. It was found that the disintegration time mean for all investigated compacts was less than 2 min, because of co-processed super disintegrant. Another finding was displayed from the obtained results that, there was a relationship between powder excipient ratio (R) and the disintegration time. The powder excipient ratio (R) was inversely proportional to the disintegration time of the compacts i.e., when the powder excipient ratio (R) increased the disintegration time of the compacts decreased ⁽⁴⁰⁾.

Table 4. Evaluation parameters of clopidogrel bisulfate liquisolid compact

| Formula Number | Hardness (Kg/cm ²) Mean± S.D. | Friability % (W/W) | Weight Variation (mg) Mean ± S.D. | Content Uniformity % | Disintegration Time (sec) Mean ± S.D. |
|----------------|--|--------------------|--------------------------------------|----------------------|--|
| LS-1 | 6.4 ± 0.264 | 0.14 | 1.065 ± 0.556 | 100.4 | 42.6 ± 2.16 |
| LS-2 | 6.46 ± 0.251 | 0.2 | 867.63 ± 0.32 | 99.58 | 55.3 ± 1.92 |
| LS-3 | 5.73 ± 0.152 | 0.28 | 722.83 ± 0.29 | 95.83 | 68.5 ± 3.87 |
| LS-4 | 5.6 ± 0.115 | 0.53 | 620.53 ± 1.32 | 100.8 | 80.6 ± 2.51 |
| LS-5 | 5.3 ± 0.153 | 0.23 | 980.53 ± 0.51 | 97.5 | 61.3 ± 2.28 |
| LS-6 | 5.86 ± 0.15 | 0.26 | 784.03 ± 0.55 | 97.05 | 83.1 ± 4.14 |
| LS-7 | 4.73 ± 0.23 | 0.42 | 653.2 ± 0.721 | 101.6 | 95.6 ± 3.62 |
| LS-8 | 4.53 ± 0.67 | 0.45 | 559.23 ± 0.68 | 95.41 | 101.9 ± 3.35 |
| LS-9 | 4.4 ± 0.2 | 0.52 | 830.5 ± 0.458 | 99.16 | 89.6 ± 2.18 |
| LS-10 | 4.33 ± 0.251 | 0.19 | 664.16 ± 0.42 | 96.25 | 107.8 ± 1.57 |
| LS-11 | 4.3 ± 0.173 | 0.41 | 552.8 ± 0.2 | 95 | 112.3 ± 4.72 |
| LS-12 | 4.46 ± 0.152 | 0.53 | 474.23 ± 0.25 | 97 | 126.4 ± 2.44 |
| DCT | 5.65 ± 0.45 | 0.61 | 770.06 ± 0.12 | 100.4 | 74.7 ± 2.21 |

n=3.

In-vitro dissolution test

The dissolution profiles of clopidogrel LS compacts in SIF (phosphate buffer pH 6.8 with 1% SLS) were graphically represented in figures (15 – 17). The percentage of clopidogrel bisulfate released from liquisolid compacts (LS 1 – 12) was varying from 28.3 – 92.2 % in first 10 minutes. While, 13.6 % and 24.2% of drug released from DCT and marketed tablets (Plavix®), respectively. The results indicated fast release of the drug was observed from the LS compacts. Such enhanced drug dissolution rate may be mainly attributed to the fact that this poorly water-soluble drug was already in solution in Tween 80 (molecular dispersed form). While, at the same time it was carried by the powder particles (microcrystalline cellulose-silica) of the liquisolid system. Thus, its release was accelerated due to its markedly increased

wettability and surface availability to the dissolution medium ⁽⁴¹⁾.

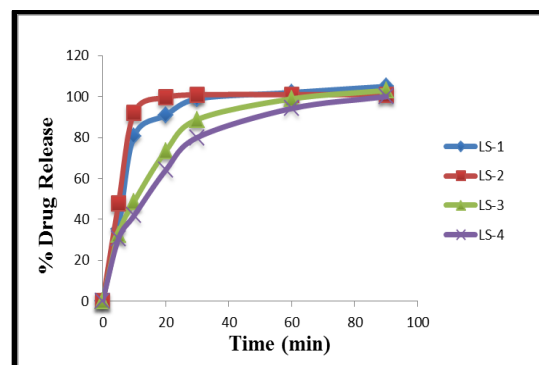


Figure 15. Dissolution profiles of clopidogrel from liquisolid compacts (LS 1 – 4) in phosphate buffer (pH 6.8) with 1% SLS at 37°C

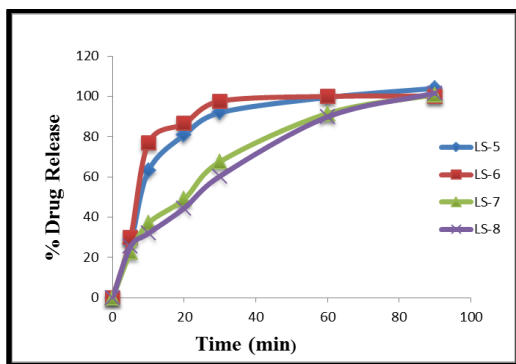


Figure 16. Dissolution profiles of clopidogrel from liquisolid compacts (LS 5 – 8) in phosphate buffer (pH 6.8) with 1% SLS at 37°C

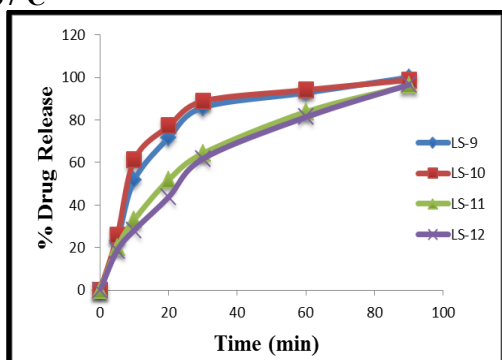


Figure 17. Dissolution profiles of clopidogrel from liquisolid compacts (LS 9 – 12) in phosphate buffer (pH 6.8) with 1% SLS at 37°C

From the calculations, the MDT and % DE for LS-2 were (5.95 min and 90%), DCT were (35 min and 34%), and for marketed tablets (Plavix®) were (30.14 min and 52%), respectively. Compacts of LS-2 represented the lowest MDT and highest %DE compared with other prepared LS formulations and it was considered as the optimum LS formula. In addition, LS-2 compacts showed better improvement in dissolution in contrast with DCT and marketed tablets (Plavix®) since, lower MDT and higher %DE values indicated that, LS-2 compacts were significantly ($p < 0.05$) enhanced dissolution rate⁽⁴²⁾.

The effect of drug concentration on the release of drug was shown in figure 18. Better dissolution was observed at huge differences in drug concentrations. However, as the drug concentration decreased, the portion solubilized and molecularly dispersed in the liquid vehicle increased thus leading to improve dissolution. In addition, the more vehicles available, the more even the distribution of the vehicle over the remaining undissolved drug particles that would help in better wetting of the drug through the dissolution stage⁽⁴³⁾.

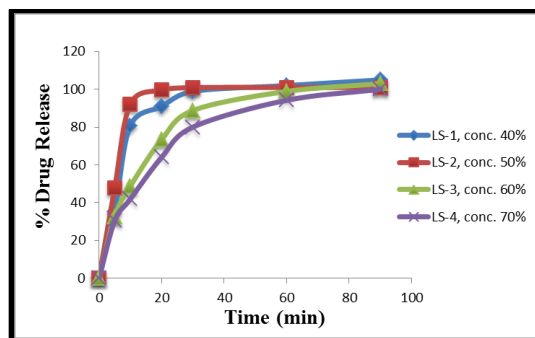


Figure 18. Effect of drug concentration on dissolution profile of clopidogrel liquisolid compacts in phosphate buffer (pH 6.8) with 1% SLS at 37°C.

On the other hand, the powder excipient ratio (R) was directly proportional to the *in-vitro* drug release i.e., when R increased, clopidogrel release also increased as shown in figure 19. This may be attributed to the high carrier (Avicel) content where it also functioned as a swellable disintegrant. In addition, the highly hydrophilic characteristic of Avicel (microcrystalline cellulose) increased the wetting of clopidogrel and enhanced its dissolution⁽⁴⁴⁾.

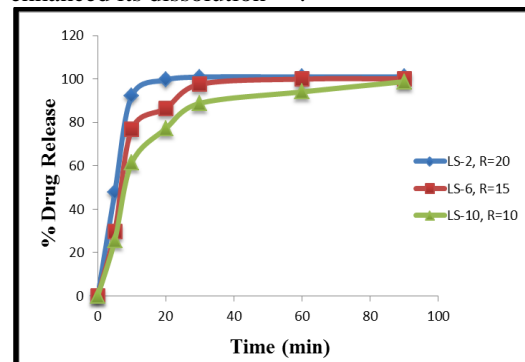


Figure 19. Effect of excipient ratio (R) on dissolution profile of clopidogrel liquisolid compacts in phosphate buffer (pH 6.8) with 1% SLS at 37°C.

The release of clopidogrel bisulfate from LS-2 compacts was compared with that of DCT and marketed tablets (Plavix®) in 0.1N HCl (pH 1.2) figure 20 and in Phosphate buffer (pH 6.8) with 1% SLS, figure 21. The difference in the percent of drug release was found to be significant ($p < 0.05$). This clearly indicated the improvement in the dissolution of clopidogrel was due to the presence of the drug in nonvolatile solvent (Tween 80) in the LS formulation. After LS compact disintegration, its primary particles suspended in the dissolution medium had the drug particles in a state of molecular dispersion. In contrary, there was a limited surface area of the plain drug exposed to the dissolution medium in

DCT and the marketed tablets (Plavix®), because of the hydrophobic nature of the drug particles. However, the higher dissolution rates observed in LS compacts could be related to a considerably larger surface area of the dispersed drug particles exposed to the dissolution medium⁽⁴⁵⁾.

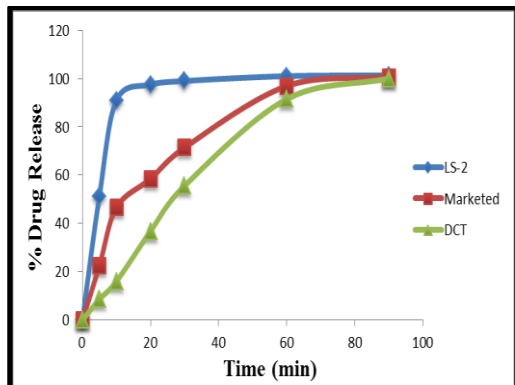


Figure 20. Dissolution profiles of liquisolid compacts LS-2, DCT and marketed tablets in 0.1N HCl (pH 1.2) at 37 °C

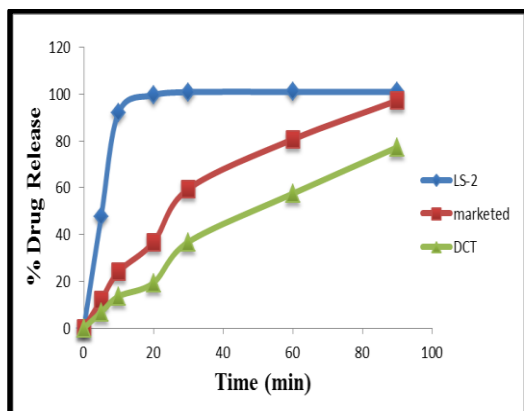


Figure 21. Dissolution profiles of liquisolid compacts LS-2, DCT and marketed tablets in Phosphate buffer (pH 6.8) with 1% SLS at 37°C.

Conclusion

The liquisolid technique succeeded to enhance the dissolution rate of the practically insoluble drug (clopidogrel bisulfate). Among the LS Compact formulas, LS-2 that was prepared by using Tween 80 as a non-volatile liquid vehicle, at the R-value of 20 and containing 50% drug concentration, possessed reasonable flow, rapid dissolution time and the highest dissolution rate. However, each of drug concentration and R-value were factors affecting the dissolution rate. In addition, LS-2 compacts exhibited the best dissolution as compared to DCT and marketed (Plavix®) tablets.

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