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# *Hibiscus rosa-sinensis* Linn., Leaves As Rate Controlling Matrix For Sustained Release Of Diclofenac Sodium: A $3^2$ Factorial Study.

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**ABSTRACT**

Diclofenac sodium is a non-steroidal anti-inflammatory drug (NSAIDs), which is commonly used for many ailments to reduce the pain especially in rheumatoid arthritis as an example. Since the biological half-life of this drug is about 1-2 hours, required multiple dosing to maintain therapeutic drug blood level. It is also less soluble in water but is readily soluble in alkaline pH. Therefore an attempt was made to formulate a sustained release formulation with increased patient compliance and decreased signs of adverse effects. The objective of this study was to formulate and evaluate the mucilage of *Hibiscus rosa-sinensis* Linn. leaves as rate controlling matrix for sustained release of diclofenac sodium tablet by direct compression method using $3^2$ full factorial design. In factorial design, amounts of dried mucilage and lactose were taken as independent factors. The percentage of drug release in 60 and 300 min and time for 80% drug release were considered as dependent variables. Shade dried *Hibiscus rosa-sinensis* Linn leaves were powdered and the mucilage was extracted and studied for percentage yield, particle size, mass loss on drying, viscosity, swelling index, bulk density, angle of repose, and compressibility. Diclofenac sodium tablets formulation was evaluated for pre and post compression parameters. Our findings suggested that the dried mucilage powder showed superior swelling capacity and excellent flow properties. Prepared tablets have acceptable hardness, friability, and uniform in weight. It was found that batch F6 fulfilled all the selected criteria. Drug release kinetics by using the above formulations agreed best to the zero-order kinetics. It was concluded that the mucilage can be used as release-retarding agent for 12 hours when the drug-mucilage build up ratio was 1:1.5. So, matrix tablet containing dried mucilage of *Hibiscus rosa-sinensis* Linn., leaves is most suitable for sustained release of diclofenac sodium.

**Keywords:** Diclofenac sodium, *Hibiscus rosa-sinensis* Linn, matrix tablets, sustained release.

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## INTRODUCTION

*Hibiscus rosa-sinensis* Linn belongs to the Malvaceae family is also known as the shoe-flower plant, China rose, and Chinese hibiscus has got many medicinal values and traditionally used for treating various ailments. The plant is available in Asia especially in tropical areas in large quantities, and the leaves contain mucilage [1, 2]. The leaves are used in traditional medicines as emollients and aperients to treat burning sensations, skin diseases, and constipation [3]. Many literature evidences showed that this plant contains cyclopropanoids, methyl sterculate, methyl-2-hydroxysterculate, 2-hydroxysterculate malvate, and β-rosasterol and are extracted by various methods. Mucilage of *Hibiscus rosa-sinensis* Linn contains L-rhamnose, D-galactose, D-galactouronic acid, and D-glucuronic acid [4]. The leaves contain carotene (7.34 mg/100 g of fresh material) and are used as cattle feed [5]. The leaves also contain moisture, protein, fat, carbohydrate, fibers, and minerals such as calcium, and phosphorus also [6]. Literature evidences stated that the *Hibiscus rosa-sinensis* Linn has been explored as a pharmaceutical excipient for many drugs.

Sustained release oral delivery systems used therapeutically to attain effective concentrations of drug in the systemic circulation over an extended period of time, which provides better patient compliance and allows a reduction of both the total dose of drug administered and the incidence of adverse side effects. There are many approaches are used for the cause and of all the different approaches, matrix systems still appear as one of the most attractive from the economic process development and scale up points of view [7].

Diclofenac sodium is a potent non-steroidal anti-inflammatory drug and rapidly dissolves in intestinal fluid reaches its maximum blood concentration ($C_{max}$) within 30 minutes. It is metabolized mainly by hydroxylation and subsequent conjugation reactions occur in the liver [8]. In healthy human beings, the mean plasma clearance of diclofenac sodium was found to be 16.0 L/h, and the mean elimination half-life of the terminal phase was 1.2–1.8 hours [9]. To reduce gastrointestinal irritation and inflammation both which are the common problems with all non-steroidal anti-inflammatory agents, effective enteric-coated dosage forms need to be developed. Commonly the food delays the absorption of the drug, which causes a non-reproducible pharmacokinetic profile, and thus the drug has no immediate therapeutic effect [10]. Drug release from hydrophilic matrices is a complex interaction between dissolution, diffusion, and the erosion mechanisms. The objective of this study was to formulate and evaluate the mucilage of *Hibiscus rosa-sinensis* Linn leaves as rate controlling matrix for sustained release of the drug diclofenac sodium by direct compression method using $3^2$ factorial study designs. In this study, the drug release mechanism was evaluated for diclofenac sodium tablets prepared with highly hydrophilic mucilage from the leaves of *Hibiscus rosa-sinensis* Linn.

## MATERIALS AND METHODS

### Chemicals

The major drug Diclofenac sodium salt was purchased from Sigma Aldrich chemicals Ltd. sodium carboxy methyl cellulose, lactose, talc, and magnesium stearate and all other chemicals were purchased from Sigma Aldrich chemicals Ltd. All the solvents and chemicals used in this study were of analytical-reagent grade. Deionized double distilled water was used throughout the study for any preparation and formulations.

### Collection of Plant material

The leaves of *Hibiscus rosa-sinensis* Linn were collected from the MAHSA University garden, Bandar Saujana Putra Campus, Selangor, Malaysia. The fresh leaves of *Hibiscus rosa-sinensis* Linn were collected and washed with water to remove dirts and debris and dried in shade. It was then powdered as fine as suitable for further study.

### Extraction of Mucilage

Powdered material was soaked in enough of water for 5 hours, boiled for 30 minutes and kept aside for an hour for complete release of mucilage into water. The mucilage was extracted using multilayer muslin cloth bag to remove the marc from the solution. Acetone was added to the filtrate to precipitate the mucilage in a quantity of three times the volume of the total filtrate (3:1 ratio). The mucilage was thus separated, dried,

in an oven at 40ºC, collected, ground, passed through #80 sieve filter paper and stored in desiccator at room temperature for further use [11].

**Physicochemical Properties *Hibiscus rosa-sinensis* Linn mucilage**

**Particle size**

The microscopic analysis in triplicates of sample was used to determine the particle size of the dried mucilage [11].

**Loss of Mass on drying**

The loss of mass on drying was determined for an appropriate quantity of dried mucilage at 105 °C for 2 hours in a hot air oven [11].

**Swelling ratio**

The study was carried out in a 100 mL stoppered graduated cylinder. The initial bulk volume of 1 g of mucilage was measured, and water was added in sufficient quantity to yield a 100 mL uniform dispersion. The sediment volume of the swollen mass was noted after 24 hours in storage at room temperature. The swelling ratio was calculated by determining the ratio of the swollen volume to the initial bulk volume. The swelling ratio was evaluated in distilled water, simulated gastric fluid (0.1 M HCl), and phosphate buffer (pH 6.8) [12].

**Bulk and tapped density**

A pre weighed quantity of dried mucilage was poured into a graduated cylinder and the volume was recorded. The cylinder was tapped until the powder bed volume reached a minimum value and the tapped volume was recorded. The bulk and tapped densities were calculated. Carr's index and Hausner's ratio were calculated from the bulk and tapped densities.

**Angle of repose**

The angle of repose (θ) was determined using the fixed-height funnel method and calculated as follows:

$$\text{Angle of repose} = \text{Tan}^{-1}\, h/r$$

where 'h' is the height of the powder heap and 'r' is the radius of the powder heap.

**Preparation of diclofenac sodium sustained release matrix tablets**

The required quantities of diclofenac sodium (60#) and dried mucilage powder (80 #) were physically admixed. Earlier the ratio of mixing was fixed by trial and error method to give the best results of listed physicochemical parameters. The powder blend was then lubricated with 1% w/w talc and 2% w/w magnesium stearate. Lubrication was done in a glass jar for 2 minutes. Each tablet contained 100 mg of the drug. The tablets were prepared by direct compression on a rotary tablet press. [13].

**Experimental**

A $3^2$ randomized full factorial study design was employed in the present work to prove the effectiveness of excipient. Two factors were evaluated, each at three levels, and experimental trials were performed at nine possible combinations. Thus this experimental design was called $3^2$ full-factorial study designs and accordingly nine formulations were prepared. In this factorial design the 2 independent variables were used. They are (i) amount of dried mucilage powder ($X_1$) and (ii) amount of lactose ($X_2$). The time required for 80% drug dissolution (t-80) and percentage drug released in 60 min (Y60) and 300 min (Y300) were selected as dependent variables [14]. The low (-1), medium (0), and high (+1) values of $X_1$ were 100, 150

and 200 mg; the low (-1), medium (0), and high (+1) values of $X_2$ were 0, 50 and 100 mg respectively. The compositions of the nine batches of the factorial design are shown in Table 1 and 2.

**Table 1: Diclofenac sodium sustained release matrix tablets with their experimental coded level of variables for $3^2$ factorial study designs**

| Batch Code | Variable Levels in Coded Form | |
|---|---|---|
| Diclofenac sodium | $X_1$ | $X_2$ |
| AF1 | -1 | -1 |
| AF2 | -1 | 0 |
| AF3 | -1 | +1 |
| AF4 | 0 | -1 |
| AF5 | 0 | 0 |
| AF6 | 0 | +1 |
| AF7 | +1 | -1 |
| AF8 | +1 | 0 |
| AF9 | +1 | +1 |

**Table 2: Translation of coded levels of diclofenac sodium sustained release matrix tablets**

| Diclofenac sodium (100 mg) | | | |
|---|---|---|---|
| Variable levels | Low (-1) | Medium(0) | High (+1) |
| $x_1$= Concentration *of Hibiscus rosa-sinensis Linn* mucilage | -1 (100 mg) | 0 (150 mg) | +1 (200 mg) |
| $x_2$= Amount of lactose | -1 (0 mg) | 0 ( 50 mg) | +1 (100 mg) |

**Multiple regression analysis**

The use of regression analysis in $3^2$ factorial study design generated polynomial equations for different models, with interacting terms and regression coefficients, useful in evaluating the responses. There are two models in which the first one is full model (non-significant terms included) and the second one is reduced model (non-significant terms excluded). In the full model study, the responses were analyzed using the quadratic equation below:

$$Y = b_0 + b_1 X_1 + b_2 X_2 + b12\, X_1 X_2 + b11\, X_1^2 + b22\, X_2^2$$

Where, Y is the response evaluated, $b_0$ is the arithmetic mean response of 9 runs and $b_1$ is the estimated coefficient of $X_1$. The main effects ($X_1$ and $X_2$) represent the average result of changing one factor at a time from its low to high value. The interaction term ($X_1X_2$) shows how the response changes when two factors are simultaneously changed. The polynomial terms ($X_1^2$ and $X_2^2$) were included to investigate nonlinearity.

In the reduced model, the non-significant terms in the quadratic equation are removed using backward regression procedure. This is quite important to study the influence of factors on the responses evaluated. The value and sign of regression coefficient ($b_1$) indicated the magnitude of influence of the particular term on the response. The regression coefficients gave the average change in a response when the particular factor was changed by a unit, when all the other terms remain constant. A positive or negative sign on the regression coefficient indicated that the factor has either positive or negative effect on the response accordingly [17, 18].

**Evaluation of diclofenac sodium sustained release matrix tablets**

**Hardness**

The hardness of the tablet was determined using Monsanto hardness tester. It was expressed in kg/cm. Three tablets were randomly picked and hardness of the tablets was determined.

**Thickness**

The thickness of the tablet was measured by using Vernier caliper. Thickness of selected tablets from each batch was measured and average readings taken in triplicate were calculated.

**Friability test**

Friability was evaluated as the percentage weight loss of 20 tablets tumbled in a friabilator (Model EF2, Electrolab) for 4 minutes at 25 rpm for 100 revolutions. The tablets were dedusted, and the loss in weight caused by the fracture or abrasion was recorded as the percentage of friability. The % friability was calculated by the following formula

$$\text{Percentage friability} = (W - W_0)/ W \times 100$$

Where, $W_0$ = initial weight ;W = weight after friability

**Weight variation test**

The weight-variation test was performed to test the uniformity of formulated tablets. For this, randomly picked twenty tablets were weighed individually, and the average weight was calculated.

***In vitro* drug release study**

The *in vitro* drug release study was carried out using an USP XXIII paddle apparatus at 37 ± 0.5 °C at 50 rpm using 900 mL of distilled water and phosphate buffer of pH 6.8 as the dissolution medium (n = 5). A 5mL sample solution was withdrawn in pre-determined time intervals, filtered through a 0.45-µm membrane filter, diluted suitably, and analyzed spectrophotometrically at 276 nm using a UV-VIS double-beam spectrophotometer (Shimadzu-UV 1700, Shimadzu, Kyoto, Japan). Equal amounts of fresh dissolution medium were replaced every time after withdrawing a test sample in a quicker session.

**Pharmacokinetic study**

The drug kinetics were used to analyze the drug release and as followed

Zero order: Cumulative % of drug released vs time

$$Q_t = Q_0 - K_{0t}$$

First order: Log cumulative % of drug remaining vs time

$$\ln Q = \ln Q_0 - K_{1t}$$

Higuchi: Cumulative % of drug released vs √t

$$Q = Kt\ 1/2$$

Korsmeyer–Peppas: Log cumulative % of drug released vs log time

$$Mt/M\alpha = Ktn$$

Where, Qi and Ki stand for the amount of drug release and kinetic release constant, respectively. Mt/Mα indicates the fractional drug release and "n" is the diffusional exponent which gives the mechanism of drug release. When n<0.5, the drug diffuses through the polymeric matrix by a Fickian (case I) diffusion mechanism. For 0.5 < n<1, an anomalous (non-Fickian) mechanism occurs; n=1 indicates a zero-order (case II) and n>1 indicates non-Fickian super case II release mechanism.

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## RESULTS AND DISCUSSION

### Analysis of Physicochemical properties of mucilage

The swelling ratio of mucilage was 9 in distilled water, 10 in simulated gastric fluid (0.1 M HCl), and 9 in phosphate buffer (pH 6.8). The swelling ratio showed that the plant mucilage is pH-independent and is nonionic. The yield of mucilage was estimated about 32% and the average particle size calculated through microscope was 190 µm.

### Analysis of Flow properties of mucilage

The flow properties of dried powder mucilage are shown in Table 3. The loss of mass on drying indicated the amount of moisture present in the material that was available to interact with other material. For dried *Hibiscus* mucilage, the loss of mass on drying was found to be 12.3%.

**Table 3: Flow properties of *Hibiscus* mucilage**

| Parameters | Dried *Hibiscus* mucilage |
|---|---|
| Bulk Density (g/cm$^3$) | 0.54±0.1 |
| Tapped Density (g/mL) | 0.80±0.08 |
| Carrs's Index (%) | 17.30±1.22 |
| Hausner's ratio | 1.15±0.14 |
| Angle of repose | 31.83°±1.47° |

The bulk density after the drug compressed with mucilage blend was found between 0.52 ± 0.22 and 0.56 ± 0.24 g/cm$^3$. This range was considered as good packing capacity. Carr's index was found between 15.84 ± 0.14 and 18.96 ± 0.24, showed that the formulation had good flow characteristics. Hausner's ratio ranged from 1.16 ± 0.28 to 1.14 ± 0.12, which indicated good flowability. The angle of repose of all the formulations was within the range of 30°30′ ± 0.52 to 34°36′ ± 0.30. i.e. the diclofenac sodiun formulation blend have fair flow properties.

The average hardness of the prepared tablets was 4.6 kg/cm2 , a value which showed good strength comparatively. The friability was < 1% (i.e., between 0.76 and 0.84%). Tablets prepared from *Hibiscus rosasinensis Linn* leaves mucilage that passed the standard for uniform in weight indicated that the drug content was homogenous.Therfore results showeed that tablets prepared from mucilage can be used as a very good directly compressible vehicle.

The preliminary batches was performed, the complete drug release was obtained within 11 hours with different proportions of *Hibiscus rosa-sinensis Linn* leaves mucilage and 100 mg of diclofenac sodium. Four criteria were established for the desired drug release profile: A release of 22–32% of the drug within the first hour. A release of 43% of the drug in < Y300 < 65% in 5 hours. A prolonged drug release of the remaining drug during next 12 hours, preferably at a relatively constant rate. A release of 80% of the drug in 378 < t80 < 578 minutes. The percentage of *in vitro* drug release of diclofenac sodium sustained release matrix tablets were shown in Table 4 represented that mucilage is suitable as a sustained release matrixing agent for a diclofenac sodium tablet.

**Tablet 4: Percentage of *in vitro* drug release of Diclofenac sodium Sustained Release Matrix Tablets.**

| Batch Code | Percentage of *in vitro* drug release | | |
|---|---|---|---|
| | $Y_{60}$ | $Y_{300}$ | $T_{80}$ |
| F1 | 30.60 | 65.36 | 378 |
| F2 | 26.26 | 65.45 | 420 |
| F3 | 22.22 | 55.82 | 398 |
| F4 | 29.66 | 60.68 | 474 |
| F5 | 26.42 | 56.42 | 542 |
| F6 | 24.42 | 43.40 | 426 |

| | | | |
|---|---|---|---|
| F7 | 32.12 | 58.60 | 469 |
| F8 | 26.62 | 48.76 | 542 |
| F9 | 26.28 | 60.26 | 578 |

The Y60, Y300 and T80 values for the nine batches (F1 to F9) showed a wide variation: 22 to 32%, 43 to 65%, and 378 to 578 min, respectively.

The values of the correlation coefficient were 0.9882, 0.9864 and 0.9880 respectively, indicated that it was a good fit. The polynomial equation used the magnitude of the coefficient and the mathematical sign it carries were used to draw the conclusion. The low value of X1X2 suggested that the interaction between X1 and X2 was not significant. Among nine batches F1 to F9 formulation, only F6 fulfilled all the four selection criteria. Therefore, batch F6 was selected as the optimized batch. The *in vitro* dissolution rate of batch F6 were compared with the ideal release profile using f2 statistics. An f2 indicated that the drug release profile of batch F6 was comparable with the ideal batch. A value of f2 ≥ 50 is necessary for similarity in dissolution profile at 10% difference. The drug release of the best batch F6 was also conducted in phosphate buffer (pH 6.8). The dissolution data of batch F6 in distilled water were compared with the dissolution data in phosphate buffer (pH 6.8). An f2 statistics of 87% indicated that the release profile of batch F6 in distilled water and phosphate buffer were comparable.

**Kinetics of drug release**

The dissolution data of the best batch (F6) were tried to fit to zero order, first order, Higuchi, Hixson Crowell and Korsemeyer and Peppas models (15, 16). The results of f2 statistics were used for selecting the most appropriate model. The least residual sum of square compared with Higuchi and Korsmeyer and Peppas models. However the superiority was statistically insignificant among these two models, as shown by the goodness of fit test (F ratio test). Accordingly the priority was given to the model with the lowest F value. Thus the results shown were best fit with (batch (F6)) the zero order equation and explained the drug release from the hydrophilic matrix of diclofenac sodium tablets (19, 20).

## CONCLUSION

The authors concluded that a novel hydrophilic excipient, such as mucilage extracted from *Hibiscus rosa-sinensis* Linn, can be used for the development of sustained release tablets. We tested this with one of the most common drug used for relieving the pain. The dried mucilage powder showed superior swelling capacity and is pH independent which will help to further exploration as a disintegrating agent, gelling agent and as modified release dosage form.

**Conflict of interest:** The authors do not have any conflict of interest.

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