



# Formulation and In-Vitro Evaluation of Dasatinib Sustained Release Capsules an Anti-Cancer Drug

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## ABSTRACT

The sustain release capsules containing Dasatinib SR and were successfully made by direct filling and dry granulation method respectively. The physiochemical evaluation results for the powder of all trials pass the official limit in bulk density, tapped density, Carr's index, Hausner's ratio, angle of repose. The capsule size for every formulation is maintained size #2 and having average weight 200mg. In the optimized F3 trial which releases the Dasatinib  $20.63\% \pm 1.06$  in 1st hour and remaining drug released upto 12 hours which is  $65.45\% \pm 1.6$ .

**Keywords:** Dasatinib, Sustained Release, Capsule, Polymers.

## INTRODUCTION

A new drug delivery system enhances the therapeutic effectiveness of incorporated medicines by continuously ensuring, monitoring delivery and targeting medicines to move from the spot. A drug delivery system aims to provide therapeutic access to narcotic drugs where there is an action of drugs in the body to achieve rapid and then maintain the concentration of drugs, for the pharmaceutical industry that is interested in a slow-release oral drug delivery system.<sup>[1-3]</sup>

Stable release tablets and capsules are usually taken once or twice daily, compared to the standard forms of a partner who may need to take several doses daily to achieve the same therapeutic effect. Typically, hard-release products provide immediate drug release that immediately reflects the expected drug effect, followed by the release of additional drug content to maintain this effect within

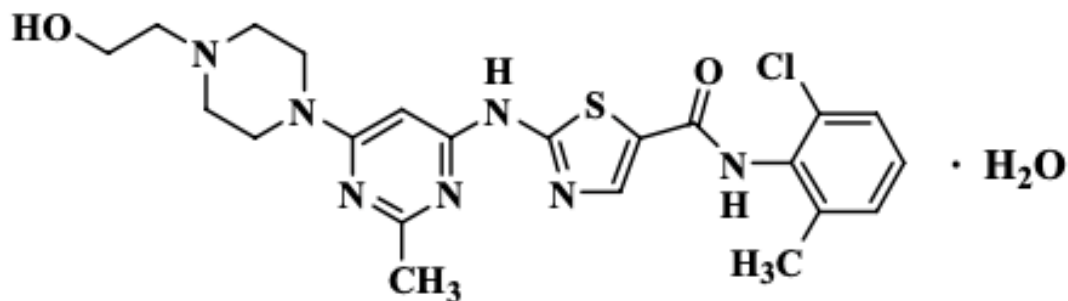
a predetermined time. Stable plasma drug levels provided by regular products always eliminate the need for overnight injections, which not only benefit patients.<sup>[4,5]</sup>

## PHARMACOKINETIC SIMULATION OF SUSTAINED RELEASE PRODUCTS

The multiple profiles of plasma over-the-counter drug profiles are equivalent to the single-chamber model considering the completion of the first order and the absorption of the drug. When we compare, maintain the release and the immediate release product, we find that a stable release product shows a higher absorption rate than a faster release product. High concentration ( $T_{max}$ ) usually lasts longer, and drug concentration ( $C_{max}$ ) decreases. If the drug formulation has to be done correctly, the AUC should be the same; parameters like  $T_{max}$ ,  $C_{max}$ , and AUC show how effective a long-term product is in vivo. For example, a product with a 3-hour  $T_{max}$  would not be very satisfactory if the product was allowed to study for 12 hours. High  $C_{max}$  is an indicator of volume loss due to uneven distribution of composition. Legal agencies have used Pharmacokinetic analysis of single and multiple-dose plasma data to evaluate multiple continuous output products. The study is effective because many products can be included in this model even if the drug has extracted originally. This analysis in which constant absorption rate may not exceed the rate of drug depletion in-vivo.

### DRUG PROFILE

Dasatinib is a selective tyrosine kinase receptor inhibitor that is used in the therapy of chronic myelogenous leukemia (CML) positive for the Philadelphia chromosome. Dasatinib was the first second-generation TKI being introduced in 2006. It works by blocking the BCR-ABL1 and other tyrosine kinases. *In vitro* analyses have shown that dasatinib is more than 300 times as potent as imatinib and 16 times as potent as nilotinib. Dasatinib inhibits many of the known mutations in BCR-ABL1 except T315I.<sup>[6]</sup>



**Figure 1:Chemical Structure Dasatinib**

## MECHANISM OF ACTION

Dasatinib, at nanomolar concentrations, inhibits the following kinases: BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, and PDGFR $\beta$ . Based on modeling studies, dasatinib is predicted to bind to multiple conformations of the ABL kinase. In vitro, dasatinib was active in leukemic cell lines representing variants of imatinib mesylate sensitive and resistant disease. Dasatinib inhibited the growth of chronic myeloid leukemia (CML) and acute lymphoblastic leukemia (ALL) cell lines overexpressing BCR-ABL. Under the conditions of the assays, dasatinib was able to overcome imatinib resistance resulting from BCR-ABL kinase domain mutations, activation of alternate signaling pathways involving the SRC family kinases (LYN, HCK), and multi-drug resistance gene overexpression.

## MATERIALS AND METHODS

Dasatinib, HPMC K100M, Guar Gum, Polyvinylpyrrolidone, Talcum Powder, Lactose Monohydrate, Hard Gelatin Capsule shell.

### Experimental Method :

In the formulation prepared, the release retardants included were HPMC K100M and Guar Gum. PVP used as binder or polymer vehicle, Lactose Monohydrate as a sweetener for optimized Sustained Release formulation.

## Direct Filling Method

- Weigh Accurately Drug + HPMC K100M/Guar Gum + PVP + Talcum powder and lactose Monohydrate pass through 36 No Sieve and mix properly for 3-5 Minutes.
- Put the above preparation in polybag and shake it for 30 minutes for uniform mixing.
- Then sifted through Sieve No 18 and finely lubricated with Lubricant.
- Fill the Powder in capsule with hands (manual filling).

**Table 1: Composition of Dasatinib sustained release capsule**

Sr. No.	Ingredients	Formulation					
		F1	F2	F3	F4	F5	F6
1.	Dasatinib (mg)	50	50	50	50	50	50
2.	HPMC K100M (mg)	70	87.5	105	-	-	-
3.	Guar Gum (mg)	-	-	-	70	87.5	105
4.	Polyvinylpyrrolidone (mg)	17.5	17.5	17.5	17.5	17.5	17.5
5.	Talcum Powder (mg)	3.5	3.5	3.5	3.5	3.5	3.5
6.	Lactose Monohydrate (mg)	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.	Q.S.

## EVALUTION STUDIES:

### Determination of bulk density and tapped density

An accurately weighed quantity of the powder (W) was carefully poured into the graduated cylinder and the volume (V<sub>0</sub>) was measured then the graduated cylinder was closed with lid, set into the density determination apparatus (Bulk density apparatus, Electrolab, Mumbai). The density apparatus was set for 500 taps and after that, the volume (V<sub>f</sub>) was measured and continued operation till the two consecutive readings were equal. The bulk density and tapped density were calculated using the following formulas:

$$\text{Bulk density} = W/V_0$$

$$\text{Tapped density} = W/V_f$$

Where

V<sub>0</sub> = initial Volume

V<sub>f</sub> = final Volume

### Compressibility index( car's Index ) & Hausner Ratio

The Compressibility index and Hausner ratio are measures of the property of a powder to be compressed. As such, they are measures of the relative importance of inter particulate interactions. In a free- flowing powder, such interactions are generally less significant, and the bulk and tapped densities will be closer in value. For poorer flowing materials, they are frequently greater inter particle interaction, and a greater difference between the bulk and tapped densities will be observed. These differences are reflected in the compressibility index and the Hausner Ratio. The compressibility index and Hausner ratio may be calculated using measured values for bulk density ( $\rho_{\text{bulk}}$ ) and tapped density ( $\rho_{\text{tapped}}$ ) as follows :

$$\text{compressibility index} = \frac{\rho_{\text{tapped}} - \rho_{\text{bulk}}}{\rho_{\text{tapped}}} \times 100$$

$$\text{Hausner ratio} = \frac{\rho_{\text{tapped}}}{\rho_{\text{bulk}}}$$

**Table 2: Acceptance criteria of flow properties**

Sr. No.	Flow Properties	Angle of Repose ( $\theta$ )	Comp. Index (%)	Hausner ratio
1.	Excellent	25-30	<10	1.00-1.11
2.	Good	31-35	11-15	1.12-1.18
3.	Fair	36-40	16-20	1.19-1.25
4.	Passable	41-45	21-25	1.26-1.34
5.	Poor	46-55	26-31	1.35-1.45
6.	Very Poor	56-65	32-37	1.46-1.59
7.	Very Very Poor	>66	>38	>1.6

### Angle of repose

The flow characteristics are measured by angle of repose. Improper flow of powder is due to frictional forces between the particles. These frictional forces are quantified by angle of repose.

Angle of repose is defined as the maximum angle possible between the surface of a pile of the powder and the horizontal plane.

$$\tan \theta = h/r$$

$$\theta = \tan^{-1}h/r$$

Where. H = height of pile

R = radius of the base pile

$\theta$  = angle of repose

## Estimation of Drug Content :

Drug content in the capsules was calculated by UV Spectrophotometric method. A sample of capsules equivalent to 100 mg was dissolved in DMSO and the volume was adjusted up to 100 ml using DMSO. Then 1 ml taken from above preparation and dissolved in 10 ml of DMSO. The solution was filtered through Whatman Filter Paper. Then the filtrate was assayed for drug content by measuring the absorbance at 326 nm after suitable dilution.

## Percentage Yield:

The percentage yield of the prepared Powder Capsule was determined by using the formula.

Percentage yield =  $\frac{\text{Practical yield}}{\text{Theoretical Yield}} \times 100$

Theoretical Yield

## In-vitro dissolution

Dissolution is the main evaluation study conducted for the estimation of the drug release from the dosage form. USP-TYPE II apparatus was selected for the study. The Capsules equivalent to 180mg of drug were weighed accurately and filled in the capsule shells. Dissolution profiles were carried out in the following media:

1. 0.1N HCL for 2 hours
2. 6.8 ph NaoH for next 10 hours.

The parameters for dissolution apparatus for all the above runs were kept constant as described below:

**Type of apparatus:** USP II.

**RPM :** 50

**Temperature:**

**N=6 samples**  $37.5^0 \pm 0.5^0 C$

## Preparation of Buffer Solutions:

**1. Preparation of pH 1.2 Buffer:** place 8.5 ml of Hydrochloric acid in 1000 ml of Distilled water.

**2. Preparation of 6.8 pH Phosphate Buffer :** 28.20 g disodium hydrogen phosphate and 11.45 g of potassium dihydrogen phosphate in 1000 ml of Distilled water.

## METHOD FOR DISSOLUTION:

A total of 6 formulations were selected for the dissolution. These formulations were taken as n=6 for the dissolution. The method dissolution is as follows.

In vitro dissolution testing was conducted on Capsules equivalent to 180mg of Dasatinib.

- Powder were filled in hard gelatin capsules shells
- USP Type I apparatus was used
- Media 900 ml 0.1N HCL and 900 ml 6.8 ph Buffer.
- RPM: 50 rpm
- Time points: 1,2,3,4,5,6,7,8,9,10,11 and 12 hours
- Estimation by UV spectrophotometry.
- ◆ The dissolution vessels must be filled with the respective dissolution media. The dissolution parameters such as temperature, stirring speed must be set before starting of the dissolution.
- ◆ As the dissolution assembly reaches the temperature, sometime must be allowed for the paddles to rotate, after which the sample should be dropped carefully and the time must be noted.
- ◆ At the prescribed time intervals, aliquots(5ml) must be withdrawn with sampling tubes, at the same time equal quantity(5ml) of dissolution medium must replaced to maintain the volume of the medium.
- ◆ Withdrawn aliquots must be suitably diluted with the dissolution medium, and analyzed spectrophotometrically.
- ◆ Drug release was calculated and tabulated.



## MECHANISM OF DRUG RELEASE

To analyze the mechanism of the drug release rate kinetics of the dosage form, the data obtained were plotted as

- To analyze the mechanism of the drug release rate kinetics of the dosage form, the data obtained w Cumulative percentage drug released Vs Time (*In-vitro* drug release plots) .
- Cumulative percentage drug released Vs Square root of time (Higuchi's plots).
- Log cumulative percentage drug remaining Vs Time (First order plots) .
- Log percentage drug released Vs Log time (Peppas plots).

### First order model:

This model has also been used to describe absorption and/or elimination of some drugs, the release of the drug which followed first order kinetics can be expressed by the equation:

$$\text{Log } C = \log C_0 - Kt / 2.303$$

Where,  $C_0$  is the initial concentration of drug,

The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of  $-K/2.303$ .

### Stability Study

Stability tests are much simpler and needed less frequently for coarse dispersion, where particle sizes and phase changes must be followed. To overcome the problem of metastable formation which are not thermodynamically stable and takes long time to separate, thermodynamic stability test are recommended. Stability was carried out as per ICH guidelines.

**Table 3: Stability Conditions**

<b>Study Condition Specification</b>	<b>Time periods</b>
40°C ± 2°C/75% ± 5% RH	30 Days
40°C ± 2°C/75% ± 5% RH	60 Days
40°C ± 2°C/75% ± 5% RH	90 Days

### **Drug-Excipient Compatibility Study :**

FTIR studies were performed on drug and the optimized formulation using FTIR (Alpha-E Bruker). The samples were analyzed between wavenumbers 4000 - 400 cm.

### **UV-Spectrometric Analysis**

The present study estimation of Dasatinib was carried out by UV-spectrometric method (UV, V-630 Shimadzu Corporation, Japan). The drug solution was scan between 400-200 nm. The  $\lambda$  max of Dasatinib in DMSO was found to be 326 nm.

**RESULT AND DISCUSSION:****Characterization of Granules:****Table 4: Physical Properties of Pre filling SR formulations**

<b>Formulation No.</b>	<b>Bulk Density (gm/cc)</b>	<b>Tapped Density (gm/cc)</b>	<b>Car's Index (%)</b>	<b>Hauser's Ratio</b>	<b>Angle of Repose (<math>\theta</math>)</b>
F1	0.3712 $\pm$ 0.011	0.4101 $\pm$ 0.025	7.27 $\pm$ 0.659	1.177 $\pm$ 0.076	29.73 $\pm$ 0.41
F2	0.3703 $\pm$ 0.05	0.4120 $\pm$ 0.026	7.57 $\pm$ 0.514	1.053 $\pm$ 0.060	25.33 $\pm$ 0.63
F3	0.3743 $\pm$ 0.015	0.4120 $\pm$ 0.05	7.43 $\pm$ 0.760	1.059 $\pm$ 0.077	27.44 $\pm$ 0.35
F4	0.376 $\pm$ 0.020	0.4270 $\pm$ 0.037	13.74 $\pm$ 0.376	1.073 $\pm$ 0.053	27.44 $\pm$ 0.52
F5	0.355 $\pm$ 0.017	0.4600 $\pm$ 0.024	15.31 $\pm$ 0.794	1.224 $\pm$ 0.011	31.34 $\pm$ 0.13
F6	0.3710 $\pm$ 0.045	0.4770 $\pm$ 0.065	17.42 $\pm$ 0.120	1.24 $\pm$ 0.020	27.26 $\pm$ 0.43

**Drug Content of Sustained Release capsule:**

The percentage drug content of all Capsule formulation was found to be in the range of 70.00  $\pm$  1.2 to 72.30  $\pm$  1.4. The showed the drug uniformly distributed in the formulation.

**Table 5: Drug Content of all formulation**

Sr. No.	Formulation Code	% Drug Content
1.	F1	70.12 ± 0.4
2.	F2	71.45 ± 0.2
3.	F3	72.20 ± 0.7
4.	F4	70.90 ± 0.1
5.	F5	71.60 ± 0.2
6.	F6	72.10 ± 0.6

The drug content of F3 formulation was found to be 72.20%. While other formulation drug content found to be less than 70.15% so it concluded that F3 formulation have more drug content as compare to others.

#### **In-vitro Dissolution Studies:**

In vitro release study of Dasatinib Sustained release Capsule were performed in the following pH media (pH 1.2) and 6.7 ph Buffer at  $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ .

- ◆ In vitro dissolution testing was conducted on Capsules equivalent to 170mg of Dasatinib.
- ◆ Powder were filled in hard gelatin capsules shells
- ◆ USP Type I apparatus was used
- ◆ Media 900 ml 0.1N HCL and 900 ml 6.7 ph Buffer.
- ◆ RPM: 50 rpm
- ◆ Time points: 1,2,3,4,5,6,7,7,9,10,11 and 12 hours
- ◆ Estimation by UV spectrophotometry.

Table 6: Dissolution Profile of Dasatinib

Time (hr)	%Cumulative Drug Release					
	F1	F2	F3	F4	F5	F6
1	15.55±1.2	14.23±1.0	20.63±1.0 6	14.97±1.2	11.13±1.4	19.73± 1.6
2	20.12±1.1	17.17±1.1	25.76±1.4 5	22.29±1.3	14.72±1.4	23.16± 1.7
3	35.54±1.3	24.35±1.2	24.59±1.4 1	34.03±1.9	19.46±1.2	25.59± 1.3
4	42.40±1.2	34.15±1.1	29.12±1.2	42.33±1.0	24.64±1.0 9	26.05± 1.3
5	45.55±1.0 6	35.65±1.2	34.36±1.1	41.05±1.6	29.21±1.3	27.09± 0.9
6	43.74±1.5	34.96±1.3	34.15±1.4	37.97±1.0	30.72±0.7	33.91± 1.2
7	47.45±1.1	40.67±1.2	36.54±1.5	34.69±1.0	27.06±1.1	35.02± 0.7
7	49.09±1.4	41.67±1.7	45.91±1.1	39.61±0.7	33.73±0.9	35.65± 0.9
9	49.17±1.2	47.54±1.4	49.49±1.7	44.22±1.0	37.71±1.1	47.16± 0.9
10	42.31±1.1	51.35±1.2	52.94±1.7	43.27±1.6	46.79±1.2	51.45± 1.0
11	51.45±1.6	54.65±1.5	57.34±1.2	44.56±1.4	47.77±1.4	55.77± 1.4
12	49.44±1.1	51.97±1.2	65.45±1.6	47.55±1.1	50.54±1.4	57.50± 1.7

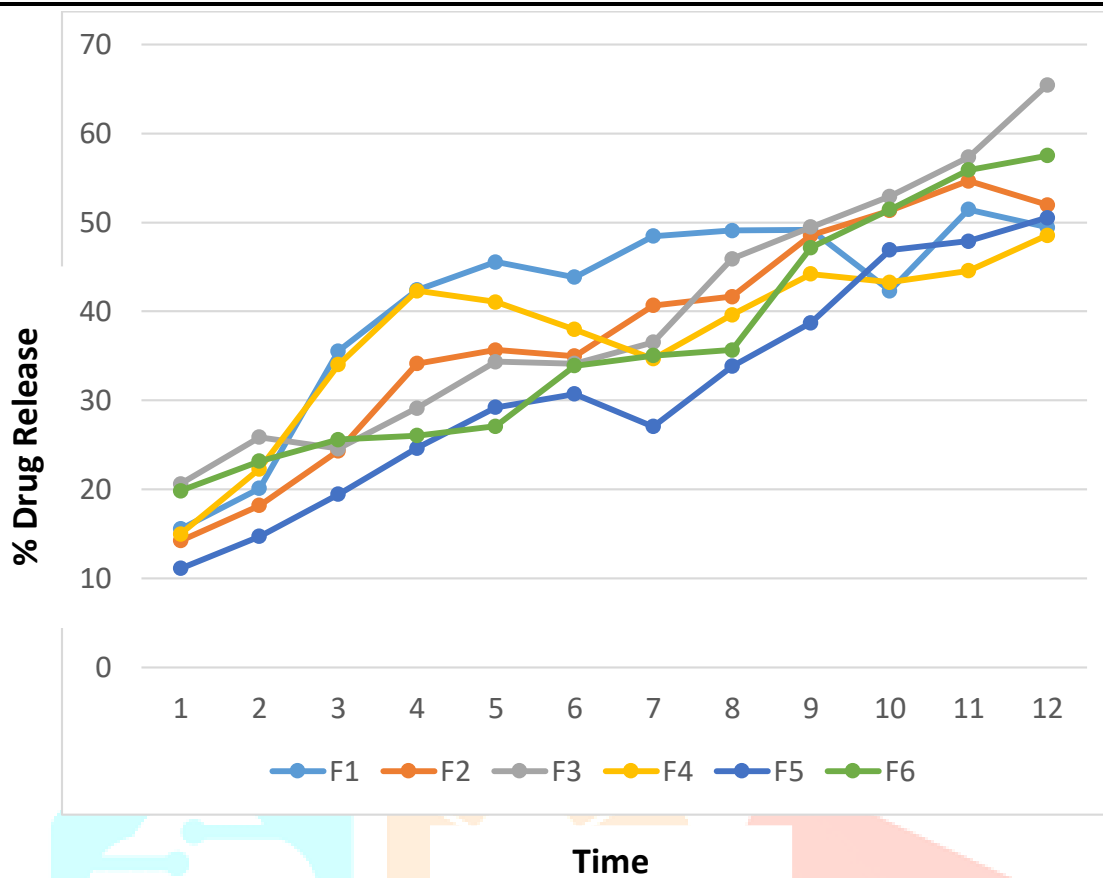


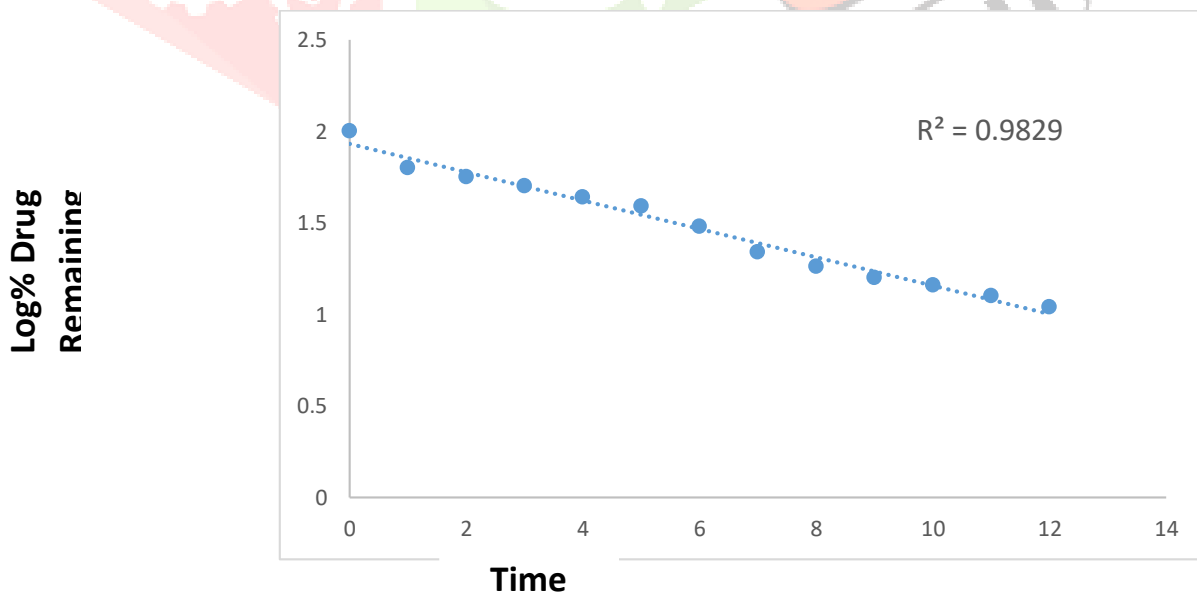
Figure 2: in-vitro Dissolution Study of Sustained release Formulation

### Release Kinetics of *in-vitro* Drug Release

The kinetics of In-Vitro drug release was determined by applying drug release data to various kinetic models such as Zero order, First order, Higuchi and korsmeyer – peppas. The result obtained represented in Table no.7 and shown in fig 3 to 7.

**Table 7: Release Kinetics of *in-vitro* drug release**

Formulation code	Zero order R <sup>2</sup>	First Order R <sup>2</sup>	Higuchi R <sup>2</sup>	Peppas R <sup>2</sup>	Best Model
F1	0.9772	0.9729	0.9759	0.9799	First Order
F2	0.9732	0.9902	0.9776	0.9799	First Order
F3	0.9901	0.9924	0.9790	0.9915	First Order
F4	0.9593	0.9614	0.9599	0.9605	First Order
F5	0.9779	0.9727	0.9797	0.9710	First Order
F6	0.9770	0.9915	0.9790	0.9901	First Order



**Figure 3: Best fit model ( first order) of formulation F1**

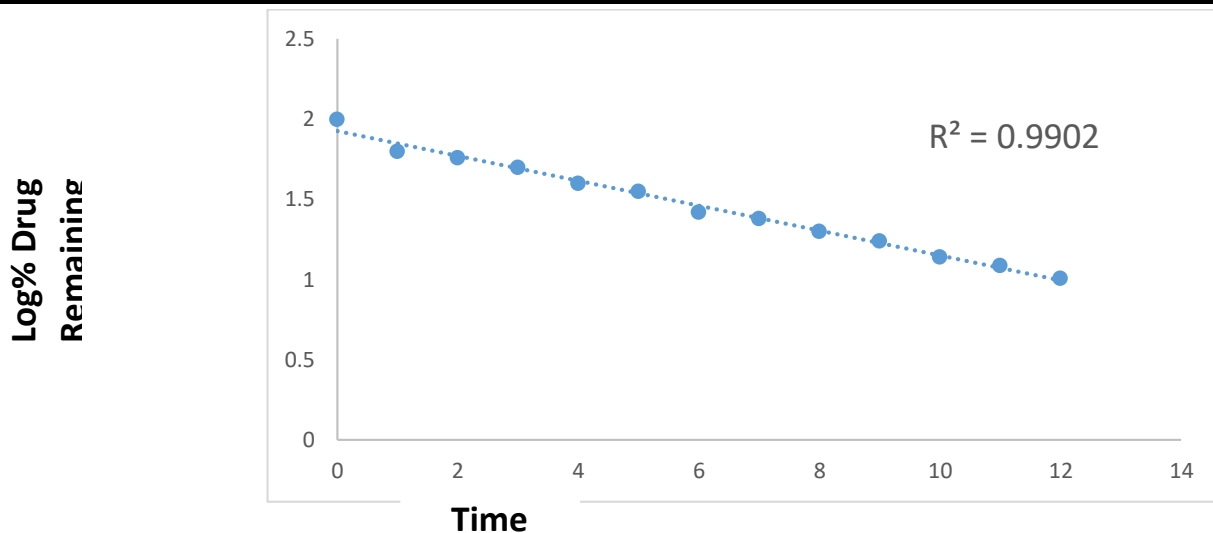


Figure 4: Best fit model (first order) of formulation F2

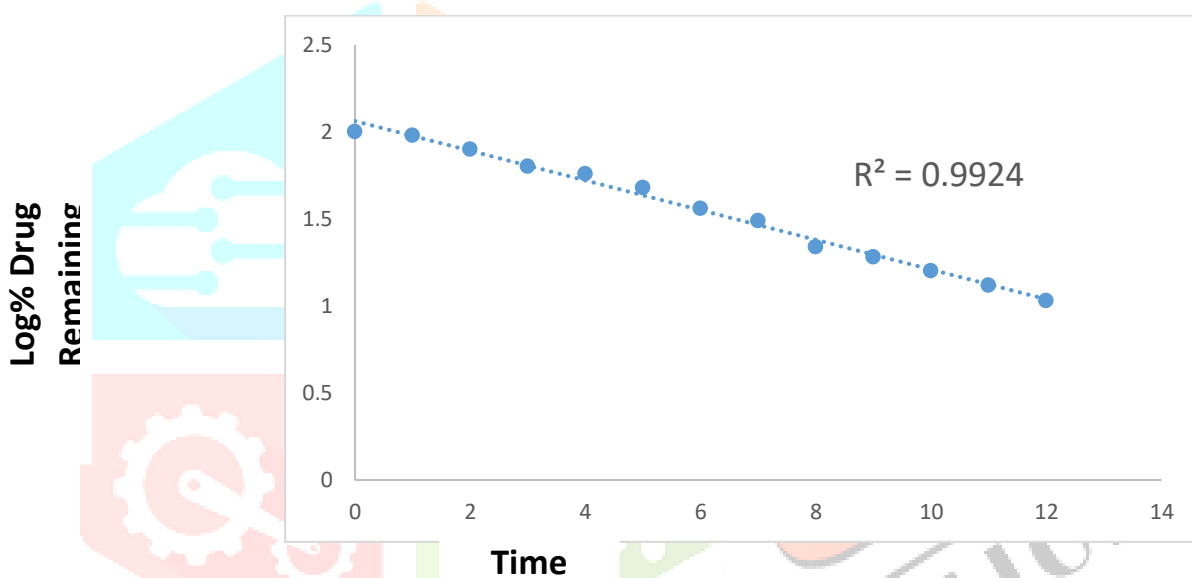


Figure 5: Best fit model (first order) of formulation F3

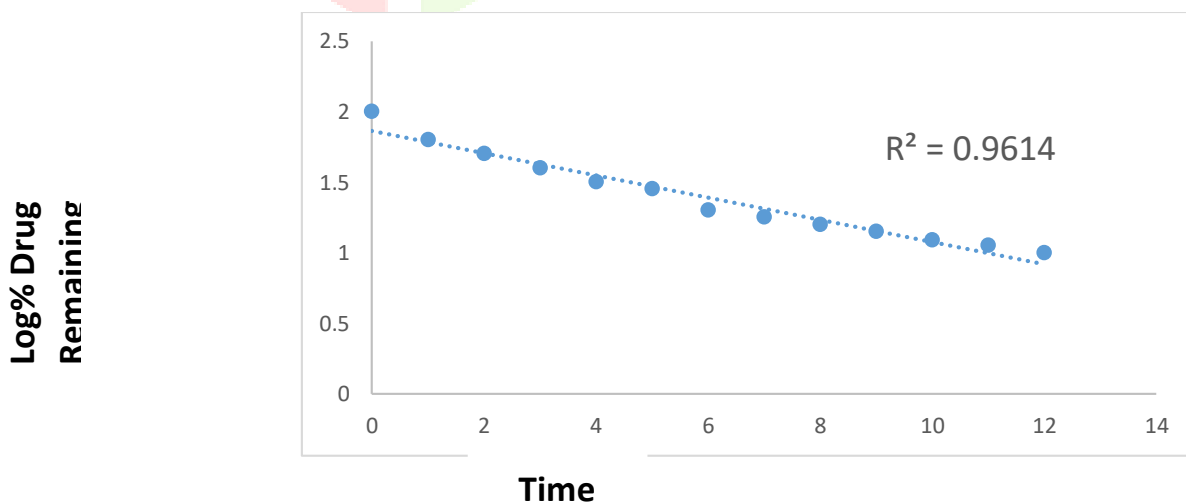
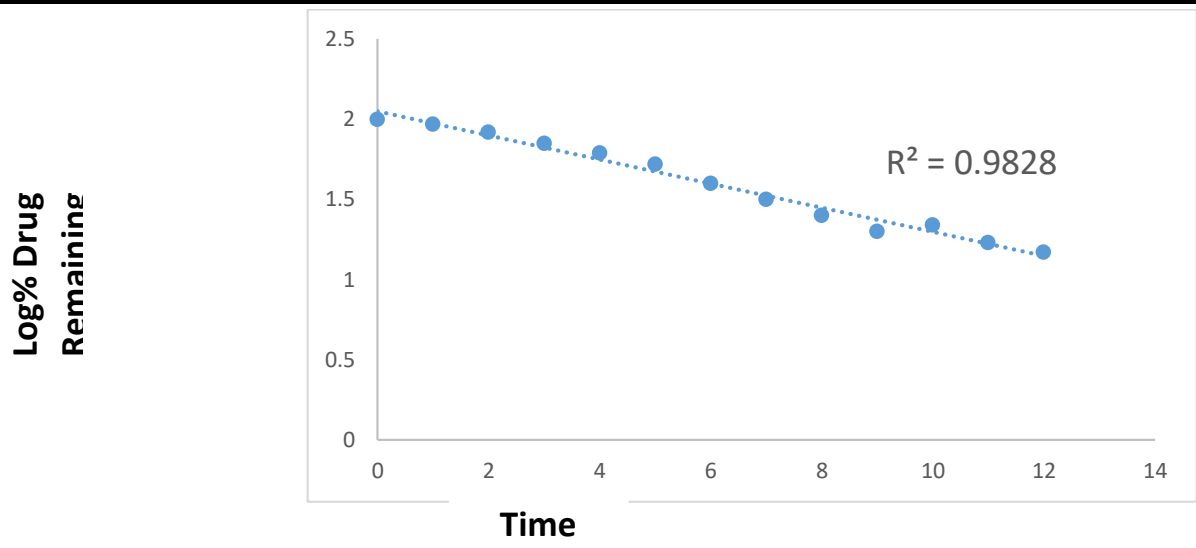
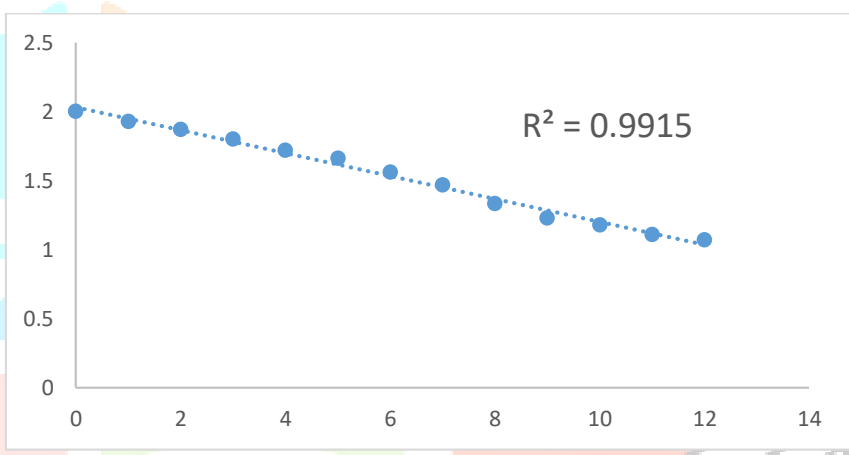


Figure 6: Best fit model (first order) of formulation F4





**Fig 7.: Best fit model (first order) of formulation F5**



**Fig 8.: Best fit model (first order) of formulation F6**

### Stability

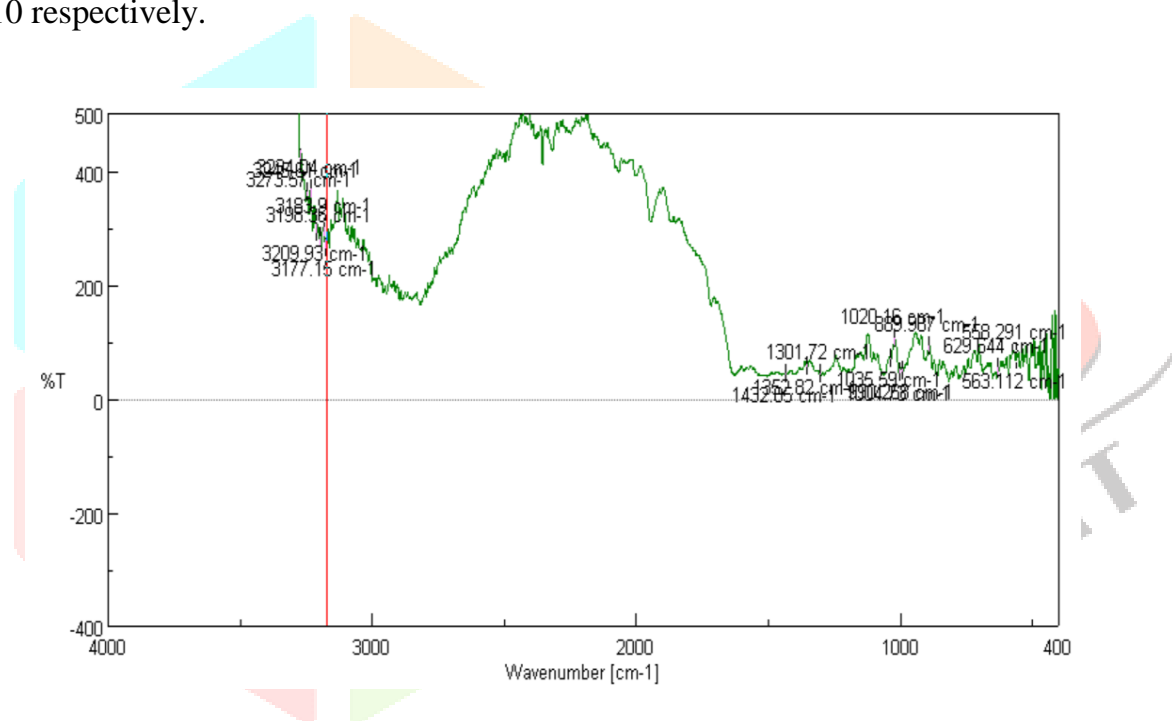
From the stability study it was found that the evaluated formulation (F3) showed there was no influence of variety of environment factors such as temperature, humidity, light and during storage condition or shelf life of Drug in table no.8.

**Table 8: Stability Study of data of F3 formulation**

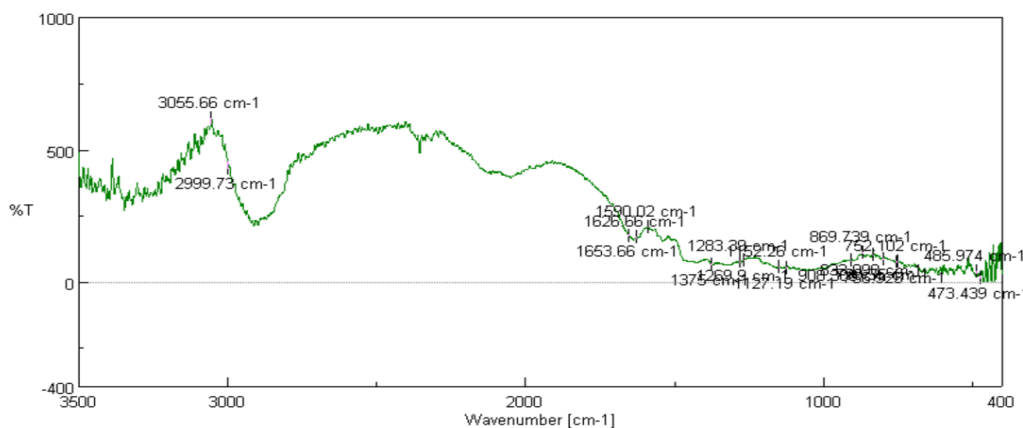
Physical Parameter	Observation		
	30 <sup>th</sup> day	60 <sup>th</sup> day	90 <sup>th</sup> day
% Drug Content	65.45	65.37	65.30

**FTIR Spectroscopy**

The FT-IR spectrum of the API was found to be similar to the standard were shown in the Figures 9 and 10 respectively.



**Figure 9: FT-IR of Dasatinib Pure Drug**



**Figure 10: FT-IR of Dasatinib with Excipients**

The FTIR spectrum for the Capsule indicates that there is no chemical interaction between the drug and the polymers used during the process of Powder capsule.

### UV-Spectrometric Analysis

The present study estimation of Dasatinib was carried out by UV-spectrometric method (UV, V-630 Shimadzu Corpraation, Japan). The drug solution was scan between 400-200 nm. The  $\lambda$  max of Dasatinib in DMSO was found to be 326 nm.

### Detection of $\lambda$ max of Dasatinib in DMSO

The  $\lambda$  max of Dasatinib in DMSO was found to be at 326 nm.

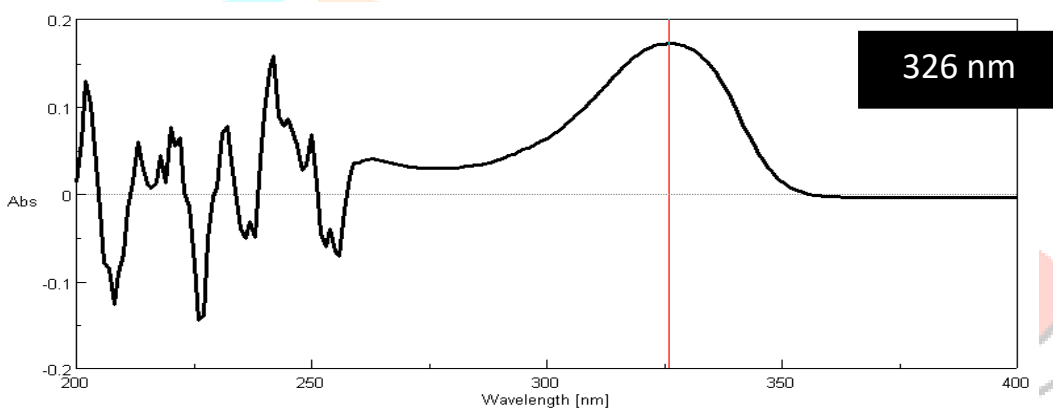
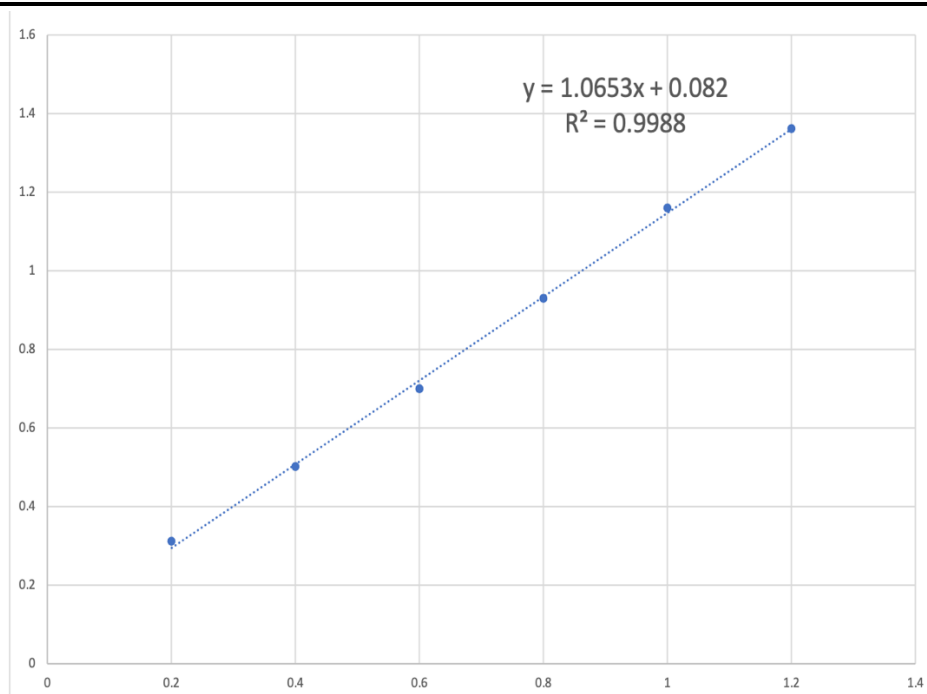


Figure 11:  $\lambda$  Max of Dasatinib in DMSO

### Construction of Calibration Curve of Dasatinib in DMSO

Table 9: Spectrophotometric data for the calibration curve of Dasatinib in DMSO

Sr. No.	Conc. ug/ml	Absorbance
1.	2	0.3111
2.	4	0.5024
3.	6	0.7001
4.	7	0.9305
5.	10	1.1602
6.	1.2	1.317



**Figure 12: Linearity Curve of Dasatinib in DMSO**

## CONCLUSION

Oral route is a promising approach for the formulation of drugs with poor aqueous solubility. In the future sight, novel development technology will enable Capsule formulation to solve more problems with other routes of administration and the oral route.

- FT-IR Spectral analysis results indicated that there was no probability of interaction between drug & excipients.
- The present study aimed to investigate the possibility of supporting the release of Dasatinib from the prepared capsule using different concentrations of polymers.
- The formulations were characterized for drug content and dissolution profile. The formulation shown good drug contain up to 82.20
- %.It shows that the drug is uniformly distributed in the Sustained release capsule.
- Based on in vitro Drug dissolution study formulation, F3 is the best-optimized formulation.
- *In-vitro* drug dissolutions study was performed on type II dissolution apparatus and due to drug release was found to be 48.55 to 65.45 %. A significantly enhanced dissolution rate was observed. Hence the formulation can show enhanced bioavailability, and moreover, the dose can be reduced.

- F3 formulation is the best fit model for the Kinetic release of the drug, which Fits into the First order model.
- Formulation F3 is optimized formulations. It shows good drug content and dissolution study. Finally, a formulated Sustained Release capsule can be considered for good anti-cancer formulation for greater bioavailability and efficacy.
- It overcomes solubility and bioavailability problems associated with the formulation. Hence, for such drugs, a capsule can be an excellent approach for such formulation.
- Formulation shows Better Half-life. The formulation can give Prolong action. This overcomes the drawback of solubility and drug release significantly, and hence; it could increase the bioavailability of Dasatinib with good efficacy

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