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Quality-by-Design based Formulation and in-vitro Evaluation of Liquisolid compacts of Axitinib

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Abstract: Axitinib, a BCS Class 2 drug was formulated into Liquisolid Compacts with an objective of to enhance solubility profile related to oral bioavailability. Liquisolid systems are free flowing, dry looking, and readily compressible powdered forms of liquid drug medications. Neusilin® US2 was used as carrier as well as coat due to its high specific surface area, porosity and oil absorption capacity. Polyethylene Glycol 400 was selected as nonvolatile solvent via solubility analysis. Quality-by-Design approach was applied by using Central Composite Design on Design Expert ® 12.0 software. Independent variables were selected viz. Concentration of the nonvolatile solvent (W %) and Carrier: Coat Ratio (R). Dependent factors were Drug release (%), Angle of Repose and Tablet Hardness (kg/cm^3). LS-9 was suggested as optimized batch by ANOVA having 99.6 % drug release, 28.1° angle of repose and 2.4 kg/cm$_3$ tablet hardness. LS-9 dissolution profile was compared with DCT (59 %) profile which demonstrated a high D_r (Drug Dissolution Rate) of LS-9 as compared to DCT. It was attributed to enhanced wetting property due to LS exposing a large surface area of AXITINIB available for dissolution. LS-9 was subjected to ageing studies at 40°C ±2°C temperature and 75 ±5% R.H. for 3 months upon which LS-9 demonstrated no major deviation in its attributes. Thus, authors concluded that Liquisolid technology serves a useful application in solubility which ultimately enhances bioavailability.
Key Words: Liquisolid Technology, Neusilin® US2, Quality-by-Design, ANOVA, In-vitro release study.

INTRODUCTION

A major reason for failure of new chemical entities in clinical development is due to their poor pharmacokinetics along with toxicity or failure to prove their efficacy at minor level. It is found that most of novel drugs after lead optimization lie in the BCS class 2 or 4 which means that they are poorly soluble in water or are practically insoluble.[1] Axitinib, a kinase inhibitor which inhibits receptor tyrosine kinases including vascular endothelial growth factor receptors (VEGFR)-1, VEGFR-2, and VEGFR-3 at therapeutic

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plasma concentrations. These receptors are implicated in pathologic angiogenesis, tumor growth, and cancer progression. It is used as a second line therapy in prophylaxis of metastatic advanced renal carcinoma. [2] Axitinib is a BCS Class 2 drug having logP value of 3.5 and aqueous solubility of 0.2 mcg/ml in pH range of 1.1-7.8. Its t_{max} is 2.5-4.1 hours with an absolute bioavailability of 58%. It's highly bound (>99%) to human plasma proteins with preferential binding to albumin and moderate binding to α1-acid glycoprotein with a t1/2 of 2.5-6.1 hours. It is a selective and a potent inhibitor of VEGF receptors. [3] Its main loophole lies in its poor aqueous solubility which was targeted to be enhanced via LS systems in this study.

Liquisolid Systems are free flowing, compressible admixtures of drug solutions or suspensions. Liquisolid systems are molecularly dispersed admixtures of liquid drug medication; hence they are dry-looking admixtures and are not exactly devoid of any moisture. Liquisolid Systems aims at enhancing the water solubility and in-vitro dissolution of poorly water soluble drugs. It is based upon incorporation of water insoluble drug into a Non-Volatile solvent in which the respective drug is fairly soluble and converting the resultant liquid drug solution or suspension into a free flowing and readily compressible powder by using carriers with high specific surface area, porous material and high liquid absorbing capacity and nanometric (10nm-5000nm) sized coating materials showing high surface adsorption.[4, 5] This study has employed Polyethylene Glycol 400 [6] as nonvolatile solvent, Neusilin® US2[7] as both carrier and coat due to its high specific surface area, high porosity and high oil absorption capacity and Sodium Starch Glycolate[8] as disintegrants.

As the drug of choice, Axitinib suffers from poor oral BA at 58% due to its low hydrophilicity; it is an excellent agent to be developed into a solubility enhancement experiment. Although liquisolid technique is studied extensively till date for dissolution enhancement, but no investigation was ever made for Axitinib. This work is based upon formulation development of Axitinib into Liquisolid technology for improving the dissolution rate and bioavailability of practically insoluble drug Axitinib. [9] Quality-by-Design approach was applied in formulation design which was designed using Central Composite Design on Design Expert® 12.0 software. Independent variables were selected viz. Concentration of the nonvolatile solvent (W %) and Carrier: Coat Ratio (R). Dependent factors were Drug release (%), Angle of Repose and Tablet Hardness (kg/cm3). A total 9 different runs were obtained which were utilized to prepare formulation design chart. [10]

MATERIALS AND METHODS

Materials

Axitinib was kindly gifted by Glenmark Pharmaceuticals Ltd., Mumbai, India, Neusilin® US2 was kindly gifted by Gangwal Chemicals Pvt. Ltd., Mumbai, India, Polyethylene Glycol 400, Methanol and Hydrochloric Acid were purchased from Rankem RFCL Ltd., Mumbai, India, Sodium Starch Glycolate and Lactose were purchased from HiMedia, Mumbai, India, Microcrystalline Cellulose (Avicel® PH102) and Magnesium Stearate were purchased from Research Lab (RL), Mumbai, India.

Methods

Solubility Analysis [11, 12]

Solubility Studies for Axitinib were performed in a variety of Non-Volatile Solvents viz. Polyethylene Glycol 200, Polyethylene Glycol 400, Propylene Glycol, Polysorbate 20, Polysorbate 80 and Glycerine. Saturated solutions of AXITINIB with each solvent were made in 10ml glass vials and were set aside in an Orbital Incubating Shaker (Remi International, India) at 32^{o}C for 24 hours at 50rpm. The solutions were filtered using a 0.45 µm filter and diluted as required for further analysis. The solutions were analyzed by UV-2450 UV-Vis Spectrophotometer (Shimadzu, Japan) at wavelength of 330nm against blank (blank contained same solvent in which drug was suspended). All the Non-Volatile Solvent solutions were diluted with methanol and analysis was carried out in triplicates. The results were extrapolated to determine the solubility of Axitinib as percent mg/ml in its saturated solution by using various solvents.

Drug-Excipient Compatibility Studies

Fourier Transfer Infrared Spectroscopy [13]

Axitinib, Polyethylene Glycol 400, Neusiilin US2®, Physical Mixture with drug and optimized liquisolid formulation were all compressed as a KBr pellet respectively for each sample at a ratio of 9:1. The prepared pellets were then scanned over range of 4000 – 400 cm^{-1} to get the IR spectra. Functional group determination was studied visually by interpreting the peaks observed in the spectra and any changes in parent peaks were observed.The FTIR experiment was conducted on FTIR- 8400S apparatus, Shimadzu, Kyoto, Japan.

Differential Scanning Calorimetry [14]

Physical Mixture of liquisolid formulation sample was prepared and sealed in a pre-washed ampoule. It was set aside in a Programmable Environmental Test Chamber, Remi Instruments Ltd. Mumbai for 28 days. Following that the sample was hermitically sealed inperforated aluminum pan and heated at constant rate of 10°C/min over thetemperature ranges of 30-300°C at 20mL/min nitrogen purging on a Mettler Toledo, UK DSC apparatus

Analytical Method Development by UV Spectroscopy [15]

The linearity experiment was carried out on two solvents viz. Methanol and pH 1.2 Hydrochloric Acid buffer. Accurately weighed 10 mg of Axitinib was dissolved in 100 ml of methanol and pH 1.2 Hydrochloric Acid buffer seperately to obtain a stock solution of 100 µg/ml. Aliquots were drawn to prepare samples ranging from concentrations of 2 mcg/ml – 25 mcg/ml as required and were transferred to 10 ml volumetric flask and the volume was adjusted up to mark with respective solvent. The absorbance of the above solutions was measured at wavelength of 330 nm. A graph of absorbance versus concentration was plotted. Polynomial equation was calculated from the plot using Microsoft Excel 2010.

Calculation of Liquid Load Factor [4, 5, 16, 17]

It is evident that a powder certainly retains only a limited amount of liquid medication while maintaining an acceptable limit of flowability and compressibility. Hence, established mathematical models are used to calculate the amount of liquid the powder can be loaded with resulting into an acceptably free flowing and readily compressible 'dry looking powder'. This parameter is known as Liquid Load Factor (Lf).

Lf_Φ (Liquid Load for acceptable Flowability) = $\Phi + \varphi/R$; where Φ is the flowable liquid-retention potential of Carrier &φ of the Coating material respectively. (1)

Lf_Ψ (Liquid Load for acceptable Compressibility) = $\Psi + \psi/R$; where Ψ is the compressible liquid-retention potential of Carrier &ψ of the Coating material respectively. (2)

R is the ratio of Carrier and Coating material to be used expressed as R = Carrier Weight (Q) / Coat Weight (q). (3)

Lf_0 (Optimal Liquid Load Factor) = Lf_Φ or Lf_Ψwhichever has a lower value (4)

Similarly weight of Carrier & Coat can be calculated by the following expression; Lf_0 (Optimal Liquid Load Factor) =Weight of Liquid Medication (W) / Carrier Weight (Q), Hence, $Q = W / Lf_0$ (5)

Since R = Carrier Weight (Q) / Coat Weight (q); q = Q/R (6)

In order to calculate an optimal Lf, it's necessary to determine 'flowable liquid-retention potential' (Φ) & 'compressible liquid-retention potential' (Ψ) values of carrier and coating materials respectively. They are determined by Liquisolid Flowability Test (LSF) & Liquisolid Compressibilty Test (LSC).It involves three steps viz. Preparation of LS admixtures, Screening of the powders and plotting the data and calculation of values using various equations.

Preparation of Liquisolid powder admixtures

Liquisolid Flowability Test and Liquisolid Compressibility Test both require the same procedure to formulate the liquisolid admixtures for screening. The procedure is explained via flowcharts in Figure 1.

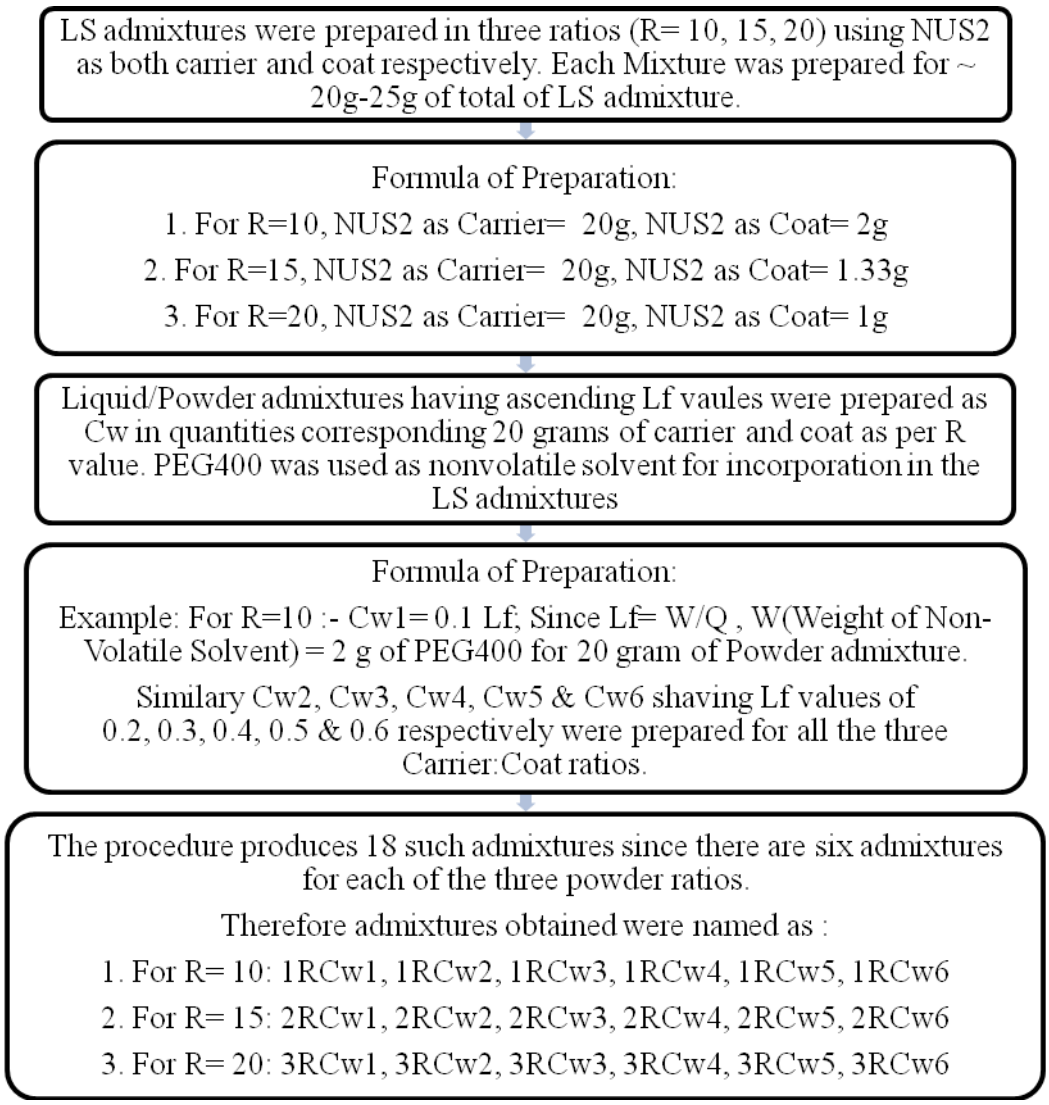


Figure 1: Preparation of liquisolid admixtures for LSC & LSF

Screening of Powders

Screening is done separately for every powder admixture. Here is where Liquisolid Flowability Test and Liquisolid Compressibility Test has its distinction

(a) Liquisolid Flowability Test

Screening was done via two procedures in the following study.

1. Angle of Slide [16, 18]

10 grams of Powder of investigation is placed on the lateral end of a polished aluminium plate and then the plate is lifted on its vertical axis until the powder aggregate slides down and that at that point, theta is recorded

up from the ground considered as angle of slide. 33 degrees is considered optimum and acceptable angle for the liquisolid powders.

2. Angle of Repose [17]

For the determination of the angle of repose, a wide-opening, glass funnel was secured with its tip at a pre-determined height above a sheet of paper placed on a horizontal surface. Twenty five grams of powder was allowed to slide slowly through the tip of the funnel resulting in the formation of the conical pile of powder. The angle of repose was calculated using the following relationship:

$tan\theta = h/r$ (7)

Where α is the angle of repose, and h and r are the height and radius of the base of the conical pile, respectively.

(b) Liquisolid Compressibility Test [4, 5]

The screening was performed using the original method described by innovator Spireas S. where tablets were compacted at plateau compression force for every ${}^{n}RCw_{n}$ and were subjected to hardness testing where hardness was recorded in kg/cm^3. Hardness was tested on a Mechanized Monsanto Hardness Tester and the hardness value recorded for the plateau compression force was selected for further statistical analysis.

Plotting of data and calculation of Φ&Ψ Values [4, 5]

(a) For Liquisolid Flowability Test

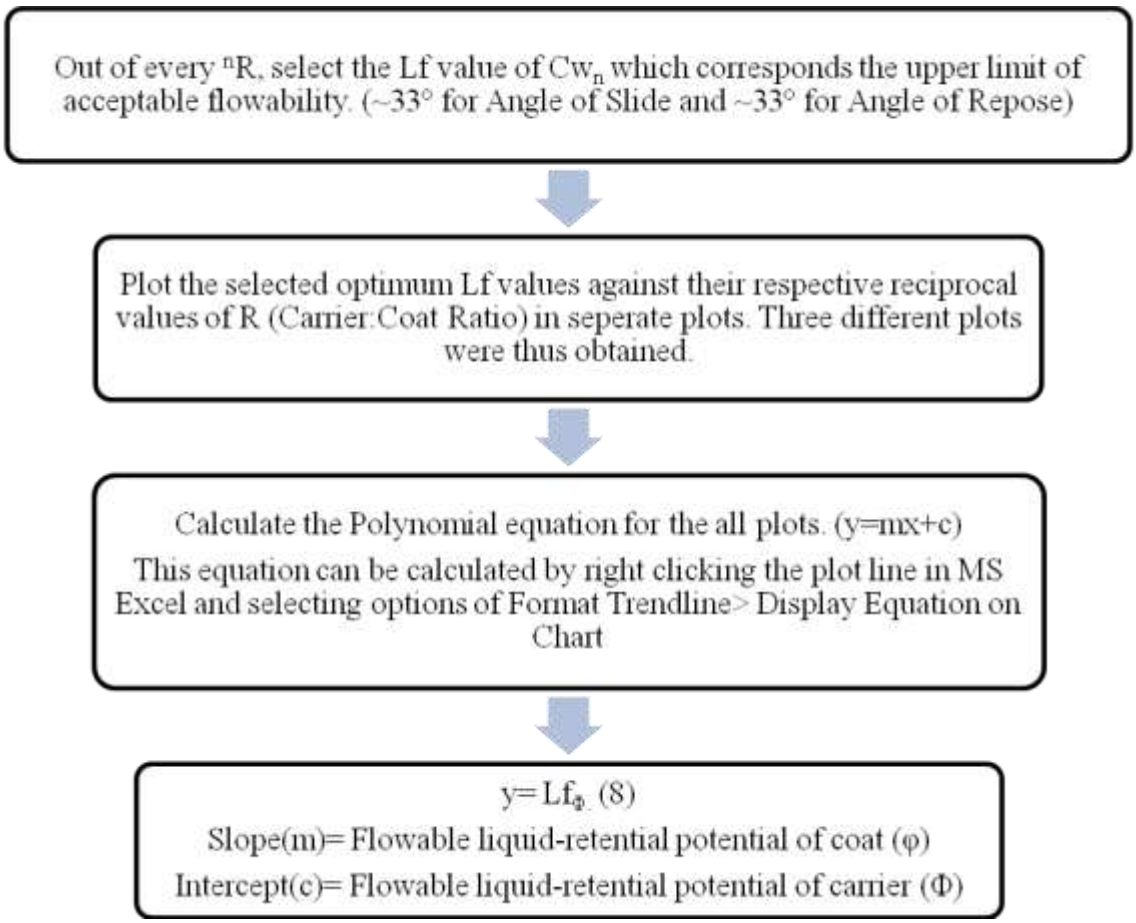


Figure 2: Liquisolid Flowability Test Calculations

(b) For Liquisolid Compressibility Test

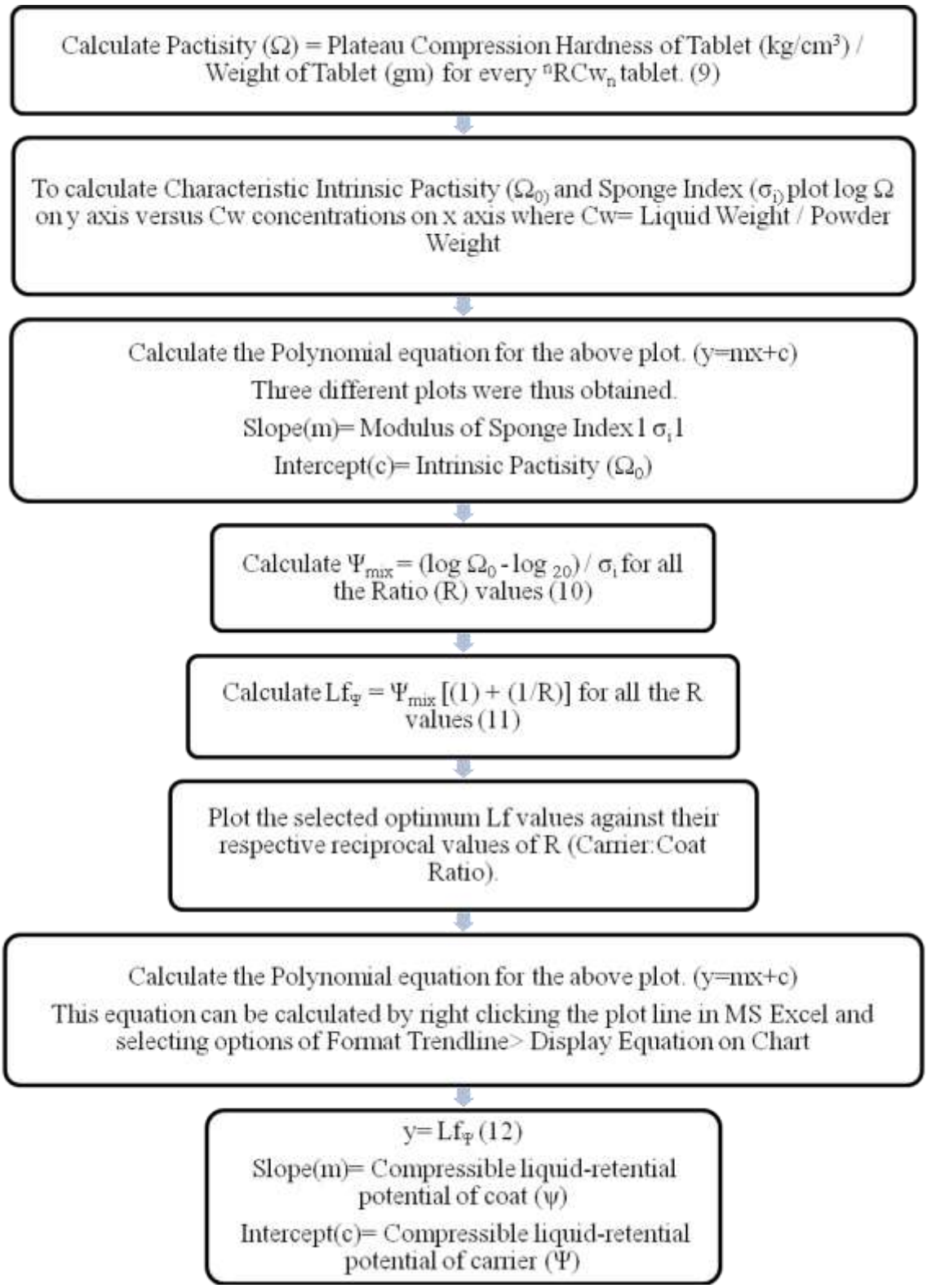


Figure 3: Liquisolid Compressibility Test Calculations

Formulation Design [10, 12]

Design Expert 12.0 software was used to create formulation design for the purpose of optimization of liquisolid tablets. Two independent factors suited the experiment's need viz. Concentration of the nonvolatile solvent (W %) and Carrier: Coat Ratio (R). Dependent factors were Drug release (%), Angle of Repose and Tablet Hardness (kg/cm^3) as these three parameters address the essence of liquisolid technology which is dissolution enhancement, flowability and compressibility of liquisolid admixtures. Central composite randomized design was applied to screen via Response Surface Methodology in the following study. Response 1 & 2 were evaluated by quadratic model and Response 3 by linear model by ANOVA, which bears the form of following equation:

$$Y = b0 + b1X1 + b2X2 + b3X3 + b4X1X2 + b5X1X2 + b6\ X1X3 + b7X12 + b8X22 + b9X32 \quad (13)$$

Where Y is the measured response; X is the levels of factors; b0 the constant and b1, b2, b3 …….b9 is the regression coefficient. X1 and X2 stand for the mail effect; X1X2 are the interaction terms they show how response changes when two factors are simultaneously charged. X12, X22 are quadratic terms of the independent variables. All the necessary calculations for Lf, R, W, Q and q were made by referring to equation 1-6.

Table 1: Formulation Chart for Axitinib Liquisolid tablets

Code	Drug (mg)	Lf	W (%)	PEG 400 (mg)	R (%)	NUS2 (mg) as carrier (Q)	NUS2 (mg) as coat (q)	SSG ~4% (mg)	Lactose (mg)	Tablet Weight (mg)
LS-1	5	0.79	5	95	10	120.25	12	9.3		241.5
LS-2	5	0.49	5	95	20	193.87	9.6	12.1		315.6
LS-3	5	0.79	15	28.33	10	35.86	3.6	2.9	100	175.2
LS-4	5	0.49	15	28.33	20	57.81	2.9	3.8	100	197.8
LS-5	5	0.95	10	45	7.928	47.36	6	4.1	100	207.5
LS-6	5	0.46	10	45	22.07	97.82	4.4	6		158.3
LS-7	5	0.59	2.928	165.76	15	280.95	18.6	18.8		489.1
LS-8	5	0.59	17.07	24.29	15	41.17	2.7	2.9	100	176.1
LS-9	5	0.59	10	45	15	76.27	5.1	5.2	50	186.6

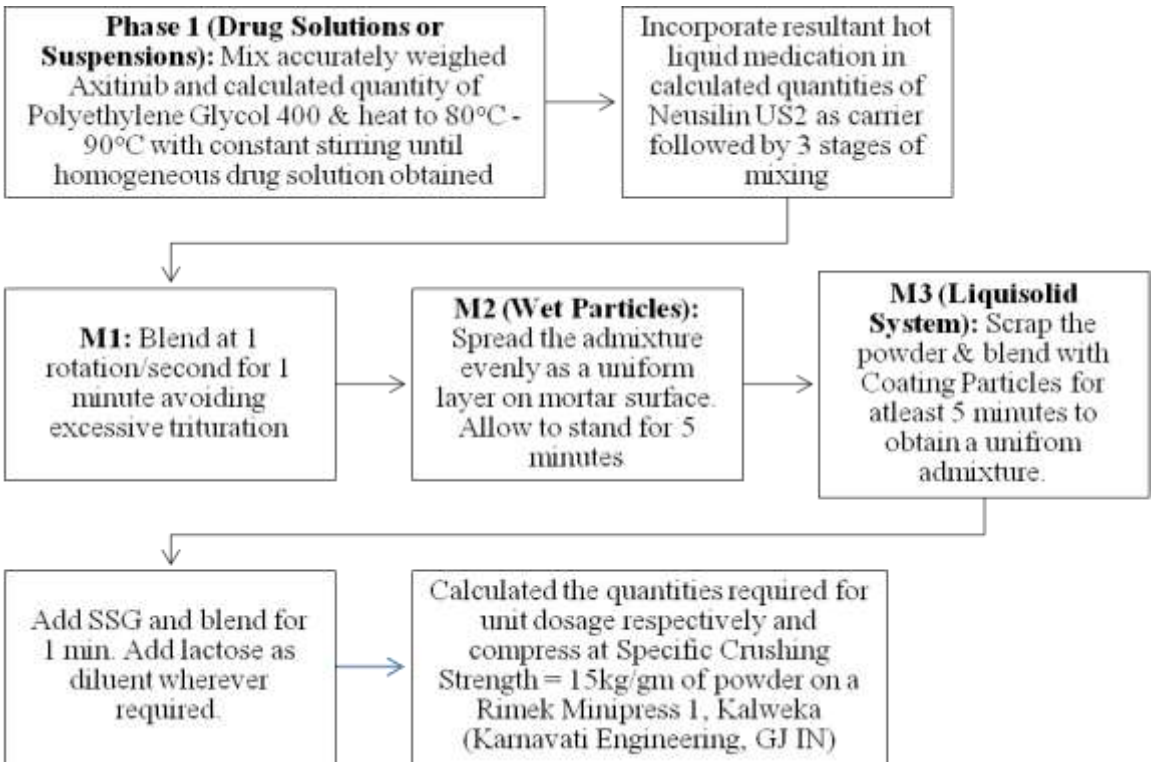
Flow property determination of prepared admixtures [18]

Flowability of all LS admixtures is assessed by determination of Carr's Index (CI) also known as percentage compressibility. The CI was calculated from the bulk and tapped densities. Bulk and Tapped density was calculated by using Tap Density Tester USP (Electrolab, Mumbai) for all the nine batches of LS admixtures. The CI is calculated according to the following equation

$$CI\% = 100\left(\frac{\text{tapped density} - \text{bulk density}}{\text{tapped density}}\right) \quad (14)$$

$$\text{Hausner Ratio} = \frac{\text{Tapped Density}}{\text{Bulk Density}} \quad (15)$$

Preparation of Tablets [4, 5,]



19]

Figure 4: Scheme for preparation of Liquisolid tablets of Axitinib

Preparation of liquisolid tablets is demonstrated in Figure 2. The directly compressed tablet was formulated by dry compression of powder mixture of drug with lactose and microcrystalline cellulose as diluents and magnesium stearate as lubricant on Rimek Minipress 1, Kalweka 10 station tablet press.

Post Compression Evaluation

Tablet Hardness [12]

Tablet hardness was tested on Mechanized Monsanto Hardness Tester for every LS Formulation along with DCT in triplicates. Tablet is placed longitudinally between the mechanized plunger and screw is thus rotated to the threshold where tablet chips or breaks down. That point is recorded as Tablet Hardness. All the readings were obtained as kg/cm^3.

Friability Test [20]

Friability was measured using Friabilator USP EF2 (Electrolab, Mumbai) by taking tablets equivalent to weight of 6.5 grams and were rotated for 4 minutes at a speed of 25rpm. Remove the tablets, remove any loose dust from them and weigh them accurately. Total percentage loss was calculated by weighing the remnant of tablets.

Disintegration Test [20]

The test was performed in the liquid medium (0.1 N HCl) in a 1-litre beaker. Disintegration Tester USP ED2L (Electrolab, Mumbai) was used for the procedure. A thermostatic arrangement was made for heating the liquid and maintaining the temperature at 37º ± 2º. The machine was operated until all the tablets lost their unit form or completely solubilized. A total of 6 tablets were screened from each batch and time reading was recorded in minutes in triplicates.

Assay [15, 21, 22]

Ten tablets of each batch were crushed producing a powder equivalent to 50 mg of Axitinib. It was suspended into 100 ml methanol, shaked until homogenized and is subjected to sonication (Ultrasonicator, Lab Hosp Corp. Mumbai) for removing any air bubbles. 10 ml of this stock solution was diluted to 100 ml methanol and 1ml of this solution is diluted upto 10 ml with methanol for final analysis via UV Spectroscopy.

Weight Uniformity of Single Dose Preparation [20]

Weight Uniformity was determined according to Indian Pharmacopoeia where 20 units of each batch were selected at random, weighed individually and average weight was calculated.

In-Vitro Drug Release (Dissolution Test) [20]

The test was conducted on a Type 1 USP dissolution apparatus (Dissolution Tester USP TDT-08L Plus, Electrolab, Mumbai). 900ml of the dissolution medium (pH 1.2 HCl buffer) was used as media. Test was carried out at 36.5º to 37.5º with a paddle rotation speed of 50rpm. Aliquots were drawn at time intervals of 5, 10, 15, 20, 30, 40, 50 and 60 minutes. Media withdrawn was immediately replaced by same amount of media to maintain sink conditions. All the aliquots were analysed spectroscopically and was carried out in triplicates. Graphs were plotted after final calculations using Microsoft Excel 2010.

X-Ray Powder diffraction analysis [6]

The Powder X-Ray Diffraction Spectra of drug was obtained using Bruker D8 Advance X-ray diffractometer with tube copper anode over the interval 5 to 60 0 of 2Θ with a Cu Kα radiation source, voltage at 40KV, current of 30mA, and a scanning rate of 2 degree/min. Optimized Liquisolid formulation and Physical Mixture was screened and was compared with the XRD of pure Axitinib.

Ageing Studies [23]

Optimized liquisolid formulation was subjected to ageing study to study the impact of any possible deterioration while ageing in certain environmental conditions. Optimized liquisolid batch was kept under accelerated stability condition at 40°C ±2°C temperature and 75 ±5% relative humidity for 3 months. Samples were withdrawn at 1, 2 and 3 month time interval and were screened for Hardness, Friability, Weight Uniformity, Disintegration time, Assay and In-Vitro Dissolution studies.

RESULTS AND DISCUSSION

Solubility Studies

Table 2: Solubility data of Axitinib

Solvent	Solubility (mg/ml)
Water	0.0002 [10, 13]
Methanol	1.38
Ethanol	0.87
DMSO	32.6
pH 1.2 HCl buffer	0.18
pH 6.8 Phosphate buffer	0.24
Propylene Glycol	0.77
Polyethylene Glycol 200	9.53
Polyethylene Glycol 400	13.7
Polysorbate 20 (Tween 20)	0.96
Polysorbate 80 (Tween 80)	0.003
Dissolution media (pH 1.2 HCL buffer)	0.18
Water	0.0002

Axitinib is practically insoluble in water. Since Axitinib has maximum solubility in Polyethylene Glycol 400 (1.37%), it was thus selected as the solvent for formulating liquid drug medications for liquisolid admixtures. Solubility of Axitinib increased as Polyethylene Glycol 400> PEG 200> T20> PG> T80> Glycerine. Axitinib was markedly more soluble in the nonvolatile solvent than dissolution media.

Drug Excipient Compatibility Studies

FTIR Studies

FTIR spectra of excipients viz. Polyethylene Glycol 400, Neusilin US2; Physical Mixture at equal quantities of Axitinib, Polyethylene Glycol 400&Neusilin US2 and optimized batch of LS-9 was studied in an overlay for testing any structural modifications caused to the formulation. Axitinib exhibits characteristic peaks at C=O (Amide –CONH) at 1635.69, C-N (Amine) at 1149.61, and C=C (Aromatic) at 1585.54. LS-9 consists of characteristic peaks at C=O (Amide –CONH) at 1651.12 which corresponds the peak of Axitinib, 1600 of Neusilin US2 and 1631.8 of Polyethylene Glycol 400 respectively. It is clearly evident that the FTIR spectrum of LS-9 follows similar lines on that of Polyethylene Glycol 400 which can be attributed to the molecular dispersion attribute of the formulation. It thus also indicates presence of Polyethylene Glycol 400 in high quantities which resulted into maximum resemblance of the LS-9 spectrum with that of Polyethylene Glycol 400. Also interesting is that the FTIR spectrum of Physical Mixture resembles Neusilin US2 spectra which can be attributed to high concentration of the excipient in the powder along with non-homogeneous solution as it was not subjected to heat treatment unlike LS-9. Silicate ion (1100-900) peaks were clearly visible in both cases at ~1100 respectively. It can thus be established that since there was no emergence of any nefarious peak in the spectrum, the materials did not show any possible interactions. Although FTIR study is not definitive for Drug-Excipient compatibility, the same can be established by further experiments using thermal analysis. An overlay is represented in Figure 5.

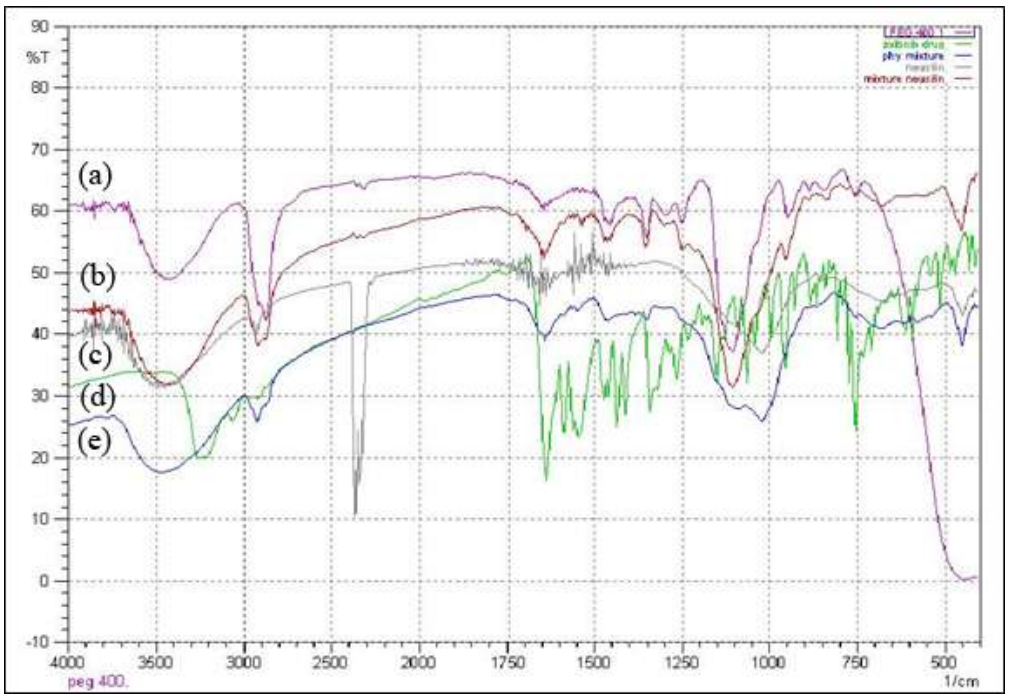


Figure 5: Overlay (starting from the top) FTIR Spectra of (a). Polyethylene Glycol 400, (b). LS-9, (c). Neusilin® US2, (d). Axitinib and (e). Physical Mixture of all ingredients.

DSC Studies

Obtained results show no significant shift in the endotherm of the physical mixture as compared to the drug. The sharpness of the endotherm is slightly evened out due to the possible loss of crystallinity or due to presence of Neusilin US2 which has a similar melting point range (251°C) [24] as of Axitinib (225°C) [21]. Hence, it is concluded that there are no possible signs of interaction between the drug and excipients. Therefore the further formulation can be carried out by using Neusilin® US2 and Polyethylene Glycol 400 as excipients. Onset of

endotherm was observed at 227.32°C, peak at 245°C and endset at 259.36°C. Only one peak was observed which was tapered at end and had no sharpness. The peak gradually falls in the melting point range of the drug Axitinib and excipient Neusilin US2. So no possible chances of interaction were seen between drug and excipients. A comparison is represented in Figure 6.

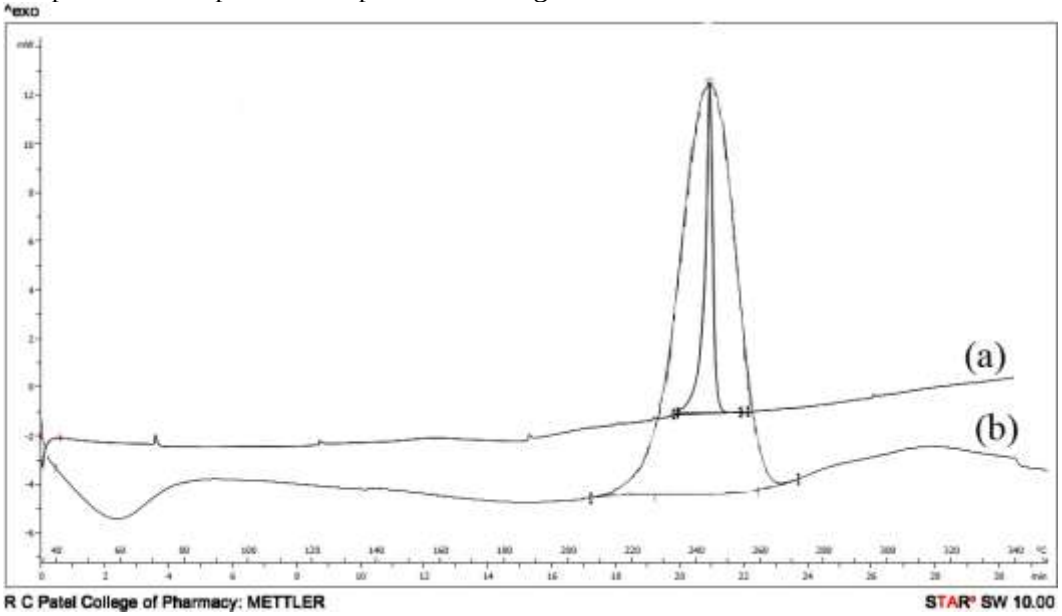


Figure 6: DSC comparison between (a) Axitinib and (b) Physical Mixture of all ingredients

Analytical Method Development

Polynomial equation was calculated with an objective of serving a means for spectroscopic analysis of the underlying experiments in the study.

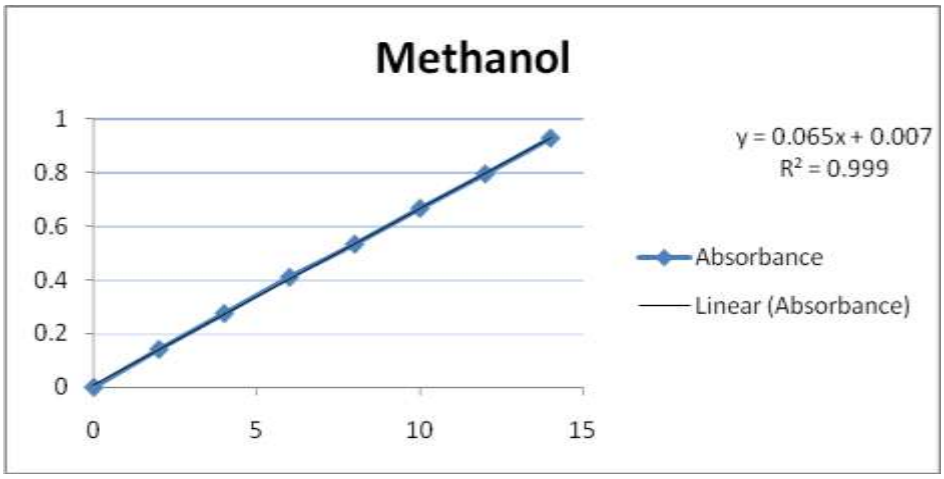


Figure 7: Concentration v/s Absorbance plot of ATB in Methanol

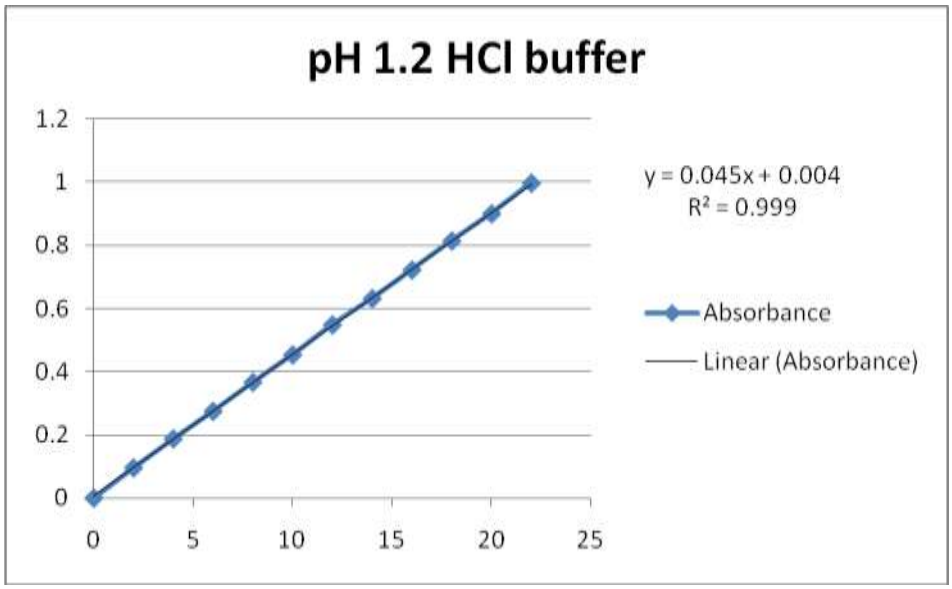


Figure 8: Concentration v/s Absorbance plot of ATB in pH 1.2 HCl buffer

Calculation of Liquid Load Factor (Lf)

The experimental values and corresponding plots were recorded. The final calculations for determination of Optimum Liquid Load factor were equated corresponding to the experimental values obtained. All the equations were sourced from [4] which is the original work of liquisolid systems by Spireas S. Equation number 1-12 were used to calculate the values.

Liquisolid Flowability Test

Experimental values obtained by Liquisolid Flowability Test experiments are listed in Table 3. (n=3; all values are written as Mean ±SD).

Table 3: Liquisolid Flowability Test Experimental Values

R1=10			R2=15			R3=20		
Lf	**θ Repose**	**θ Slide**	**Lf**	**θ Repose**	**θ Slide**	**Lf**	**θ Repose**	**θ Slide**
0.1	17.1 ±2.9	26 ±2	0.1	32.82 ±3.6	31.5 ±1	0.1	36.6 ±3.4	34.5 ±1
0.2	29 ±3.1	28 ±1	0.2	34.43 ±3.8	33 ±1	0.2	37.4 ±4	40 ±1
0.3	30.32 ±3	29 ±1	0.3	47.35 ±3.7	40	0.3	38.65 ±4.2	42
0.4	33 ±2.5	32 ±1	0.4	48.99 ±4.2	44	0.4	40.35 ±3.2	45
0.5	33.69 ±3.4	35	0.5	49.7 ±4	50	0.5	40.6 ±3	45
0.6	35.57 ±4	37	0.6	49.29 ±3.6	50			

w.r.t. to steps provided in Figure 2, such Lf value should be selected in which the experimental values comply with the upper limits of the conducted experiment. Hence Lf= 0.4, 0.2 & 0.1 values were selected for R1, R2 & R3 respectively as θ Repose and θ Slide values fall in the desired range of values for Angle of Slide and Angle of Repose experiments respectively. It is made clear that upper limit of these values is independent to be chosen by the formulator and same can also be replaced by any other flowability experiment respectively.

Table 4 and Figure 9 conclude the plot of Lf v/s 1/R for Liquisolid Flowability Test as directed in Figure 2. [4, 5]

Table 4: 1/R v/s Optimum Lf plot values for Liquisolid Flowability Test

1/R	Lf
0.1	0.4
0.066	0.2
0.05	0.1

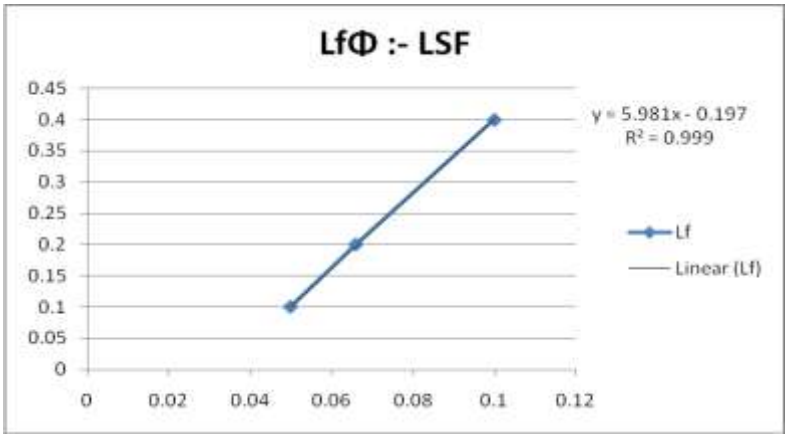


Figure 9: 1/R v/s Optimum Lf plot for Liquisolid Flowability Test

$Lf_{\Phi}$was calculated according to the equation 1. Lf_{Φ} values for R= 10, 15, 20, 7.92 and 22.07 were 0.795, 0.596, 0.496, 0.952 and 0.468 respectively.

Liquisolid Compressibility Test

Experimental values obtained by Liquisolid Compressibility Test experiments are listed in Table 27(a, b, c). Pactisity (Ω) was calculated by Equation 9. Lf_{Ψ} was calculated by Equation 11.

Table 5: Liquisolid Compressibility Test Experimental Values

R1=10				R2=15				R3=20			
Lf	**Plateau Compression Hardness**	**Pactisity (Ω)**	**log Ω**	**Lf**	**Plateau Compression Hardness**	**Pactisity (Ω)**	**log Ω**	**Lf**	**Plateau Compression Hardness**	**Pactisity (Ω)**	**log Ω**
0.1	9	160.71	2.2060429	0.1	9.4	155.22	2.19094768	0.1	10.6	132.5	2.12221588
0.2	8	121.21	2.08353845	0.2	8.4	119.17	2.07616694	0.2	8.8	116.27	2.06546767
0.3	6.6	86.84	1.93871981	0.3	6.2	95.69	1.98086655	0.3	6	115.48	2.06250678
0.4	5.2	60.46	1.78146814	0.4	5.4	75.48	1.87783189	0.4	5.6	96.88	1.98623413
0.5	4	46.51	1.66754634	0.5	3.6	64.12	1.80699351	0.5	2.8	75.18	1.87610232
0.6	3.4	40.86	1.61129836	0.6	3	41.85	1.62169546	0.6	2.8	42.36	1.62695595
Lf_{Ψ} for R= 10 = 0.895				**Lf_{Ψ} for R= 15 = 0.994**				**Lf_{Ψ} for R= 20 = 1.139**			

w.r.t. to steps provided in Figure 3, graph plot of 1/R v/s Lf is demonstrated in Table 6& Figure 10. Since plateau compression force was selected as upper limit for the values, Lf values tend to have a higher value than that of Liquisolid Flowability Test values. Although if required, the same experiment can also be carried out with a low hardness value for the same.

Table 6: 1/R v/s Optimum Lf plot values for Liquisolid Compressibility Test

1/R	Lf
0.1	0.895
0.066	0.994
0.05	1.139

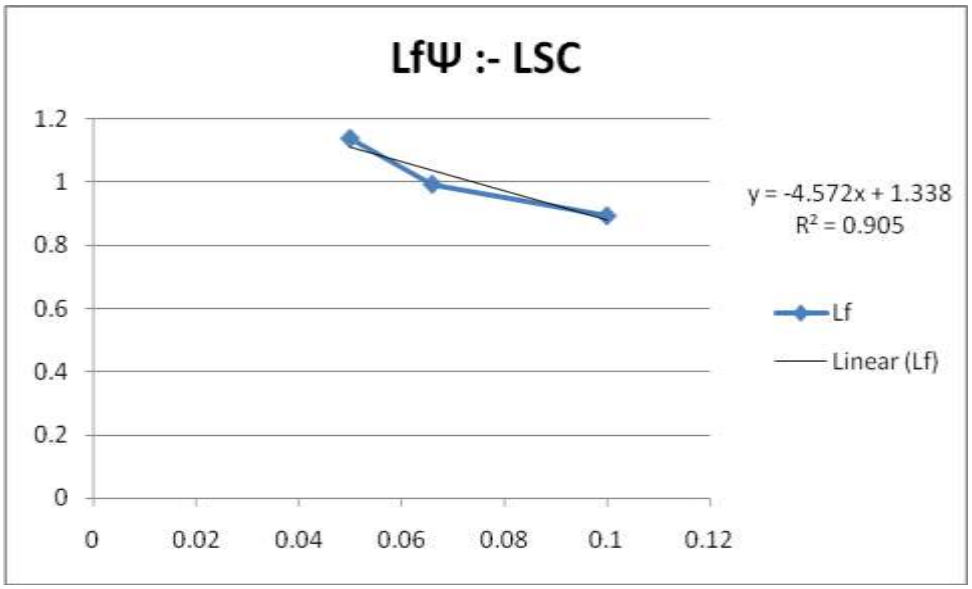


Figure 10: 1/R v/s Optimum Lf plot for liquisolid compressibility test

Lf_{Ψ} was calculated according to the equation 2.Lf_{Ψ}values for R= 10, 15, 20, 7.92 and 22.07 were 1.796, 1.643, 1.567, 1.915 and 1.545 respectively.

Selection of Optimum Liquid Load Factor (Lf_0)

Since, $Lf_0 = Lf_{\Phi}$if $Lf_{\Phi} < Lf_{\Psi}$or Lf_{Ψ}if $Lf_{\Psi} < Lf_{\Phi}$Hence, Lf_{Φ}values were considered for formulation design.

Precompression Parameters

Results are represented in tabular form in Table 7. (n=3; all values are written as Mean ±SD)

Table 7: Precompression Parameters

Formulation Code	Bulk Density (g/cm^3)	Tap Density (g/cm^3)	Compressibility Index (%)	Hausner's Ratio	Angle of Repose (θ)
LS-1	0.22 ±0.1	0.32 ±0.2	33	1.45	31±2.8
LS-2	0.20 ±0.2	0.30 ±0.2	33	1.5	31.8±3.2
LS-3	0.21 ±0.1	0.30 ±0.2	30	1.43	29.3±2.4
LS-4	0.21 ±0.1	0.31 ±0.2	32.25	1.47	32.4±2.9
LS-5	0.22 ±0.1	0.30 ±0.1	26.66	1.36	26.9±3

LS-6	0.23 ±0.2	0.33 ±0.2	30.30	1.43	32.6±4
LS-7	0.20 ±0.3	0.29 ±0.4	31.03	1.45	30.2±3.1
LS-8	0.21 ±0.4	0.33 ±0.6	36.36	1.57	30.5±2.8
LS-9	0.22	0.31 ±0.1	29.03	1.4	28.1±2.2
DCT	0.29 ±0.3	0.40 ±0.3	27.5	1.37	26.5 ±2.8

Whilst all the LS formulations show passable to poor flowability and compressibility, LS-9 is the best formulation in terms of flowability and compressibility. This loophole can be attributed to the amounts of presence of nonvolatile solvent which leaves a humid environment inside the admixtures which thus results into poor flowability. The DCT mixture had good flow properties and good compressibility.

Postcompression Parameters

Results are represented in tabular form in Table 8. (n=3; all values are written as Mean ±SD)

Table 8: Postcompression Parameters

Formulation Code	Hardness (kg/cm^3)	Friability (%)	Disintegration (min:sec)	Drug Content (%)	Average Weight of Tablets (mg)	Dissolution achieved (%)
LS-1	2.2±0.2	0.5 ±0.1	4:10 ±0:30	95.8 ±0.4	240.4 ±6.2	93.2±2
LS-2	2.6±0.2	0.6 ±0.2	5:20 ±0:20	98.2 ±0.6	315.6 ±8.3	95.4±2.5
LS-3	2.6±0.4	0.6 ±0.1	4:30 ±0:25	94 ±0.2	177.8 ±5.4	89±3
LS-4	2.6±0.4	0.7 ±0.1	5:40 ±0:35	91.8 ±0.9	195.9 ±4.2	87.1±3
LS-5	2.6±0.4	0.9 ±0.1	5:05 ±0:30	96.9 ±0.8	208 ±5.7	94.2±4.2
LS-6	2.4±0.2	0.5 ±0.3	4:55 ±0:40	99.2 ±1.2	158.9 ±3.2	96.3±4.3
LS-7	2±0.2	0.7 ±0.1	7:55 ±1:30	88.4 ±1.9	485 ±15.3	76.8±5.1
LS-8	2.8±0.4	0.8 ±0.2	3:50 ±0:50	94.5 ±1.4	174.2 ±6.8	86.5±2.8
LS-9	2.4±0.2	0.7 ±0.2	4:25 ±0:45	100.8 ±2.0	185.1 ±4	99.6±2.8
DCT	3.6±0.2	0.5 ±0.3	6:45 ±0:55	94.2 ±0.8	106.9 ±3.4	59 ±1.8

All the liquisolid tablets were compressed upon the standard direction. DCT tablets were compressed at appropriate hardness. No leakage of Polyethylene Glycol 400 was observed in tablets after compression. Tablet Friability and Weight variation values were in accordance with the standard limitations as directed by the Indian Pharmacopoeia.Content Uniformity was complied with the standard guidelines of 85% - 115% of the Indian Pharmacopoeia. [20]

Design of Experiment by QbD Response Surface analysis

The data obtained as coefficients in the polynomial equation showed excellent fitting in quadratic models for R1 and R2 and linear model for R3 where significant P values were obtained for ANOVA model and insignificant values were obtained for model lack of fit. Evaluation of correlation coefficient showed R2 value ranging between 0.598 and 0.89.

Effect of Formulations Variables on In-Vitro Drug Release

Quadratic model was suggested for R1 analysis. The Model F-value of 6.68 implies the model is significant. The P value was obtained for B^2 as 0.007 which is a significant model term. A negative Predicted R^2 of -0.2314 implies that the overall mean (93.58) may be a better predictor of the response than the current model. The ratio of Adeq Precision of 6.490 indicates an adequate signal. This model can be used to navigate the design space. The coded equation was obtained as follows

In-Vitro Drug Release (%) = 99.6 + 0.408731 * A + 0.152234 * B + -1.025 * AB + -1.49375 * A^2 + -8.29375 * B^2.

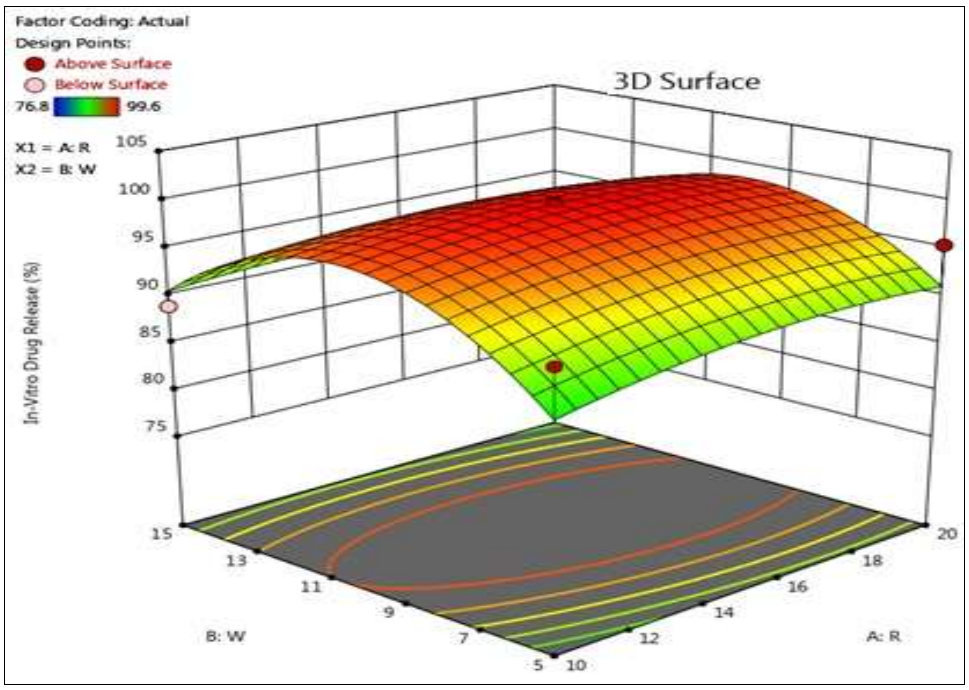


Figure 11: 3D Response Surface Methodology graph for R1

It can be well observed that Carrier: Coat Ratio (R) had a consequential effect upon the drug release whereas amount of liquid medication (W) had a little effect as compared to R. A median value was optimized which infers that a very high or very low R and W value lowers the Drug release.

Effect of Formulation Variables on Angle of Repose

Quadratic model was suggested for R2 analysis. The Model F-value of 11.36 implies the model is significant. The P value was obtained for A (0.0014), A^2 (0.01), B^2 (0.003) were significant model terms. The ratio of Adeq Precision of 7.980 indicates an adequate signal. This model can be used to navigate the design space. The coded equation was obtained as follows

Angle of Repose (θ) = 28.1 + 1.49513 * A + -0.084467 * B + 0.575 * AB + 1.09375 * A^2 + 1.39375 * B^2. .

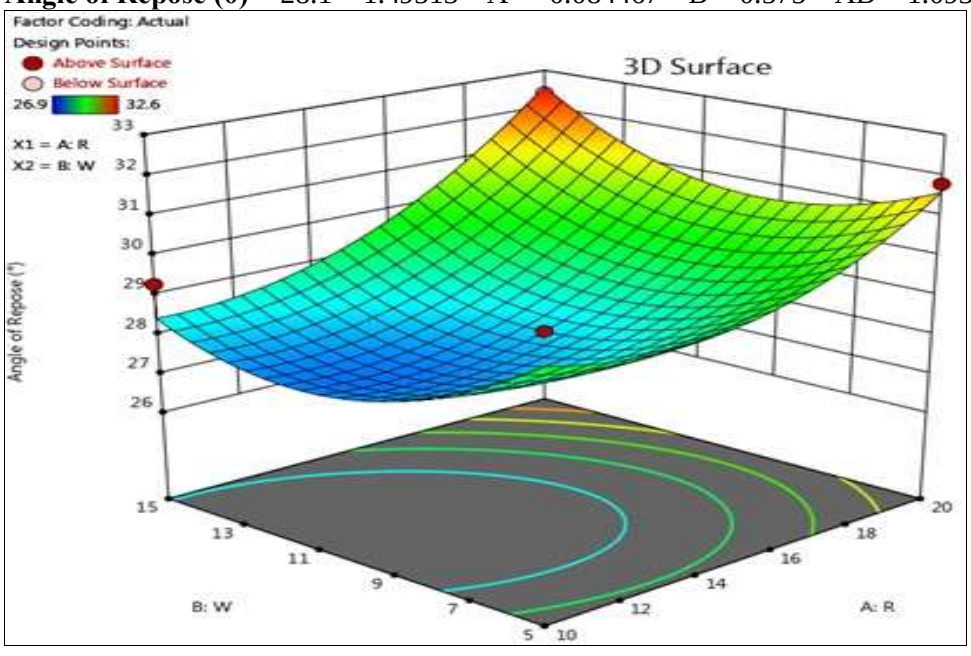


Figure 12: 3D Response Surface Methodology graph for R2

It can be well observed that Carrier: Coat Ratio (R) had a consequential effect upon the angle of repose whereas amount of liquid medication (W) showed no effect as compared to R. Flowability is compromised if R value is greater which leads to incomplete adsorption of PEG 400 from the carrier surface.

Effect of Formulations Variables on Hardness

Linear model was suggested for R3 analysis. The Model F-value of 7.47 implies the model is significant. The P value was obtained for B as 0.007 which is a significant model term. The ratio of Adeq Precision of 8.021 indicates an adequate signal. This model can be used to navigate the design space. The coded equation was obtained as follows

Hardness (kg/cm^3) = 2.44615 + 0.0146447 * A + 0.191421 * B.

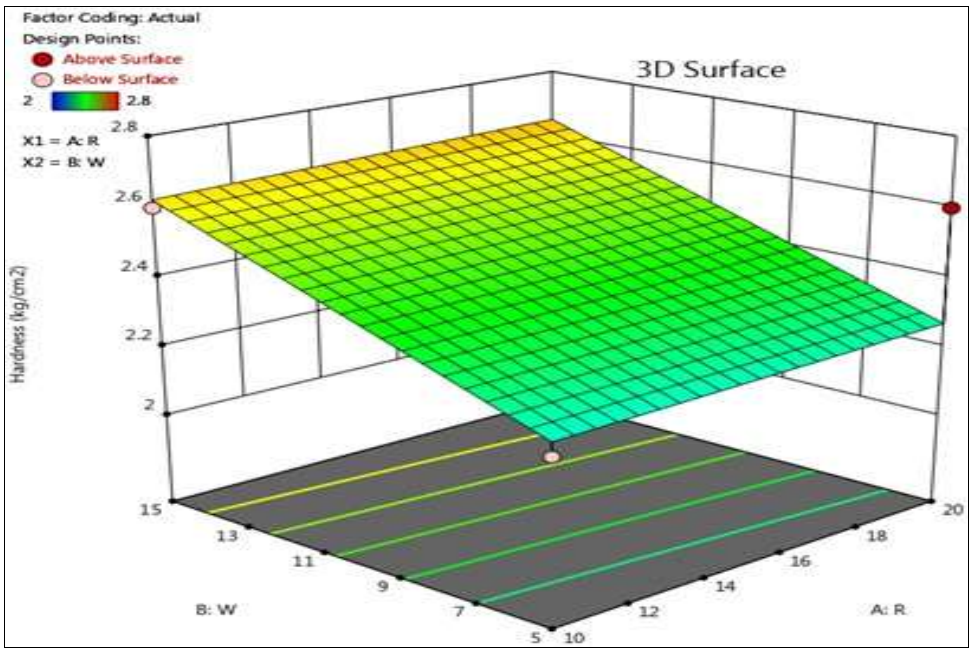


Figure 13: 3D Response Surface Methodology graph for R3

It can be well observed that Carrier: Coat Ratio (R) had a little effect upon the Hardness whereas amount of liquid medication (W) had major effect as compared to R. It can be explained via inferences occurred during study where increasing amounts of liquid medication induced humidity and thus lowering the hardness of the tablet.

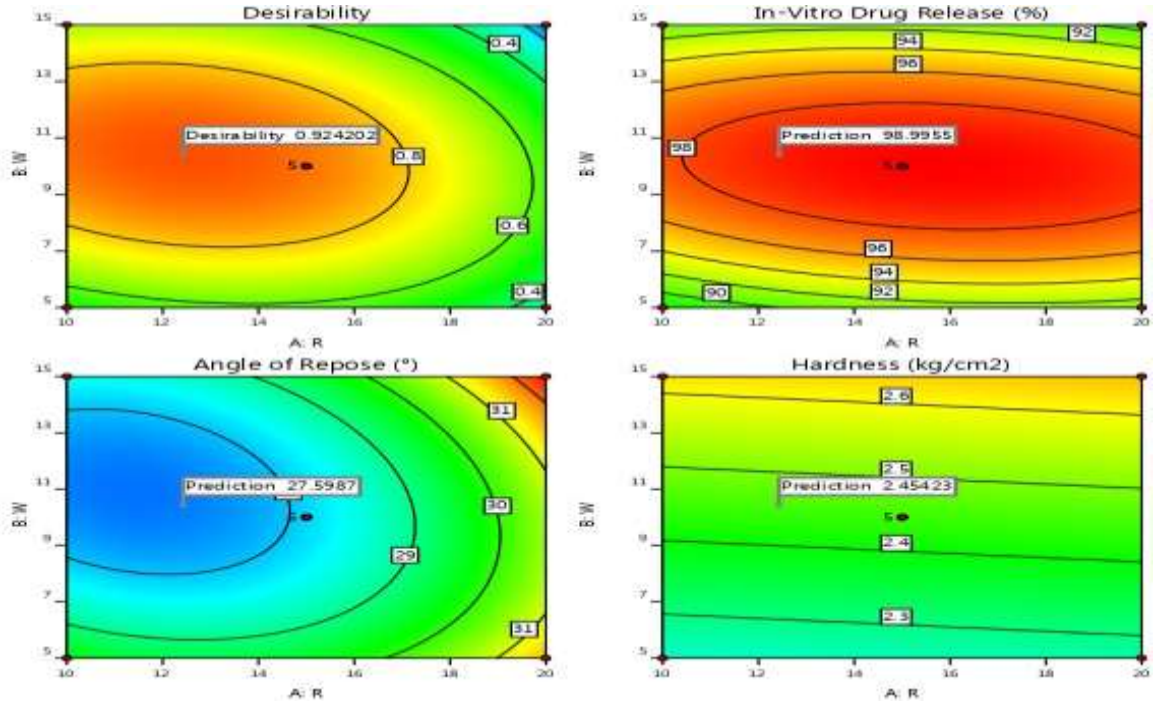


Figure 14: Contour plots for all responses.

In-Vitro Dissolution Test Results For Axitinib Liquisolid Tablets

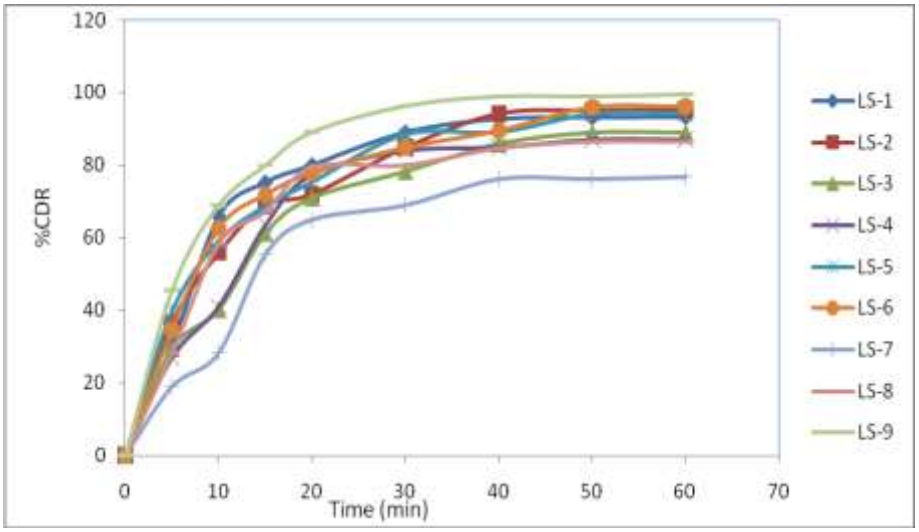


Figure 15: In-Vitro Dissolution test Time v/s % Cumulative drug release plot for LSTs

All the LSTs demonstrated excellent dissolution profiles except for LS-7 which released only 76.8 % of the total drug whilst rest of the formulations released more than 85% of the drug. LS-9 released maximum drug at 99.6%. LS-7 dissolution profile can be attributed to its extremely high liquid content. LS-9 was selected for comparison with conventional tablets due to its greater coverage of the Time v/s % Cumulative drug release plot and also due to maximum dissolution achieved profile. Futher explanations are made in section 9. (Dissolution Profile Comparison).

Dissolution Profile Comparison between Optimized (LS-9) Tablet and DCT

Table 9: In-Vitro Dissolution Test data comparsion between LS-9 and DCT

Time (min)	% Drug Released	
	LS-9	DCT
0	0	0
5	45.6 ±3.1	19.3 ±2.8
10	69 ±5	28.5 ±3
15	80 ±3	32.5 ±2.5
20	89.2 ±3.3	45 ±2.1
30	96.4 ±3.9	49.2 ±2.2
40	99 ±4.6	54.2 ±3.8
50	99 ±3.4	56 ±4.1
60	99.6 ±2.8	59 ±1.8

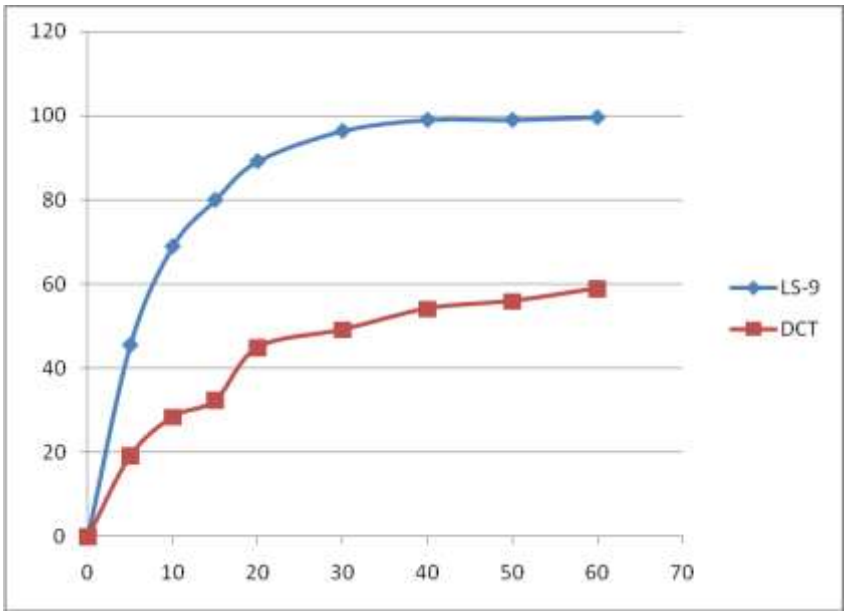


Figure 16: In-Vitro Dissolution test Time v/s % Cumulative drug release plot comparison between LS-9 and DCT

The drug dissolution profiles of LS-9 and DCT of Axitinib are compared in Figure 16. D_r (mcg/min) for 30 minutes of dissolution stands at 53.55, 13.66 and 16.16 mcg/min for LS-9 and DCT respectively. Dissolution rate was exponentially higher for LS-9 as compared to DCT. Also, at any given point of time, drug release from LS-9 was superior to the conventional tablets. According to the Noyes-Whitney equation [25, 26] and the "diffusion layer model" dissolution theories, the dissolution rate of a drug (DR) is equal to:

$$D_R = \frac{D}{h} A(Cs - C) \quad (16)$$

Where, h = thickness of the stagnant diffusion layer formed by the dissolving liquid around the drug particles, D = diffusion coefficient of the drug molecules transported through it, A = surface of drug available for dissolution, Cs = saturation solubility of the drug in the dissolution medium, and C = drug concentration in the bulk of the dissolving medium. [25, 26, 27] Dissolution Experiment was carried out at identical parameters for all the LS and DCT formulations in pH1.2 HCl buffer media (900 ml) on a Type-1 USP apparatus at 50rpm paddle speed. Since, it was conducted on a constant setting, Diffusion coefficient (D) and Thickness of stagnant diffusion layer (h) stayed constant for all the tablets. However, concentration gradients (C_s-C) differ in every

test due to the amount of drug differing in the stagnant dissolution layer. It can be speculated that in case of LS-9, the drug is suspended in Polyethylene Glycol 400 (10%). It establishes enhanced wetting of drug particles which in turn enhances the concentration gradient of the LST. Also, the LST contains the drug in a state of molecular dispersion as it is already suspended into a nonvolatile solvent in a homogeneous environment, whereas DCT merely disintegrates into micronized drug particles. LST formulation thus provides the drug with enhanced wettability and enhanced surface area exposure with the dissolution media as compared to DCT. Neusilin US2 also can be credited here due to its massive specific surface area which allows the drug to establish more contact with the dissolution media. Hence, the hypothesis that increased surface area (A) along with higher concentration gradient in the stagnant layer due to exposure of molecularly dispersed Axitinib particles appears to be fundamentally acceptable. Due to Polyethylene Glycol 400 present with Axitinib in every LST, Saturation solubility of drug may have a relative enhancement in minute quantities which may not alter the solubility of Axitinib at a large scale, but can be able to alter the surface contact interface between media and drug particles allowing more drug in stagnant layer of the particles. Hence, here the cosolvency concept applies where amounts of Polyethylene Glycol 400 diffusing along with Axitinib act as cosolvent with the pH 1.2 HCl buffer media in the stagnant layer (h). Due to this exposure, concentration gradient shoots up automatically thus explaining the enhanced dissolution rate of LS-9 as compared to DCT.

XRD Analysis

Physical Mixture at equal quantities of Drug: PEG 400 + NUS2 resulted into Graph 2. A little loss of crystalinity is observed as the characteristic peaks of drug at 8.93°, 12.03°, 15.76°, 19.32°, 21.69°, 25.01°, and 26.39° are still present in the Physical Mixture XRD but have diminished intensity. This can be attributed to presence of PEG 400 which solubilizes Axitinibthus diminished intensity is observed. An additional diffraction peak can be observed at 31.91° and 33.56° which can possibly be of Silicate and Aluminium ions present in Neusilin® US2.

A complete loss of crystallinity is observed in LS-9. Only three diffraction peaks are recorded viz. 18.85°, 20.3° and 33.66° having an extremely diminished intensity. Two inferences can be drawn via this result. One is that drug has turned amorphous which is on similar lines with solubility enhancement of the drug. Although it is to be noted that never ever for once it has been recorded in literature that solubility enhancement by liquisolid occurs via amorphousization of drug as it works purely upon cosolvency concept. Also it is noted that the drug did not showed any complete loss of crystalinity in graph of XRD of Physical Mixture. Second inference is that the LS-9 formulation content proportion can be noted. It contains 2.6 % w/w Drug, 73.18 % w/w powder excipients and 24.2 % w/w of nonvolatile solvent. Due to a very miniscule fraction of drug content in LS-9 (2.6%) as compared to Physical Mixture (50%) it can be inferred that it was unable to be screened via the X-Ray diffractometer. This can be also confirmed by studying the nature of excipient Neusilin® US2 used. Neusilin® US2 is amorphous silicate powder which has a very high specific surface area with high porosity. It has the ability to entrap drug molecules inside its porous structure which is one of the speculations why Axitinib molecules were unable to be detected in the LS-9 graph. As drug was present in half the Physical Mixture, it was detected very easily with retention of nearly all its diffraction peaks. Hence, it can be concluded that drug did not lose its crystalinity. A representative comparison between all the XRD graphs is shown in Figure 17.

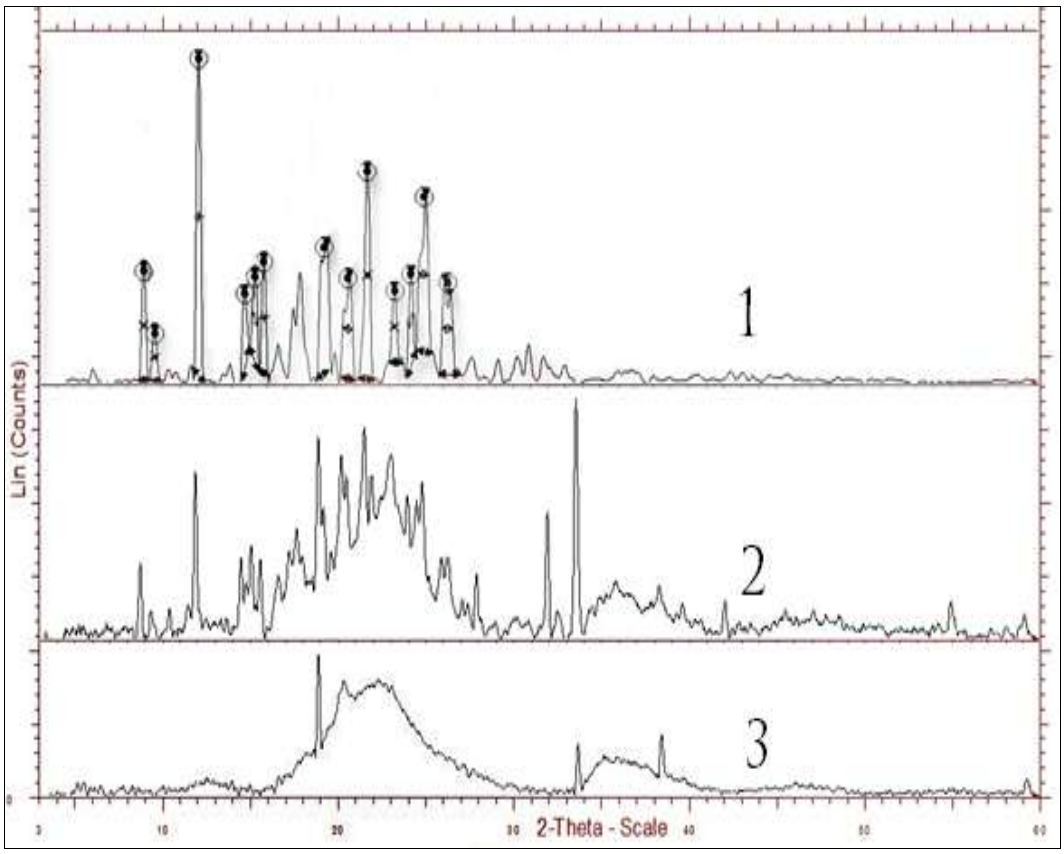


Figure 17: XRD Comparison: 1. Axitinib, 2. Physical Mixture and 3. LS-9

Ageing Studies

LS-9 ageing studies were conducted under accelerated stability condition at 40°C ±2°C temperature and 75 ±5% relative humidity for 3 months. Results are listed in Table 10. (n=3; all values are written as Mean ±SD).

Table 10: Ageing Studies results

Evaluation parameters	Initial Reading	1 Month	2 Month	3 Month
Hardness (kg/cm^2)	2.4±0.2	2.2 ±0.2	2.2 ±0.2	2.2 ±0.2
Friability (%)	0.7 ±0.2	0.8 ±0.2	0.9 ±0.1	0.9 ±0.1
Tablet Weight (mg)	185.1 ±4	184.8 ±3.6	184.6 ±3.2	184.1 ±3.7
Disintegration time (min:sec)	4:25 ±0:45	4:40 ±0:30	4:35 ±0.55	5 ±0:50
Drug content (%)	100.8 ±2.0	98.2 ±3.4	99.4 ±4.8	97.6 ±3.8
% Dissolution achieved	99.6±2.8	102.4 ±3.8	98.2 ±4.4	100.8 ±2.2

No major difference was observed in any of the tests for LS-9 indicating that it held its stability for a period of 3months.

CONCLUSION

Solubility enhancement of Axitinib was successfully performed using LS technology. LS-9 was selected as optimized batch by QbD approach using Central Composite Design. An exponential difference was observed between the dissolution profiles (99.6 and 59 for LS-9 and DCT respectively) of LS-9 as compared to DCT. Also high D_r (53.55 and 13.66 mcg/min for LS-9 and DCT respectively) was observed for LS-9 as compared to DCT. Usage of Neusilin US2 had advantages such as a high Lf (0.59) as compared to literature values of

conventionally used Avicel® PH-102. Overall Liquisolid technology proves itself to produce unit dosage forms combined with solubility enhancement of BCS class 2 drugs.

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