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Formulation And Evaluation of Ibrutinib Nanosuspension

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Abstract

The aim of the present work is to develop Oral Nanosuspension of Ibrutinib by Nano Precipitation method using various Carriers & Surfactant such as Urea, Pluronic F127, PVP K-30 and SLS various formulation as well as process parameters were optimized in order to achieve desirable size and saturation solubility. Ibrutinib is an inhibitor of Burton's tyrosine kinase which is used for the treatment of chronic lymphocytic leukemia. Evaluation of the prepared Nanosuspension was done with respect to percentage yield, drug content, entrapment efficiency, viscosity, Sedimentation volume, Surface morphology study (SEM), particle size, zeta potential, saturation solubility, in-vitro diffusion study and release kinetics. All the evaluations were passed within the limits in that formulation NS12 having more potential and its show best results in all Characterization when compared to other formulations. The Zeta potential value for the optimized formulation (NS12) was found to be 0.2 mv which was found to be within the acceptable limits. The Average particle size of Nano suspension of optimized formulations (NS12) which is in 1:1 ratio with PVP K-30 was found to be 185.42 nm. From the invitro studies, we can say that optimized formulation NS12 shows best drug release of 99.65±1.84% within 30 minutes whereas all the other formulations didn't release high amount of the drug. The drug release from the Nanosuspension was explained by the using mathematical model equations such as zero order, first order, and equation methods. Based on the regression values it was concluded that the optimized formulation NS12 follows zero order kinetics with super case-II transport mechanism.

Keywords: Ibrutinib, PVP K-30 and SLS, FTIR, SEM, PSD and Zeta Potential.

Introduction

Ibrutinib is a small molecule medication that binds to Bruton's tyrosine kinase protein irreversibly, preventing B-cell growth and survival.[1] The B-cell receptor pathway, which is often abnormally active in B-cell malignancies, is inhibited by blocking BTK[2]. When ibrutinib is taken with meals, its exposure almost doubles, which reduces the medication's effectiveness and safety [3]. It is commercially available in capsular dosage form with extremely high dosages (140 mg per ml) a day that cause significant adverse effects on the gastrointestinal tract due to its low solubility and hepatic first-pass effects. Therefore, ibrutinib has to be formulated in an improved oral form with increased bioavailability and effectiveness [4].

October 2014 saw the EMA approve ibrutinib [5], and November 2014 saw Health Canada approve it as well).[6] In August 2017, [7] it was licensed for the treatment of a number of illnesses, including Waldenström's macroglobulinemia, chronic graft versus host disease (cGVHD), and chronic lymphocytic leukemia (CLLNotably, in August 2017,[8] ibrutinib was authorized by the FDA as the first therapy for pediatric cGVHD.

The nanoprecipitation process has several benefits, including being a simple, quick, and effortless approach to use. This procedure involves dissolving the medication in an organic solvent, such as methanol, ethyl acetate, acetone, or acetonitrile. One of two methods is used to evaporate the organic solvent: constant stirring or pressure reduction. It was discovered that the kind of carrier, carrier concentrations, and homogenizer speed all affected particle size.

The current study prepares nanosuspension by the nanoprecipitation technique, in which a drug is dissolved in a solvent and then introduced to a non-solvent, causing the precipitation of small drug particles. Polymer and surfactant stabilize the system.[9]

The current research aimed to improve lbrutinib's solubility by creating nanosuspensions of the ibrutinib by using the nanoprecipitation technique, using a variety of carriers and surfactant (surface active agent), and then evaluating all of the finished formulations.

Materials and Methodology

Drug and Excipients:

Ibrutinib was a purchased from Xenon Pharma Pvt Ltd, New Delhi. Urea, Pluronic F 127, PVP K-30, SLS and Ethanol was purchased from Loba chemie, Mumbai. Distilled water was purchased from Narmada chemicals, Hyderabad.

Method of Preparation of Ibrutinib Nanosuspensions

Table No.1 Formulation table of Ibrutinib loaded Nano suspensions using by Nano Precipitation method

Ingredients	Class/ use	NS1	NS2	NS3	NS 4	NS 5	NS6	NS7	NS8	NS9	NS10	NS11	NS12
Ibrutinib (mg)	**Anti neoplasticagent**	140 mg	140 mg	140 mg	140 mg	140 mg	140 mg	140 mg	140 mg	140 mg	140 mg	140 mg	140 mg
Urea (mg)	**Carrier**	35 mg	70 mg	105 mg	140 mg	-	-	-	-	-	-	-	-
Pluronic-F127 (mg)	**Carrier**	-	-	-	-	35 mg	70 mg	105 mg	140 mg	-	-	-	-
PVP K30 (mg)	**Carrier**	-	-	-	-	-	-	-	-	35 mg	70 mg	105 mg	140 mg
SLS (mg)	**Surfactant**	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg	10 mg
Ethanol (ml)	**Organicsolvent**	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml	2ml
Ratio	**Drug: Carrier**	1:0.25	1:0.5	1:0.75	1:1	1:0.25	1:0.5	1:0.75	1:1	1:0.25	1:0.5	1:0.75	1:1
Wate (ml)	**Aqueous Solvent**	30ml	30ml	30ml	30ml	30ml	30ml	30ml	30ml	30ml	30ml	30ml	30ml

Nanosuspension of Ibrutinib was prepared by precipitation method with various carriers and drug. [10] At first the weighed amount of Ibrutinib was taken and dispersed into the beaker containing Ethanol which acts as organic solvent. This drug and ethanol solution are termed as organic phase. Now the carriers Urea, Pluronic F 127 and PVP K-30 was dissolved in water and add surfactant (SLS) to this aqueous solution. We can label as aqueous phase. This solution was kept on magnetic stirrer for uniform mixing. Addition of organic solvents by means of a syringe positioned with the needle directly into carrier/surfactant containing water (aqueous phase). After 1 hour, the solution was kept in sonicator for about 30 mins. Then formed Nanosuspensions were collected by filtration and dried.[11] (Table No.1)

Evaluation Parameters of Nano Suspensions

Percentage yield:

Percentage practical yield of Ibrutinib Nanosuspensions is calculated to know about percentage yield, thus it helps in selection of appropriate method of production. Practical yield was calculated as the weight of Ibrutinib Nanosuspensions recovered from each batch in relation to the sum of starting material.

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

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

Drug content:

An accurately measured nanosuspension equivalent to 10mg of drug was taken in 100ml volumetric flask and diluted to 100ml with methanol. (To prepare the stock solution of 100µg/ml). The amount of drug determined spectrophotometrically at 257 nm. Single Beam Spectrophotometer (YIS-294).

$$\%DC = \frac{\textit{Total amount of drug added} - \textit{amount of drug in suspension}}{\textit{total amount of drug added}} x100$$

Entrapment efficiency:

The 140 mg of the Ibrutinib weight equivalent Nanosuspensions was analyzed by dissolving the sample in 10ml of dichloromethane. After the drug was dissolved 10ml of clear layer of dissolved drug is taken. There after the amount of drug in the water phase was detected by a UV-Spectrophotometric

method at 257 nm (U.V Spectro photometer).The concentration of the drug is determined with the help of calibration curve. The amount of drug inside the particles was calculated by subtracting the amount of drug in the aqueous phase from the total amount of the drug in the Nanosuspensions.[12]

Viscosity:

The rheologic parameters of the prepared suspensions, in terms of Viscosity, were determined by use of the steady shear method, Measuring the "non-Newtonian viscosity". Rheology of all Nanosuspensions was performed with a RVT Brookfield viscometer from Choksi Lab. (Indore, M. P.) All measurements were performed after Eliminating all thixotropy from the suspension.

Sedimentation volume:

The suspensions were stored individually in a 50ml measuring cylinder for 8hours at room temperature. Observations were made at every hour up to 8hours. The sedimentation volume (F) was then calculated using the following equation:

$$\mathbf{F = \frac{Vu}{Vo} * 100}$$

where, Vu is the ultimate volume of the sediment and Vo is the original volume of the suspension.

Scanning electron microscopy:

The morphological features of Ibrutinib nanosuspension are observed by scanning electron microscopy at different magnifications.

Particle Size analysis:

The particle size of the formulated nanosuspension batches was determined by using the Motic digital microscope. The particle size of the batches was recorded in micrometer. The formulations were diluted with an appropriate volume of phosphate buffer solution (6.8 pH) The measurements were carried out three times where the mean value was used.

Invitro drug release kinetic studies:

Kinetic model had described drug dissolution from nano suspension where the dissolved amount of drug is a function of test time. In order to study the exact mechanism of drug release from the nanosuspension, drug release data was analyzed according to zero order, first order, Higuchi square root, Korsmeyer- Pappas model. The criteria for selecting the most appropriate model were chosen on the basis of goodness of fit test.[19,20]

The entrapment efficiency (%) of drug was calculated by the following equation.

$$\% \text{ Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Freely dissolved drug}}{\text{Total amount of drug}} x100$$

Zeta potential:

The zeta potential of the dilute silver nanoparticles formulation (1:2500 v/v) homogenized evaluated with zeta meter system measured with zetasizer. (Malvern, Nano Series ZS90, Malvern Instruments, Ltd., UK). The studies were performed in triplicate at 25 °C. [13]

Diffusion study:

The invitro release of various nanosuspension formulations were performed by dialysis bag diffusion technique. Dialysis tubing will act as dialysis sac. (Sigma dialysis membrane MW 12000 Da). Length of dialysis tube is 4 - 5 cm., The sac was then emptied and 1 ml of the formulated liquid nanosuspension was accurately transferred into the sac, which served as the donor compartment. The sac was once again examined for leak and then suspended in the stoppered vessel containing 100 ml 6.8 pH Phosphate Buffer, which behave as the receptor compartment [14,15,16]. The Media temperature should be 370 ± 0.5ºC at 500 rpm speed. At predetermined time intervals,3 ml of the sample was withdrawn from the receptor compartment and analyzed for the quantity of drug released. Fresh buffer was used to replenish the receptor compartment at each time point. The samples were withdrawn at 5,10,15,20,25,30,45 and 60 minutes. The diffusion studies and sample analysis were carried out for all the developed formulations. Collected samples were suitably diluted with 6.8 pH Phosphate Buffer and analyzed at 257 nm using 6.8 pH Phosphate Buffer as blank by using a UV spectrophotometer. The cumulative % drug release was calculated and graphs were plotted against time Vs % cumulative drug release.[17,18]

Results and Discussions

Percentage yield:

All the Nanosuspension formulations was showed the Percentage yield 85.74±0.75% to 96.84±0.87%. The Optimized Formulation NS12 Was found to be 96.84±0.87% of yield which was having more yield when compared to the remaining formulations. (Table No.2)

Drug content:

All the Nanosuspension formulations was showed the Drug content was increased gradually from 86.54±0.71% to 96.84±0.87%. The Optimized Formulation NS12 Was found to be 98.82±0.85%% of yield which was having more yield when compared to the remaining formulations. (Table No.2)

Entrapment efficiency:

All the Nanosuspension formulations was showed the Entrapment efficiency was increased gradually from 87.23±0.84% to 97.57±0.48. The Optimized Formulation NS12 having less drug loss. So, it found to be 98.82±0.85%%, which was having more yield when compared to the remaining formulations. (TableNo.2)

Table No.2 Evaluation parameters of Nanosuspensions

Formulations	Percentage yield (%)	Drug Content (%)	entrapment Efficiency (%)	Viscosity (cps)
NS1	85.74±0.75	89.24±0.84	95.23±0.85	0.857
NS2	87.18±0.41	86.54±0.71	90.15±0.74	0.641
NS3	85.68±0.94	90.84±1.29	89.45±0.51	0.574
NS4	89.21±0.47	96.47±0.75	95.63±0.47	0.445
NS5	88.67±1.03	88.68±0.68	87.23±0.84	0.894
NS6	91.21±0.68	93.47±0.45	92.14±0.74	0.754
NS7	90.26±0.65	91.84±0.62	90.26±0.85	0.687
NS8	94.58±0.58	95.67±0.74	94.15±0.47	0.489
NS9	91.28±0.28	97.64±0.65	96.24±0.61	0.784
NS10	93.48±0.74	96.84±0.74	95.17±0.74	0.624
NS11	92.84±0.59	95.74±0.98	97.28±0.51	0.562
NS12	96.84±0.87	98.82±0.85	97.57±0.48	0.342

Viscosity:

All the Nanosuspension formulations was showed the Viscosity in between 342 cps -894 cps. The Optimized Formulation NS12 having less drug loss. So, it found to be 98.82±0.85%%, which was having more yield when compared to the remaining formulations. the optimized formulation shows the less viscosity with 0.342 cps. (Table No.2)

Sedimentation volume:

The Nanosuspension formulations was followed the Sedimentation volume. In this evaluation, the formulations from NS1 to NS12 was found in between 100-42%. In this, the sedimentation volume for the Optimized formulation NS12 with the 1 ratio of PVP K30 was found to be 86% at the end of 8th hour shown the good flocculation of particles in the suspension. (Figure No.1)

Scanning Electron Microscopy:

The Surface and shape the nano suspension was examined under the Scanning electron microscope. The surface structure of optimized formulation was observed by scanning electron microscopy at different magnifications. In this SEM, the nanosuspension particles are appeared within slightly spherical in shape and particle size was reduced up to 200nm. (Figure No.2)

Particle size analysis:

The Optimized formulation was subjected to Particle size analysis. So average particle size was found to be 185.24 nm which was determined by using the Motic digital microscope. So, the particle size was decreased by using this nano precipitation technique. (Figure No.3)

Zeta Potential:

The Zeta potential of the optimized formulation by using polymers PVP K30 as carrier, and SLS as surfactant, which demonstrate the Zeta potential value for the optimized Formulation (NS12) which shows the significant results of 0.2 mv that indicates positive charge because the positive charge of carrier which indicates good stability of the formulation. (FigureNo.4)

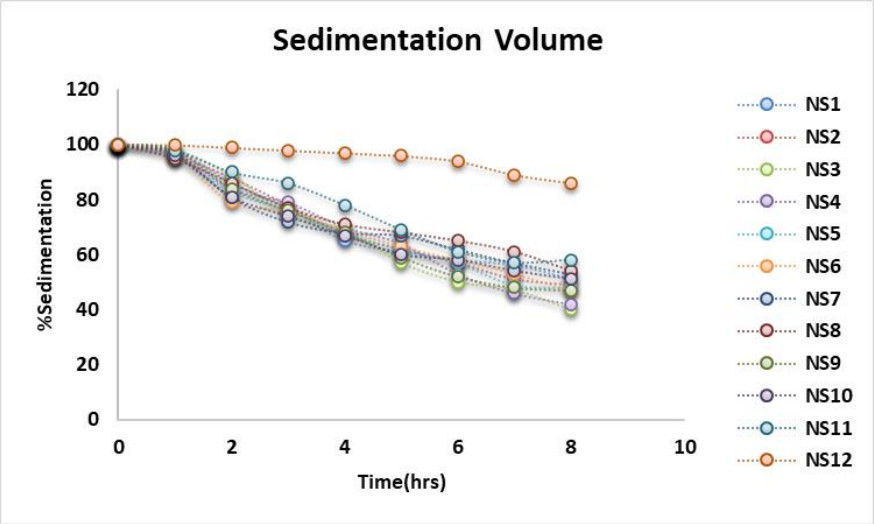

Figure No.1 Sedimentation volume regrading nanosuspension formulations

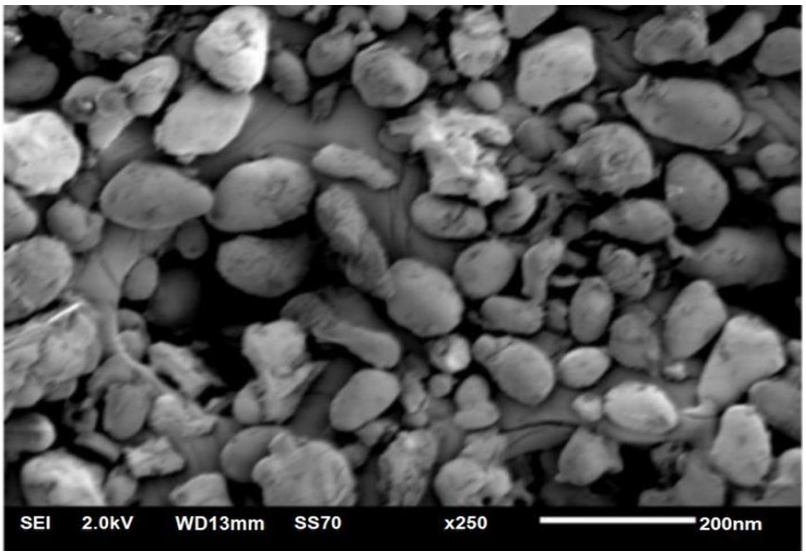

Figure No.2 Scanning electron microscopy of optimized formulation

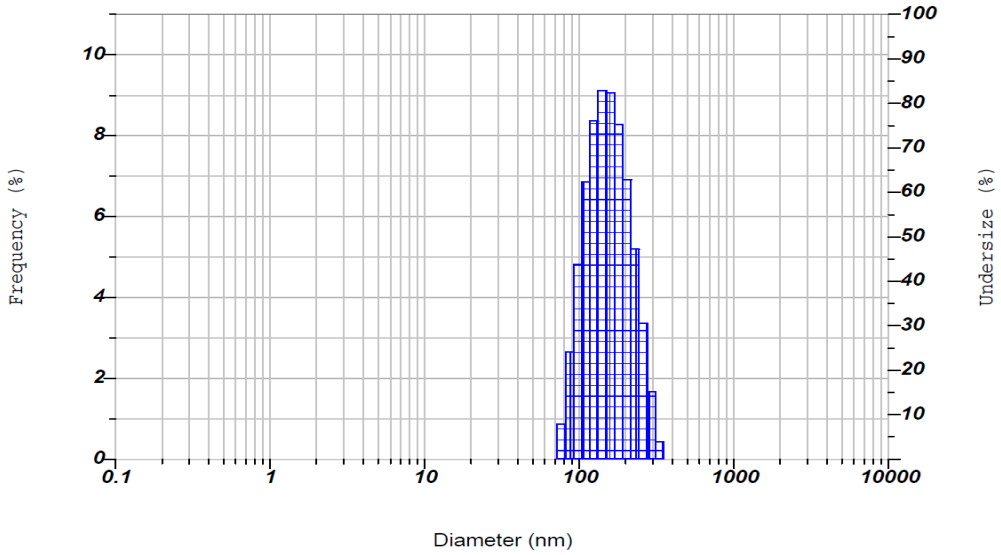

Figure No.3 Particle size analysis of optimized formulation

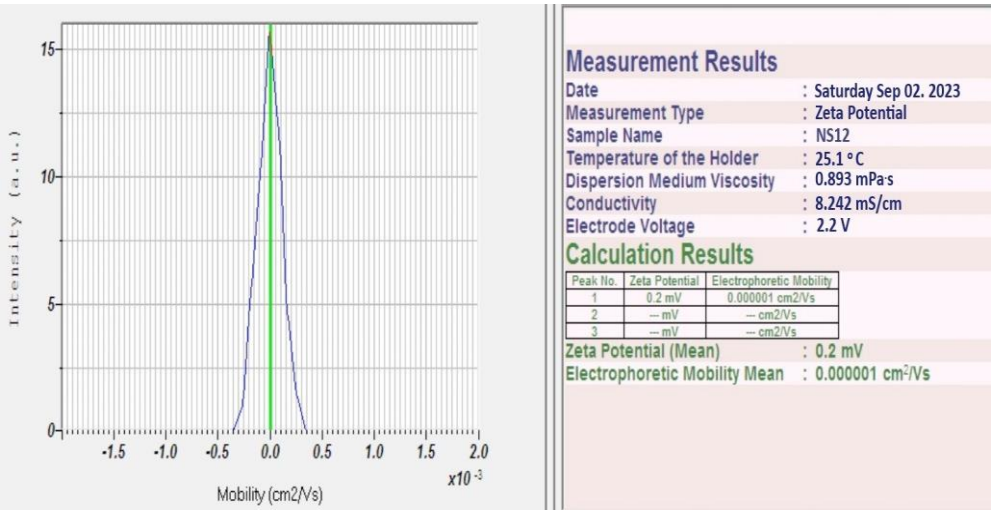

Figure No.4 Zeta potential of optimized formulation

Diffusion Studies:

The Invitro Diffusion studies which was obtained by the Invitro Diffusion technique shows the drug release of Nanosuspensions in various intervals of Time i.e 0, 5, 10, 15, 20, 30, 45 and 60 minutes. In this Formulation NS1 release the 87.12±1.47% of drug at the end of 60minutes, NS2 release the 89.07±1.36% of drug at the end of 60 minutes, NS3 release the 94.21±1.69% of drug at the end of 60minutes, NS4 release the 97.18±1.85% of drug at the end of 30 minutes, NS5 release the 78.25±1.85% of drug at the end of 60 minutes, NS6 release the 86.78±1.74% of drug at the end of 60minutes, NS7 release the 85.69±1.28% of drug at the end of 60minutes, NS8 release the 98.47±1.84% of drug at the end of 30minutes, NS9 release the 77.48±1.85% of drug at the end of 60minutes, NS10 release the 87.26±1.78% of drug at the end of 60minutes, NS11 release the 93.65±1.85% of drug at the end of 60minutes and NS12 release the 99.65±1.84% of drug at the end of 30minutes.

So, the formulation NS12 shows the best results at the end of 30 minutes. Hence NS12 was considered to be optimized formulation. (Figure No.5)

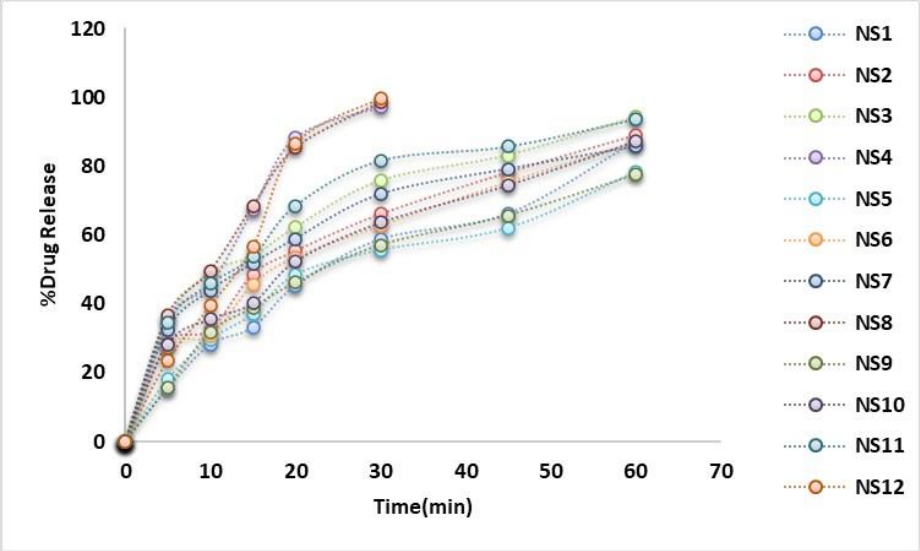

Figure No.5 Invitro diffusion studies of Nanosuspension formulations

Release kinetics:

The drug release from the Nanosuspension was explained by using mathematical model equations such as zero order, first order, Higuchi and Peppas model. Based on the regression values it was concluded that the optimized formulation NS12 follows zero order kinetics with super case-II transport mechanism. (Figure No.6,7,8,9) and (Table No.3).

Zero Order

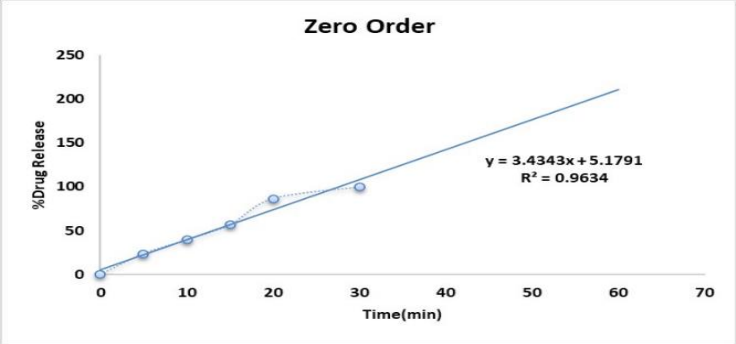

Figure No.6 Zero order release profile of formulation NS12

First order

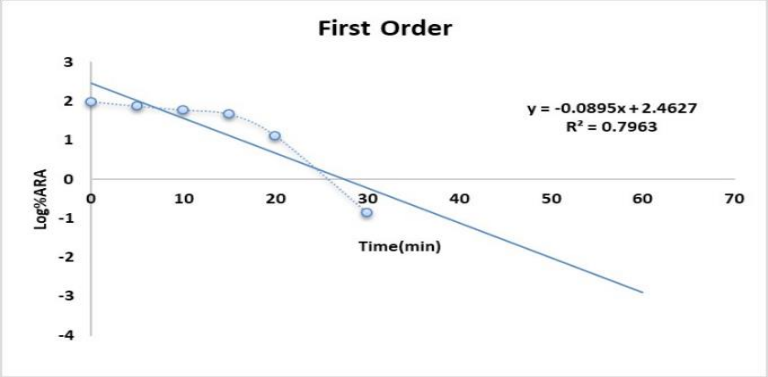

Figure No.7 No First order release profile of formulation NS12

Higuchi Plot

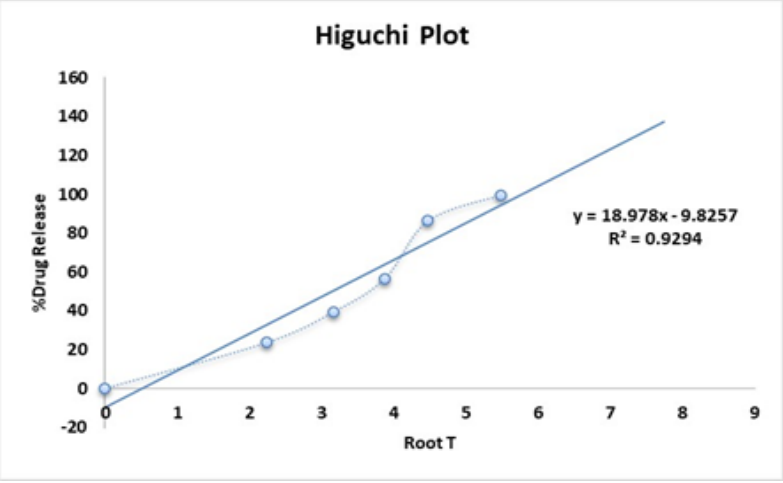

Figure No.8 Higuchi model of formulation NS12

Korsmeyer-Peppas model

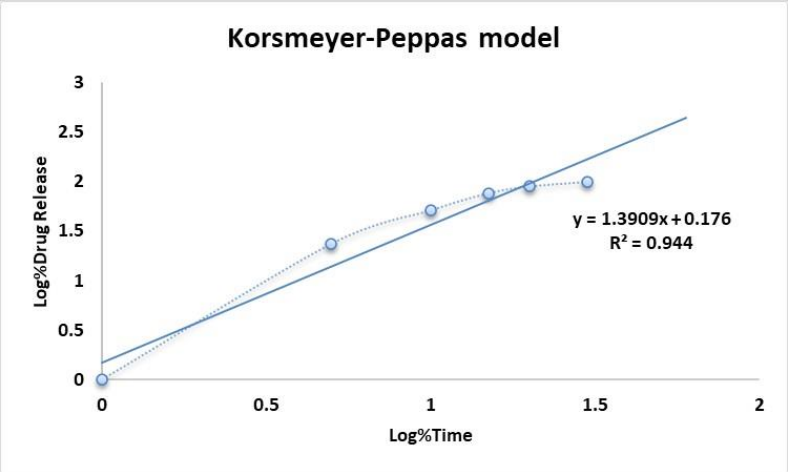

Figure No.9 Korsmeyer-Peppas model of formulation NS12

Table No.3 Kinetic release values of Optimized formulation NS12

Cumulative % Drug Release	Time (min)	Root T	Log % Release	Log Time	Log % Remains	Drug remaining
0	0	0	-	-	2	0
23.64	5	2.236	1.373	0.698	1.882	2.236
51.43	10	3.162	1.711	1	1.782	3.162
76.61	15	3.872	1.884	1.176	1.675	3.872
89.49	20	4.472	1.951	1.301	1.130	4.472
99.65	30	5.477	1.9984	1.4771	0.853	5.477

Summary and Conclusion

In present research studies regarding Nanosuspensions of Ibrutinib which prepared by Nano Precipitation method by various carriers and surfactant follows the evaluation parameters as percentage yield, drug content uniformity, percent entrapment efficiency, viscosity, sedimentation volume, scanning electron microscopy, particle size analysis, zeta potential, in-vitro release and drug release kinetics. The Evaluation parameters like Percentage yield, Drug content, Entrapment Efficiency, Viscosity and Sedimentation volume for the Nanosuspension formulations are being conducted and, in all parameters, NS12 formulation yields the best results. The formulation NS12 was shown the best result in every parameter with the invitro diffusion shows the maximum drug release within 30 minutes and the particle size also decreased to nanometers. In release kinetics also the Optimized Formulation NS12 follows zero order kinetics with super case-II transport mechanism.

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