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Development and Characterization of Tyrosine Kinase Inhibitors Loaded Polymeric Nanoparticles for The Anti-Cancer Activity

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ABSTRACT: The present study aimed to develop and characterize Dasatinib-loaded polymeric nanoparticles using the nanoprecipitation technique to achieve sustained drug release and enhance anticancer efficacy. Natural polymers such as Arabic gum, gum rosin and locust bean gum were employed as stabilizing agents for nanoparticle formulation. The prepared nanoparticles exhibited high drug entrapment efficiency and particle sizes ranging from 220.2 to 604.1nm. SEM and TEM analyses revealed that the nanoparticles possessed a smooth surface with irregular morphology. The in-vitro drug release profile demonstrated a sustained and controlled release of Dasatinib over a period of 48hr. furthermore, in vitro cytotoxicity and cellular uptake studied using MDA-MB-231 and K562 cell lines confirmed that the optimized formulation (NP4) showed enhanced cellular internalization and a markedly higher cytotoxic effect compared to the pure drug stability studies conducted over three months indicated no signification alterations in physicochemical properties, confirming formulation robustness. Overall, the developed Dasatinib-loaded polymeric nanoparticles demonstrated promising potential as a sustained-release drug delivery system for effective cancer therapy.

KEYWORDS: Dasatinib, Polymeric Nanoparticles, Nanoprecipitation, Sustained release, Natural polymers, Anti-cancer activity.

I. INTRODUCTION

NANOPARTICLES

Nanoparticle-based drug delivery systems have emerged as a promising approach in modern pharmaceuticals research for enhancing the therapeutic performance of various bioactive compounds[1]. Solid polymeric nanoparticles are submicronic colloidal carriers, generally ranging in size from 10 to 1000nm, that are composed of biodegradable and biocompatible macromolecular materials[2]. These systems possess the ability to encapsulate, absorb or conjugate active pharmaceutical ingredients (APIs) through mechanisms such as dissolution, entrapment, surface adsorption, or encapsulation within the polymeric matrix[3].

The physicochemical characteristics of polymeric nanoparticles such as particle size, surface charge and morphology play a critical role in determining their drug release kinetics, stability and bioavailability[4]. Depending on the preparation method and polymer composition nanoparticles can be designed as nanospheres or nanocapsules, each offering distinct drug release and protection profiles[5]. These versatile carriers provide several therapeutic advantages, including controlled and sustained drug release, improved solubility of poorly water-soluble drugs, targeted delivery and reduced systemic toxicity[6]

Nanoprecipitation Method

Using this technique, the organic solvent will diffuse in the hydrophilic media with or without the assistance of a surfactant, and the polymer will precipitate out of it. Usually, a water-miscible solvent is used to dissolve the polymer, which causes the polymer to precipitate and form a nanoscale particle. This phase is now stirred into the hydrophilic media together with a stabiliser, or surfactant. The rapid diffusion process facilitates the deposition of polymer on the organic-aqueous solvent interface, a feature that results in the development of nanoscale structures. In the initial stage of this procedure, nanoparticulate production can be improved by phase separation is carried out using a solvent system that is fully miscible. Using this method, nanocapsules might

also be created by adding a little amount of non-toxic oil to the organic phase. This method can only be applied to water miscible solvents where there is sufficient diffusion rate to cause spontaneous emulsification[7].

Leukemia

Leukaemia is a type of cancer that originates in the blood-forming tissues of the body, including the bone marrow and lymphatic system. It exists in multiple forms, with certain types more prevalent in children, while others are primarily seen in adults. This malignancy typically targets white blood cells, which play a crucial role in the immune response. Under normal conditions, white blood cells grow and divide in a controlled manner, aligning with the body's physiological needs. However, the bone marrow of leukaemia patients creates aberrant white blood cells that are defective in their usual function[8].

Tyrosine kinase inhibitors (TKI)

TKIs are useful for the focused therapy of a number of cancers. The first medication to be used in clinical oncology was imatinib; medications like gefitinib, erlotinib, sorafenib, sunitinib, and dasatinib came after it. A powerful small-molecule, second-generation BCR-ABL multitarget kinase inhibitor is dasatinib. Except for those with the T315I mutation, dasatinib can impede the growth and kinase activity of BCR-ABL mutant and wild-type cell lines that are resistant to imatinib. In vivo Research has indicated that dasatinib exhibits 325 times greater potency against unmutated BCR-ABL compared to imatinib, and 16 times greater potency versus nilotinib, another BCR-ABL kinase inhibitor. Dasatinib is a selective inhibitor of tyrosine kinase receptors, primarily used in the treatment of chronic myelogenous leukaemia (CML) in patients harboring the Philadelphia chromosome fusion. The first second-generation TKI to be released in 2006 was dasatinib.38In order to approach sustained release action, dasatinib is consequently chosen as the ideal choice for the creation of polymeric nanoparticles by the Nanoprecipitation process using biodegradable polymers such as gum rosin, gum Arabic, and locust bean gum[9].

MATERIALS & METHODS

Dasatinib(Gift sample from Shilpa Medicare limited, Bangalore), Poloxamer 188(Sigma Aldrich), Mannitol(S D Fine-Chem Limited, Mumbai), Gum rosin(S D Fine-Chem Limited, Mumbai), Locus bean gum(S D Fine-Chem Limited, Mumbai), Gum Arabic(Merck Life Science India Pvt. Ltd), Acetone(SDFCL, Mumbai), Methanol(SDFCL, Mumbai), Dimethyl sulfoxide (DMSO) (Sigma Aldrich), Dicholoro methane(Sigma Aldrich), Dimethyl formamide(Sigma Aldrich),

PREFORMULATION STUDIES.

Description

The dasatinib sample's physical characteristics, including its colour, odour, and powder texture, were assessed.

Determination of solubility

The solubility of the pure dasatinib medication was assessed using a variety of solvents, including water, methanol, and ethanol.[10-11]

Determination of λ max

A 7.4 pH phosphate buffer was used to make a solution of dasatinib containing conc. 10µg/ml, and a Shimadzu (UV-1601) Spectrophotometer was used to take the UV spectrum. The 200–400 nm range was used to scan the solution[12-13].

Conformation of Dasatinib drug by using FT-IR

A small amount of dasatinib was taken and subjected to infrared spectra in Shimadzu kept at an ambient temperature of 25.0±0.5oC. The spectra were recorded by placing the sample carefully and scanning the sample in region of 4000-400cm-1 to determine various functional groups. The obtained IR spectrum of dasatinib was recorded[14].

Preparation of Polymeric nanoparticles

Formula for the preparation of polymeric nanoparticle by Nanoprecipitation method

Table no 1: Formula for the preparation of polymeric nanoparticle by Nanoprecipitation method.

S.I	Ingredients	Formulation Code					
		NP1	NP2	NP3	NP4	NP5	NP6
1	Dasatinib (mg)	100	100	100	100	100	100
2	Gum Arabic (mg)	250	350	-	-	-	-
3	Gum rosin (mg)	-	-	250	350	-	-
4	Locust bean gum (mg)	-	-	-	-	250	350

5	Methanol (ml)	10	10	10	10	10	10
6	Distilled water (ml)	100	100	100	100	100	100

To create solution I, ethanol was used to dissolve the polymer and precisely weigh the DST. To create solution II, Poloxamer 188 was dissolved in distilled water simultaneously. Solution I was incorporated to Solution II little by bit. The mixture was then subjected to homogenization at 15,000 rpm for a duration of 10 minutes. For five minutes, the dispersion was sonicated (15 cycles with a 30-second break). To remove the methanol from each formulation, it was magnetically agitated at 100rpm for six hours. The mixture was then centrifuged at -20°C for 20 to 30 minutes, discarding the Surprenant liquid to produce pellets. The pellet was pre-frozen at the pre-glycolic route at -40°C after being redispersed in a cryoprotectant solution containing 0.5% mannitol. It was lyophilized at -40°C for 24 hours and -80°C for 4 hours (primary and secondary drying). To prevent moisture, the produced spongy nanoparticle was kept in an airtight container[15,16,17].

CHARACTERIZATION

Solubility studies

Examination of polymeric nanoparticles' solubility. A solubility test was conducted on the produced polymeric nanoparticle using distilled water, methanol, ethanol, and DMF, in accordance with Dasatinib profile. 10ml of solvent were used to dissolve 1 mg of the corresponding sample, which was then sonicated for two minutes[10-11].

Drug content (DC)

10 mg of Dasatinib containing nanoparticle was equivalently weighed and The sample was dissolved in phosphate buffer at pH 7.4, followed by continuous stirring. Subsequently, the solution underwent sonication for 10 minutes and was then diluted to fall within the appropriate Beer's law concentration range. Absorbances were observed at 323 nm by using UV spectrophotometer[18].

Drug entrapment efficiency:

After preparing 80 millilitres of 7.4 pH buffer, nanoparticles equivalent to 5 milligrams of dasatinib were introduced. The container was sealed with aluminum foil and left undisturbed overnight. stirred and warmed for up to fifteen minutes at 40°C. I added the remaining 20 millilitres of buffer. Additional aliquots were made in accordance with Beer's range, and spectrophotometric absorbance measurements were made at 323 nm[19].

Fourier transforms infrared spectroscopy (FT – IR) analysis

FT-IR analysis was performed on the purified medication and the nanoparticles that were produced by mixing with potassium bromide (KBr) following a baseline correction with dried potassium bromide to verify compatibility. Pellets were compressed to a pressure of roughly 5 x 106 Pa in an evacuated die to create transparent, clear discs that had a diameter of 2 cm and a thickness of 0.2 cm. At room temperature, spectra from 4000 cm-1 to 400 cm-1 were recorded using an integrated Fourier transform spectrometer (Bruker, USA)[20].

Particle size, PDI and Zeta potential

Using DLS for size analysis and a combination of LDV The mean particle size, polydispersity index (PDI), and zeta potential of the nanoparticles obtained from both preparation methods were measured using a particle size analyzer (Nano ZS, Malvern Instruments, UK) equipped with Phase Analysis Light Scattering (PALS) technology. These two values provide PDI[21].

Transmission electron microscopic studies (TEM)

The PNP from GR2 was subjected to high-resolution transmission electron microscopy using a JEOL JEM 2010F UHR running at 200 kV. After that, the suspension of nanoparticles in polyol was put on the transmission electron microscope grid's amorphous carbon membrane and let to evaporate at room temperature. To remove the majority of the organic compounds, the grid underwent another heat treatment at 150 °C while under an extremely high vacuum. This treatment significantly increased high-resolution photos without changing the crystallinity of the particles. Using the SAISAM and TAMI applications, along with a digital camera (Microvision Instruments), we analyzed the TEM images to determine the particle size.[22]

Scanning Electron microscopic studies (SEM)

After sputtering a thin layer of gold over the produced nanoparticles from formulation, the microstructure was examined using an EDAX equipment (Jeol 6390LA/OXFORD XMX N) coupled to a scanning microscope operating at a 20 kV acceleration voltage[23].

Differential scanning calorimetry (DSC)

Using a heating rate of 10 K/min from 20°C to 300°C in the temperature range of 25ml/min under nitrogen flow, DSC thermograms of pure drugs and nanoparticles were recorded for 5 -15 mg of samples. Prior to sealing, a perforated lid was placed on each aluminum crucible containing the sample. The reference was an aluminium crucible empty.

In vitro drug release study

The equilibrium dialysis membrane method, or diffusion study, was used to conduct a drug release study on PNPs. For two hours, the study was conducted in a 1.2 pH buffer, and from the conclusion, it was conducted in a 7.4 pH buffer solution. After taking 10 mg of dasatinib equivalent PNP, 10 ml of methanol was used to dissolve it. This suspension/solution was dialyzed against a 1.2 pH buffer for two hours in a diffusion setup using gelatin paper as the equilibrium membrane. A 2millilitre sample was taken out at predetermined intervals, and the absorbance at 323 nm was measured. Dialysed against a 7.4 pH buffer after two hours, 2 millilitres of material were taken out, and the absorbance was measured at 323 nm[24].

Anti-cancer activity principle

It is now commonly acknowledged that tetrazolium salt reduction is a trustworthy method for assessing cell proliferation. Metabolically active cells reduce the yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl2)-2,5-diphenyl tetrazolium bromide) to produce reducing equivalents like NADH and NADPH, partially due to the action of dehydrogenase enzymes. Using spectrophotometric techniques, the resultant intracellular purple formazan may be solubilised and measured. The assay evaluates both the proliferation rate of cells and the reduction in cell viability induced by metabolic activities leading to necrosis or apoptosis

Procedure for Determining Cell Cytotoxicity:

A 96-well flat-bottom microplate was used to seed the cells, and they were kept there for the entire night at 37˚C, 95% humidity, and 5% CO2. Various concentrations of pure drug and DST PNPS (100, 50, 25, 12.5, 6.25, 3.125µg/ml) were administered. Another 48 hours were spent incubating the cells. Following two PBS washes, each well contained 20µL of the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) staining solution. The plate was then incubated at 37˚C. Following a 4-hour period, 100µL of DMSO was introduced into each well to dissolve the formazan crystals, and the absorbance was measured using a microplate reader at 570 nm.

Formula:

Surviving cells % = Mean OD of test compound / Mean OD of negative control ×100 Using graph pad prism version 5.1, we calculated the IC50 values of Dasatinib and F1 Apoptosis by Flow cytometer.

Procedure:

A 96-well flat-bottom microplate was used to seed the cells, and they were kept there for the entire night at 37˚C, 95% humidity, and 5% CO2. Various concentrations of pure drug and DST PNPS (100, 50, 25, 12.5, 6.25, 3.125µg/ml) were administered. Another 48 hours were spent incubating the cells. Following two PBS washes, each well contained 20µL of the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) staining solution. The plate was then incubated at 37˚C. Following a 4-hour period, 100µL of DMSO was introduced into each well to dissolve the formazan crystals, and the absorbance was measured using a microplate reader at 570 nm[25].

Comparative pharmacokinetic drug release study

Using the models developed by Koresmeyer and Poppa and Higuchi, a comparative drug release study is conducted.

Higuchi's Model:

Diffusion-based drug release from matrix devices is explained as follows The classical diffusion of Higuchi equation: Q = [DЄ /ι (2A – ЄCs) Cst] ½

where,

Q = Amount of drug release at time ‘t’

D = Diffusion coefficient of the drug in the matrix. A = Total amount of drug in unit volume of matrix. Cs = Solubility of drug in the matrix.

Є = Porosity of the matrix. ι= Tortuosity.

T = Time (hrs at which q amount of drug is released).

Above equation can be simplified as

if we assume that ‘D’, ‘Cs’ and ‘A’ are constant. Then equation becomes

Q = kt1/2

The drug was released via diffusion mechanism when the data is analysed using equation, i.e. cumulative drug release versus square root of time, which produces a straight line. The incline equals 'K'. The aforementioned equation is verified using in vitro release data in an Excel sheet model. or the KinetDS3 program was employed.

Koresmeyer Equation / Peppas Model

The release data was also fitted to the well-known exponential equation, which is frequently used to characterise the drug release behaviour from polymeric systems, in order to investigate the mechanism of drug release from the liposomal solution[26].

Stability studies.

To calculate the amount of medication lost from the polymeric nanoparticles, stability experiments were conducted. For the optimized formulations produced by each approach, a stability analysis was conducted. The formulations were separated into three sets of samples and kept at room temperature (29˚C) in a refrigerator at 2℃. The results of the in vitro drug release research and entrapment efficiency after storage at room temperature and 2°C were used to predict the stability of the formulations. Samples were estimated every month for a duration of three months[27].

RESULTS

Estimation of Dasatinib

UV – visible Spectrophotometric method

The was subjected to UV-Visible spectrophotometric method showed λmax at 323nm using methanol as solvent.

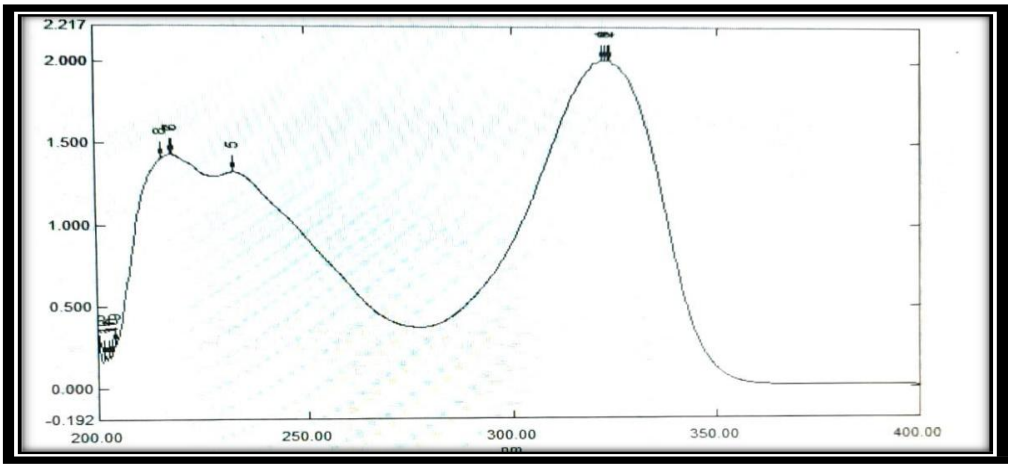


Fig.01. λmax of Dasatinib at 323nm

Solubility of nanoparticles

Table 01: Solubility of polymeric nanoparticles in different solvents.

Solvent name	Nanoparticles solubility
Distilled water	Insoluble
Methanol	Soluble
Ethanol	Insoluble
DMSO	Sparingly soluble

FT- IR analysis

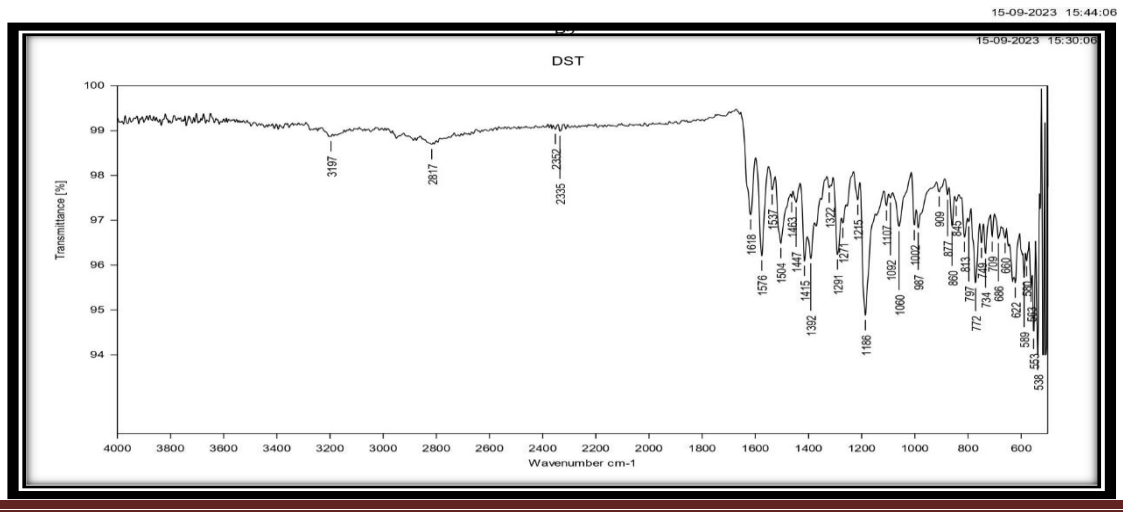


Figure 02: FT-IR Spectrum of Dasatinib pure drug

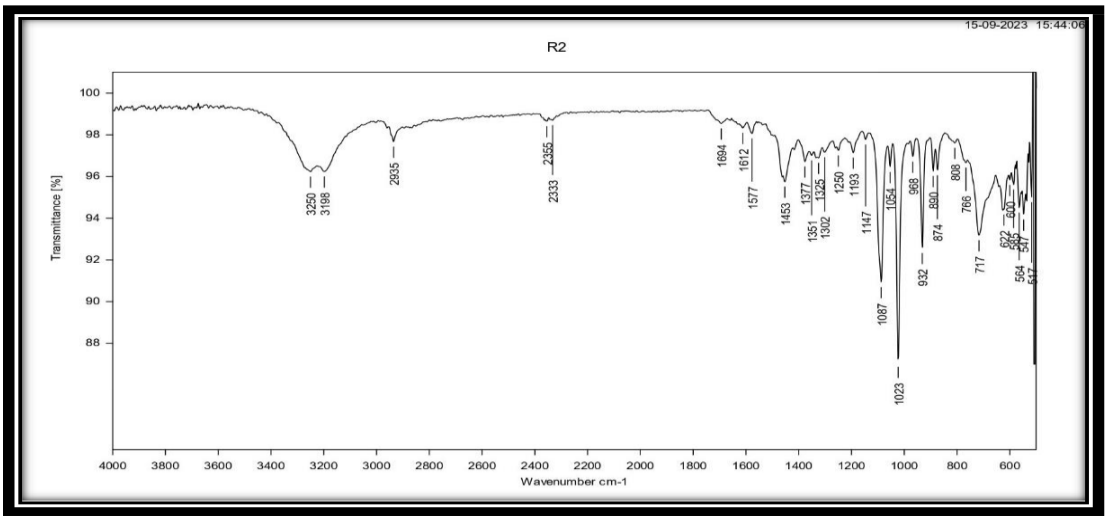


Figure 03: FT-IR Spectrum of Formulation NP1

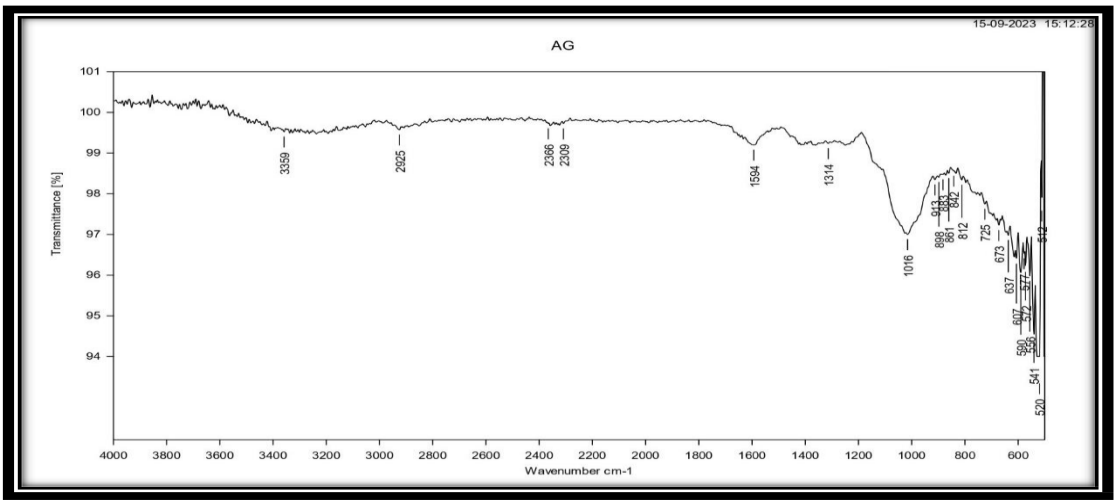


Figure 04: FT-IR Spectrum of NP3 formulation

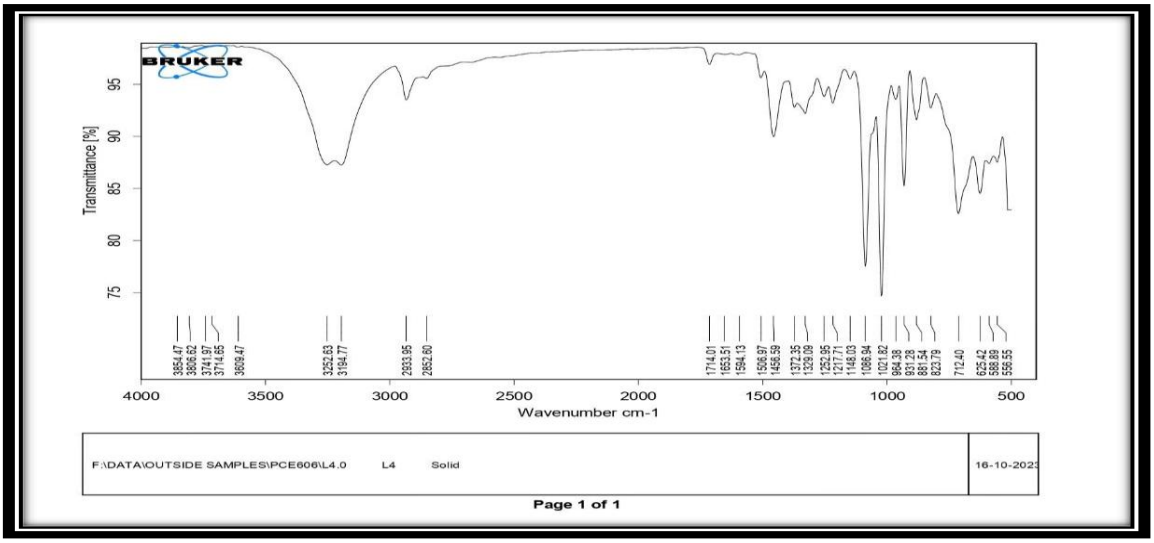
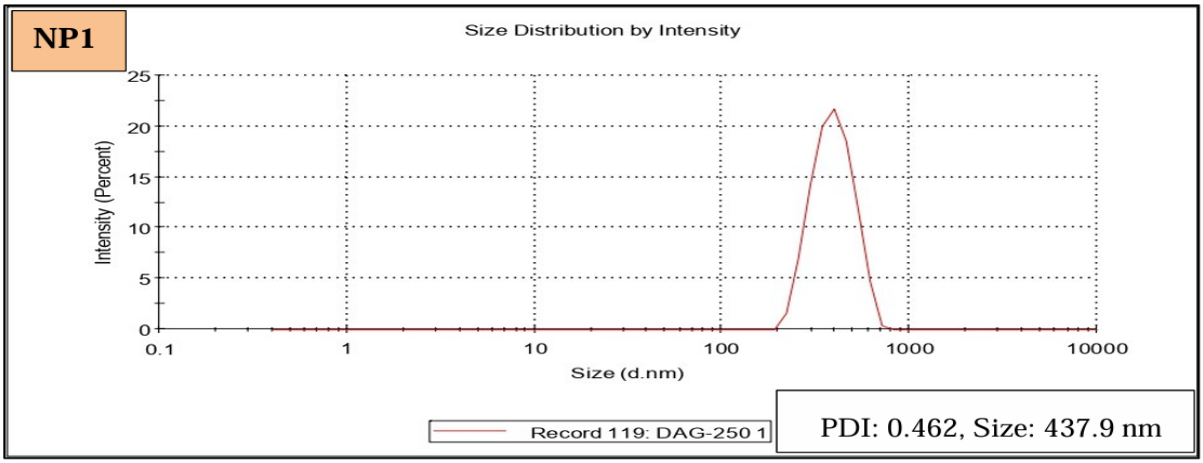


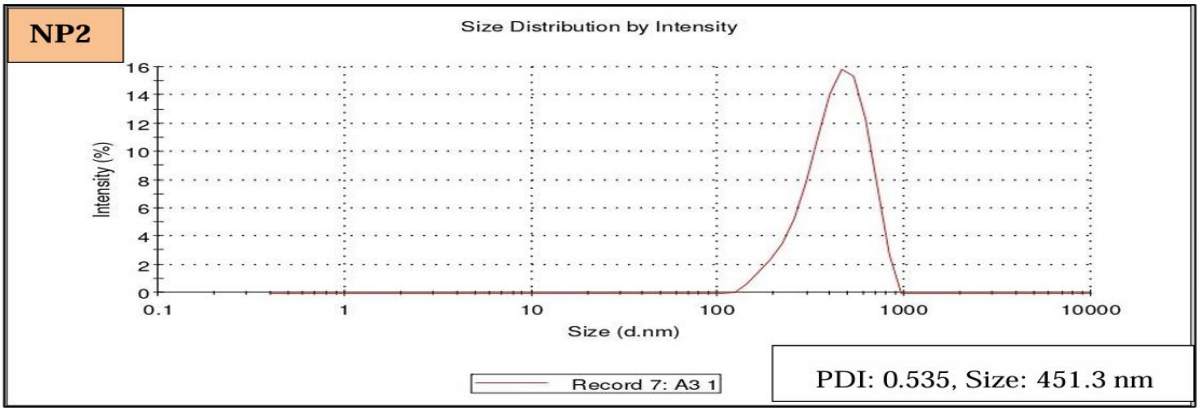
Figure 05: FT-IR Spectrum of NP5 formulation

Table 02: FT – IR interpretation of drug and nanoparticles.

SL.NO	IR SPECTRUM	PEAKS (cm^{-1})	FREQUENCY	GROUPS	VIBRATIONS
1	Dasatinib	1566	700-1500	Aromatic C=C	Bending
		3049	3030	Aromatic C – H	Stretching
		1156 1216.4	1250 - 1000	Aromatic C – H	In plane bending
		1452	1500 – 1400	Aromatic C – C	Stretching
		1400.04	1400 – 1000	iphaticC – F	Stretching
2	NP1	3414.35 3353.3 3213.24	3550 – 3200	Alcohol O – H Intermolecular bonded	Stretching
		2935	2916 – 2936	CH_2	Stretching
		1693.53	1900 – 1600	Carbonyl C = O	Stretching
		1444	1500 – 1400	romaticC – C	Stretching
3	NP3	3250	3350 – 3070	-OH	Stretching
		3198	3516 – 2936	-NH	Stretching
		1694	1842 – 1266	CONH	Stretching
		2935	3105 – 2000	romatic C-CL	Stretching
4	NP5	3250	3350 – 3070	H bonded N –H	Stretching
		2935	2916 – 2936	CH2	Stretching
		1326	1342 – 1266	Aromatic	Stretching
				amine	
		1256		C – N	

Determination of Particle size, PDI and Zeta potential.





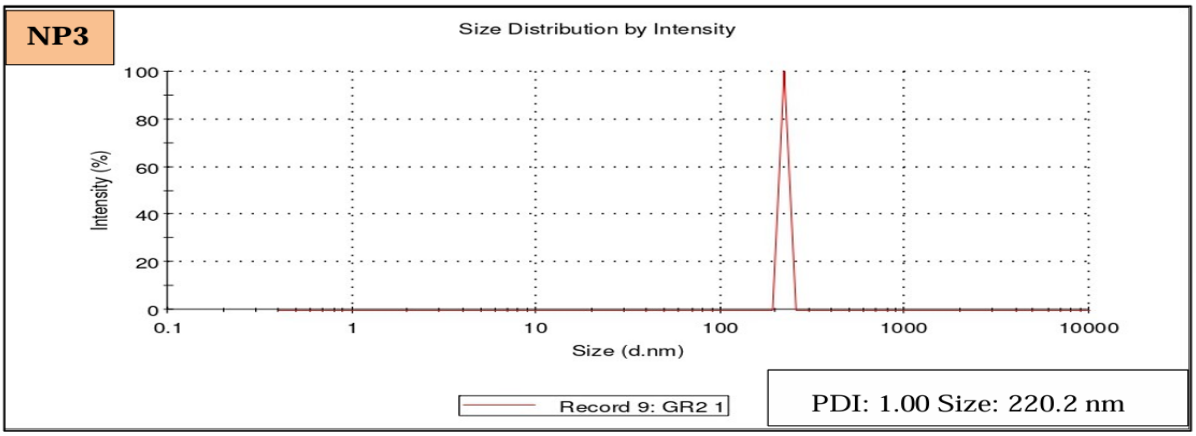
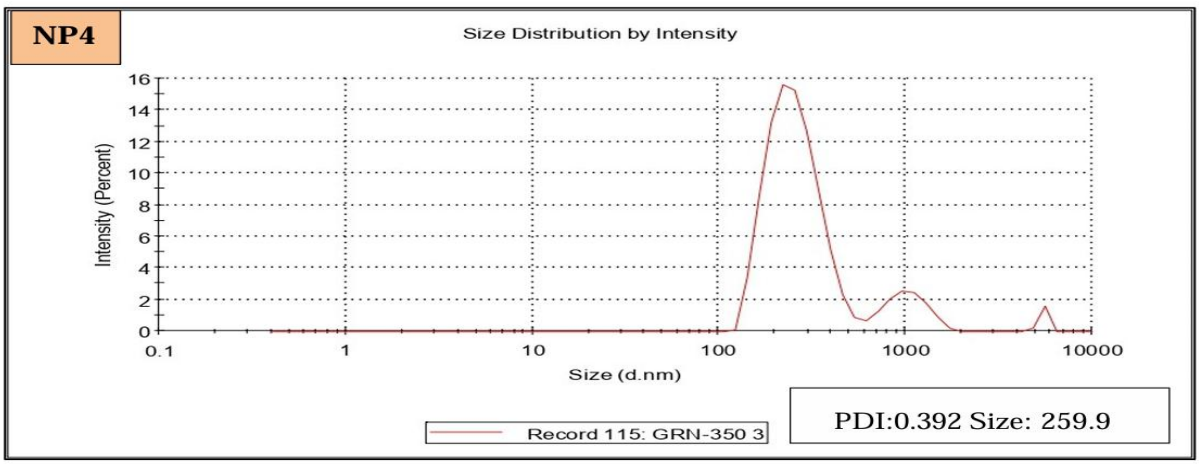
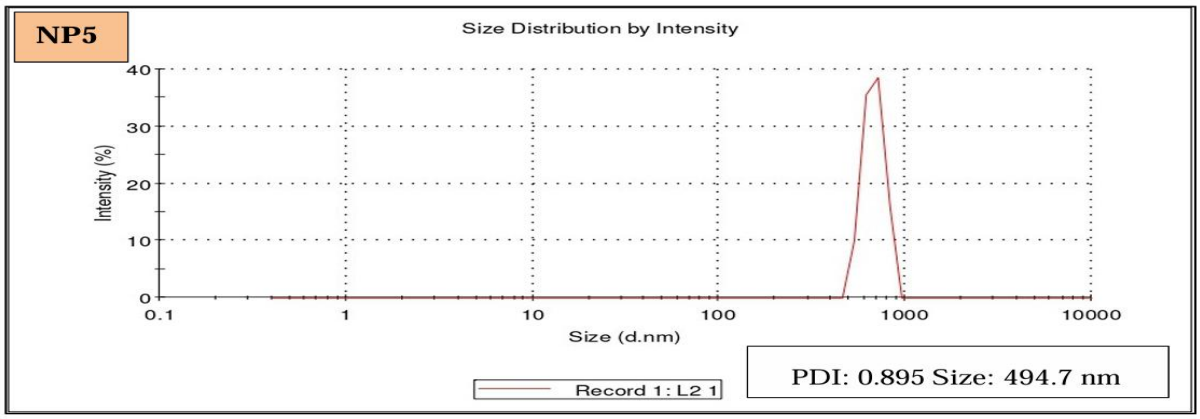


Fig.06. Size distribution intensity of Polymeric Nanoparticles 1. NP1 2. NP2 3. NP3





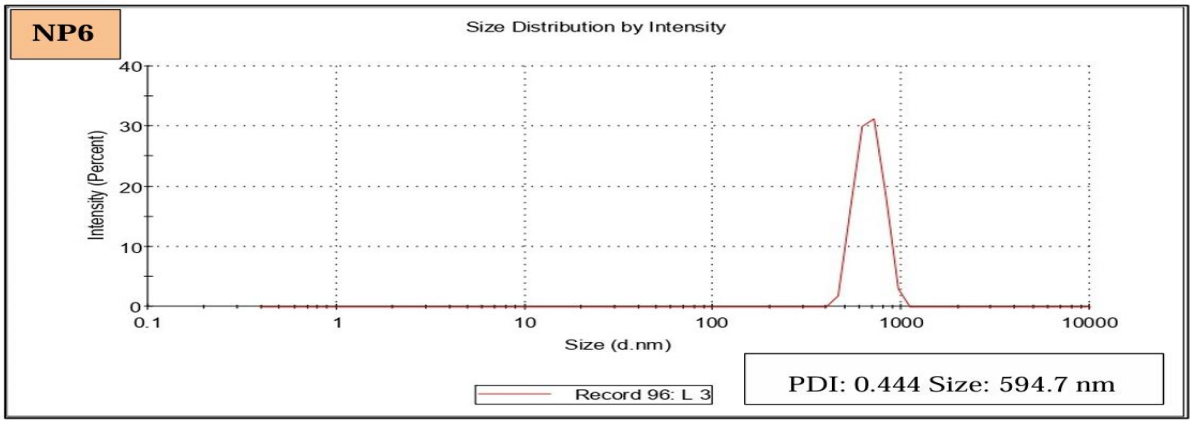


Fig.07. Size distribution graph of formulation 1.NP4 2. NP5 3.NP6

Zeta Potential

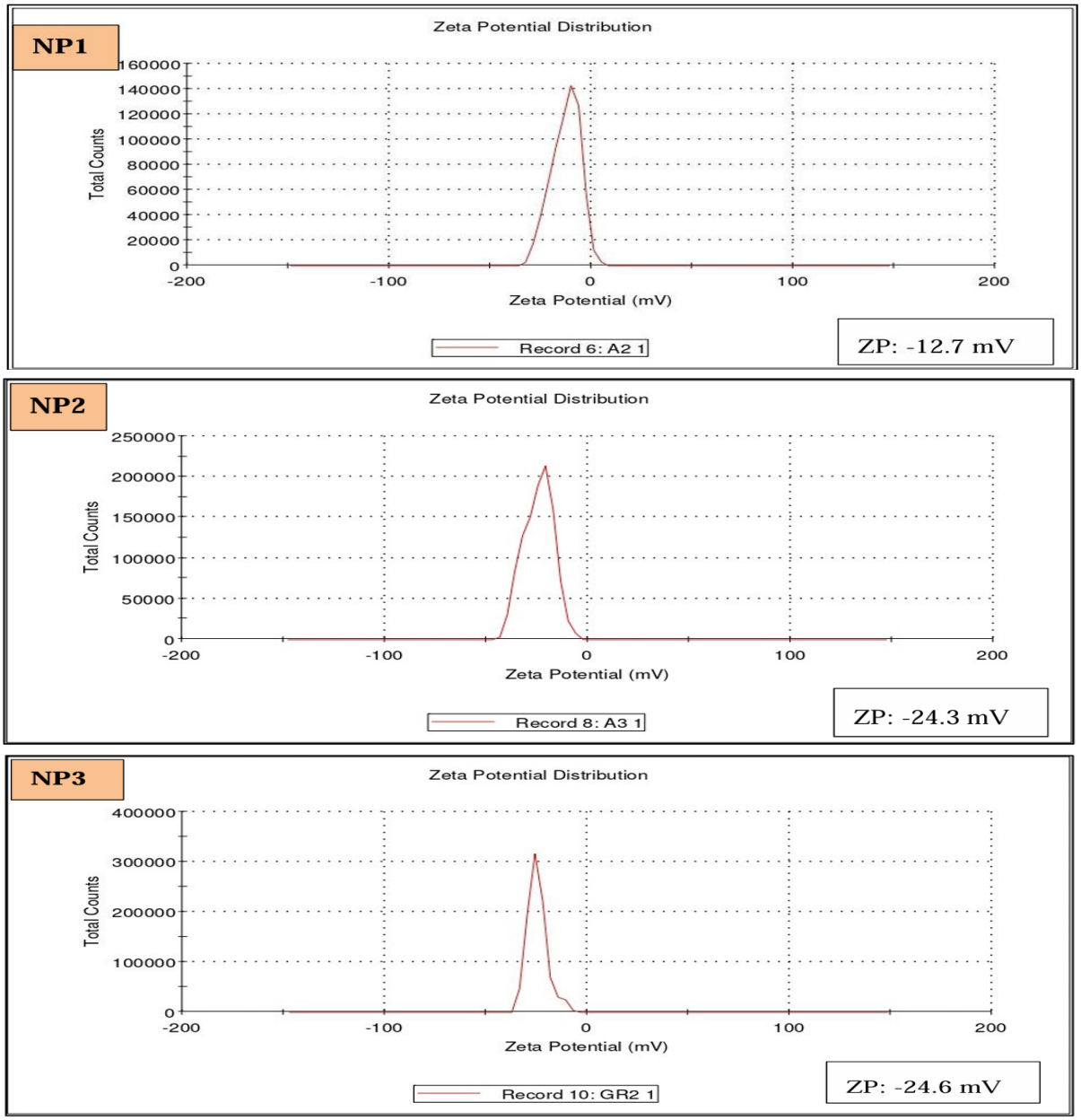


Fig.08. Zeta potential graph of formulations1.NP1 2. NP3 3.NP3

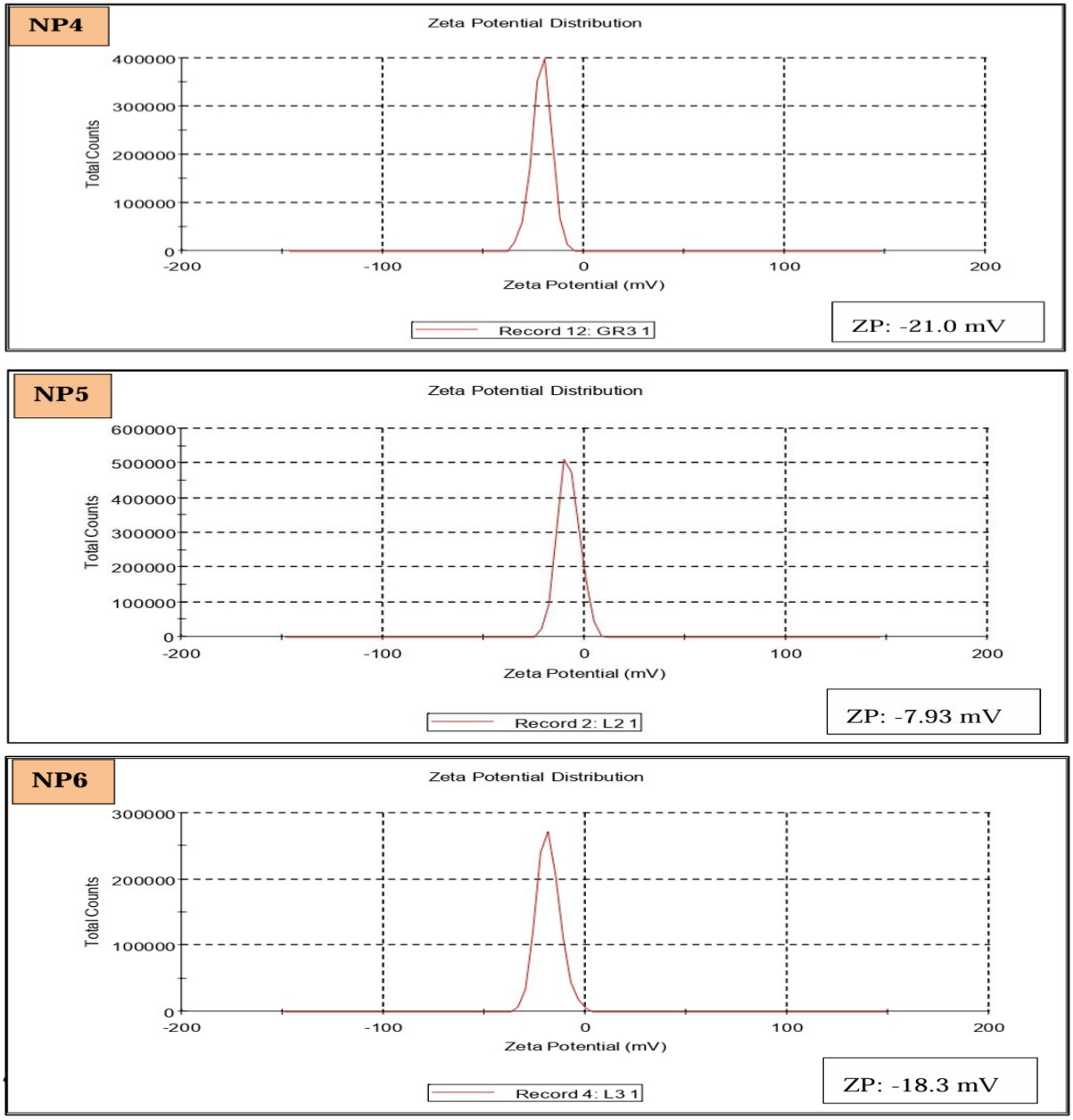


Fig.9. Zeta potential graph of formulations1.NP4 2. NP5 3.NP6

Table no 03: Measurement values of particle size, PDI, Zeta potential and DEE

Serial no	Formulation	Particle size in nm	PDI	Zeta potential in mV	% of Drug content	% of DEE of Efficiency
01	NP1	437	0.462	-12.7	64%	73.7%
02	NP2	451.3	0.535	-24.3	72%	77%
03	NP3	259.9	1.00	-24.6	82%	65%
04	NP4	220.2	0.392	-21.0	88%	84%
05	NP5	494.7	0.895	-7.93	60%	73%
06	NP6	601.4	0.444	-18.3	82%	82%

Differential scanning calorimetry.

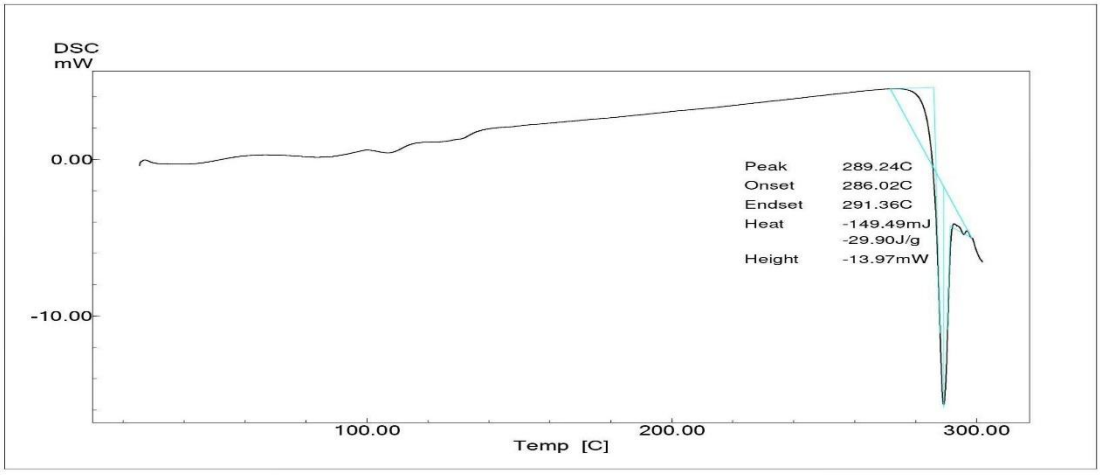


Fig.10. DSC thermograph of Dasatinib

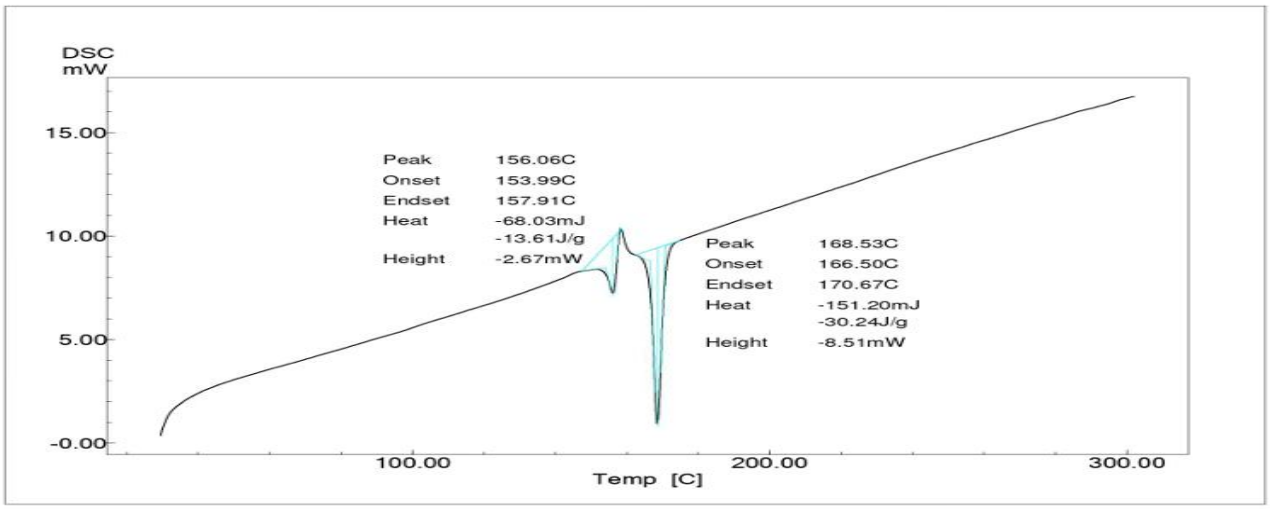


Fig.11. DSC thermograph of Formulation NP4

IN-VITRO DRUG RELEASE STUDY

Table no:04. In-vitro diffusion profile of formulation NP1 to NP6 in 1.2pH and 7.4 pH buffer

Time in Hrs	NP_1	NP_2	NP_3	NP_4	NP_5	NP_6
01	7.15	5.975	4.34	1.78	9.61	5.67
02	14.18	11.47	8.88	3.82	18.57	9.98
03	16.85	14.92	11.99	4.97	22.66	13.49
04	17.54	16.18	14.99	5.08	24.10	15.71
05	20.27	21.11	18.47	5.97	27.87	18.08
06	23.23	25.22	22.47	7.27	32.19	20.07
07	25.87	29.42	24.95	9.17	34.64	22.25
08	27.98	32.40	30.17	10.18	355.72	25.01
10	38.92	59.76	52.92	24.23	54.32	41.95
12	42.57	70.27	73.23	40.27	57.48	45.07
14	53.48	72.12	75.32	44.21	60.18	49.09
18	56.11	74.11	78.31	47.12	64.13	53.02
24	61.25	78.23	81.16	50.12	68.32	58.12
48	79.05	85.02	93.13	69.75	76.25	81.6

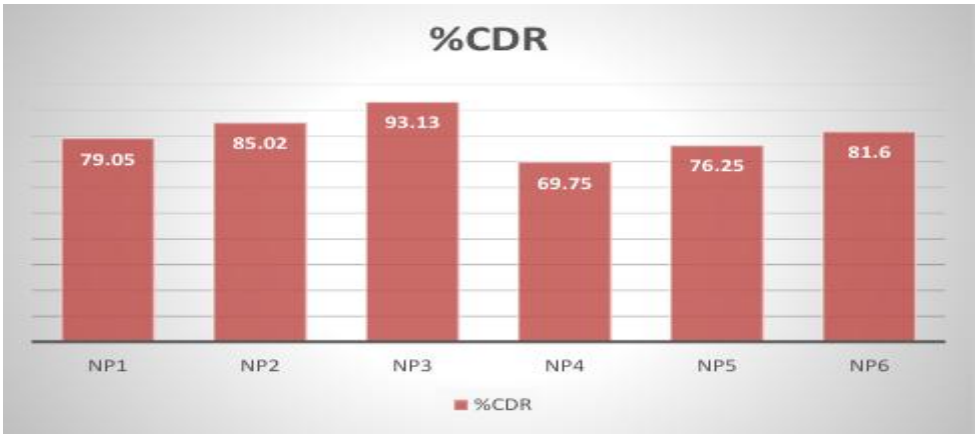


Fig.12: Graph of % cumulative drug release for formulation NP1-NP6

Table 05: Pharmacokinetic study of polymeric nanoparticles

Formulation code	Zero Order. (R2 value)	Higuchi.(R2 value)	Peppas. (R2value)	Best Fit Model.
NP1	0.9744	0.9366	0.9743	Both zero order and peppas
NP2	0.9895	0.8522	0.9939	Peppas
NP3	0.931	0.8389	0.9902	Peppas
NP4	0.9968	0.5563	0.9852	Zero order
NP5	0.9744	0.9366	0.9743	Both zero order and peppas
NP6	0.9310	0.8389	0.9902	Peppas

ANTI-CANCER ACTIVITY

MTT Assay K562

Table no:06: MTT Assay of NP4 vs K562 cell line.

Concentration in µg/ml	% of cell death	
	DST	Formulation NP4
Untreated	100	100
3.125	39.8	28.83
6.25	53.49	32.02
12.5	60.64	64.71
25	65.82	80.56
50	68.72	88.61
100	75.02	89.79

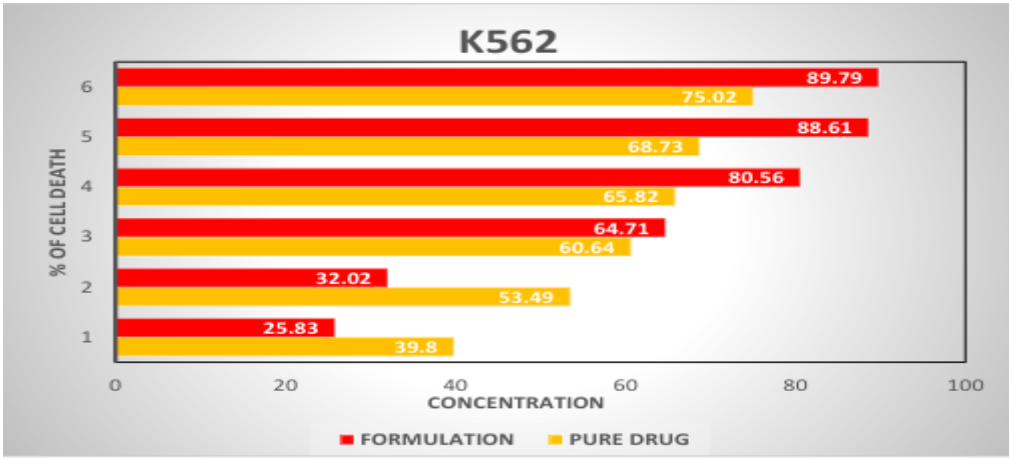


Fig .13. Percentage cell Death of K562 cell line vs NP4

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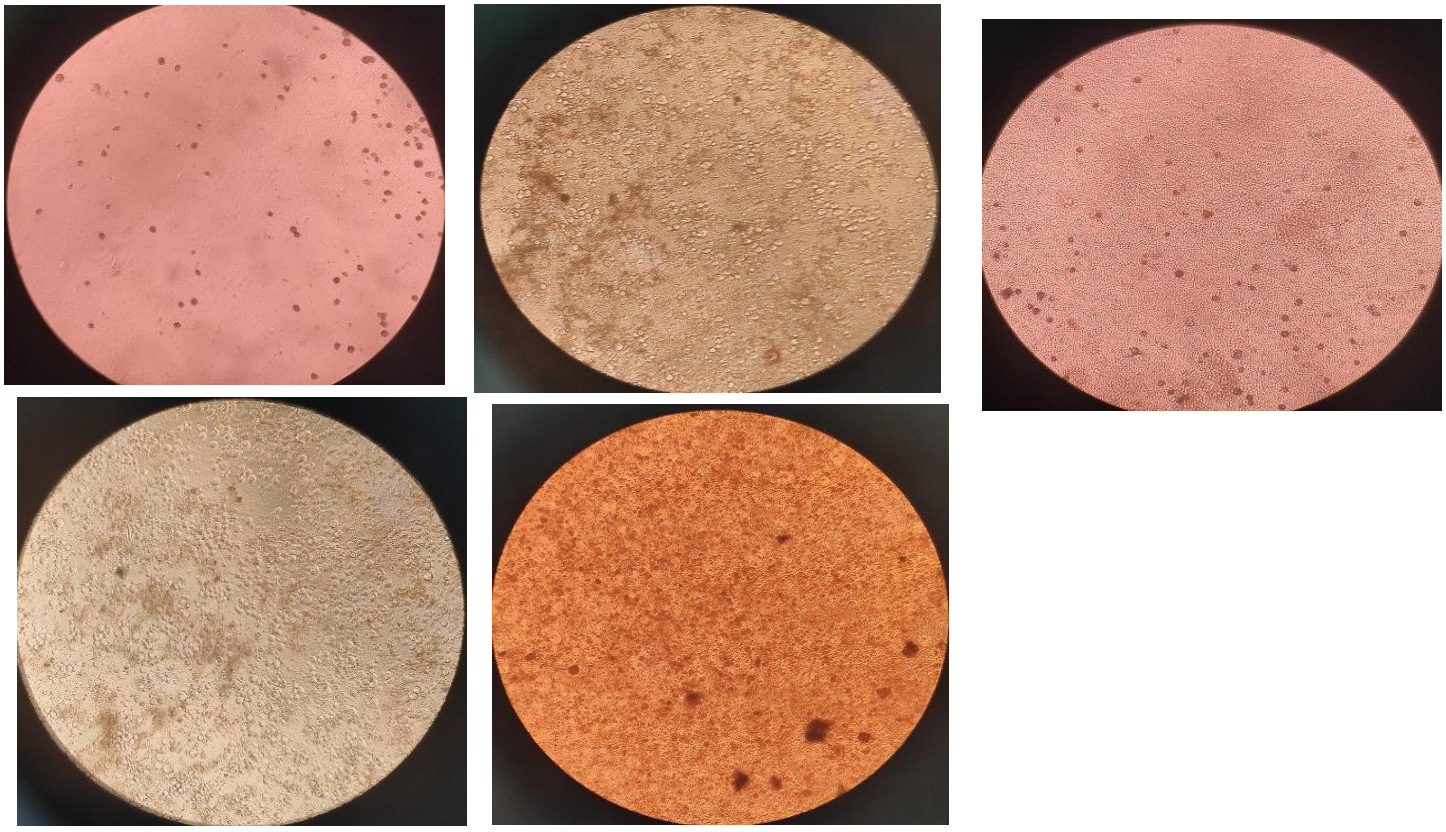

Fig 14. K562 cell line

MTT Assay MDA MB 231

Table no:07: MTT Assay of NP3 vs MDA MB 231 cell line.

Concentration in µg/ml	% of cell death	
	Pure drug	Formulation NP4
Untreated	100	100
3.125	51.75	30.52
6.25	53.75	38.59
12.5	55.87	59.4
25	56.59	74.41
50	58.39	86.28
100	88.4	90.76

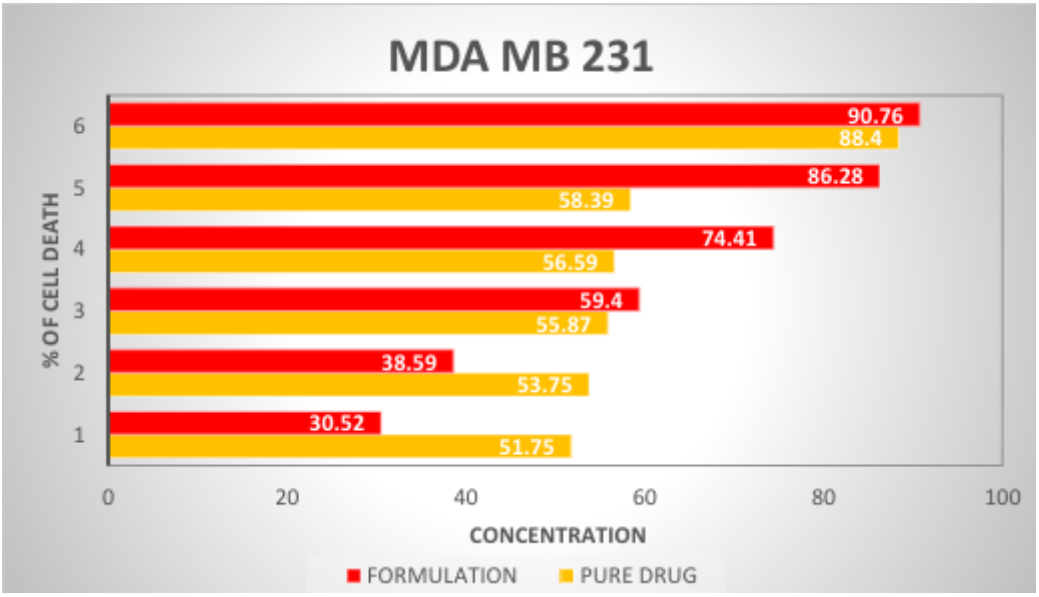

Fig 15. Percentage cell Death of K562 cell line vs NP4

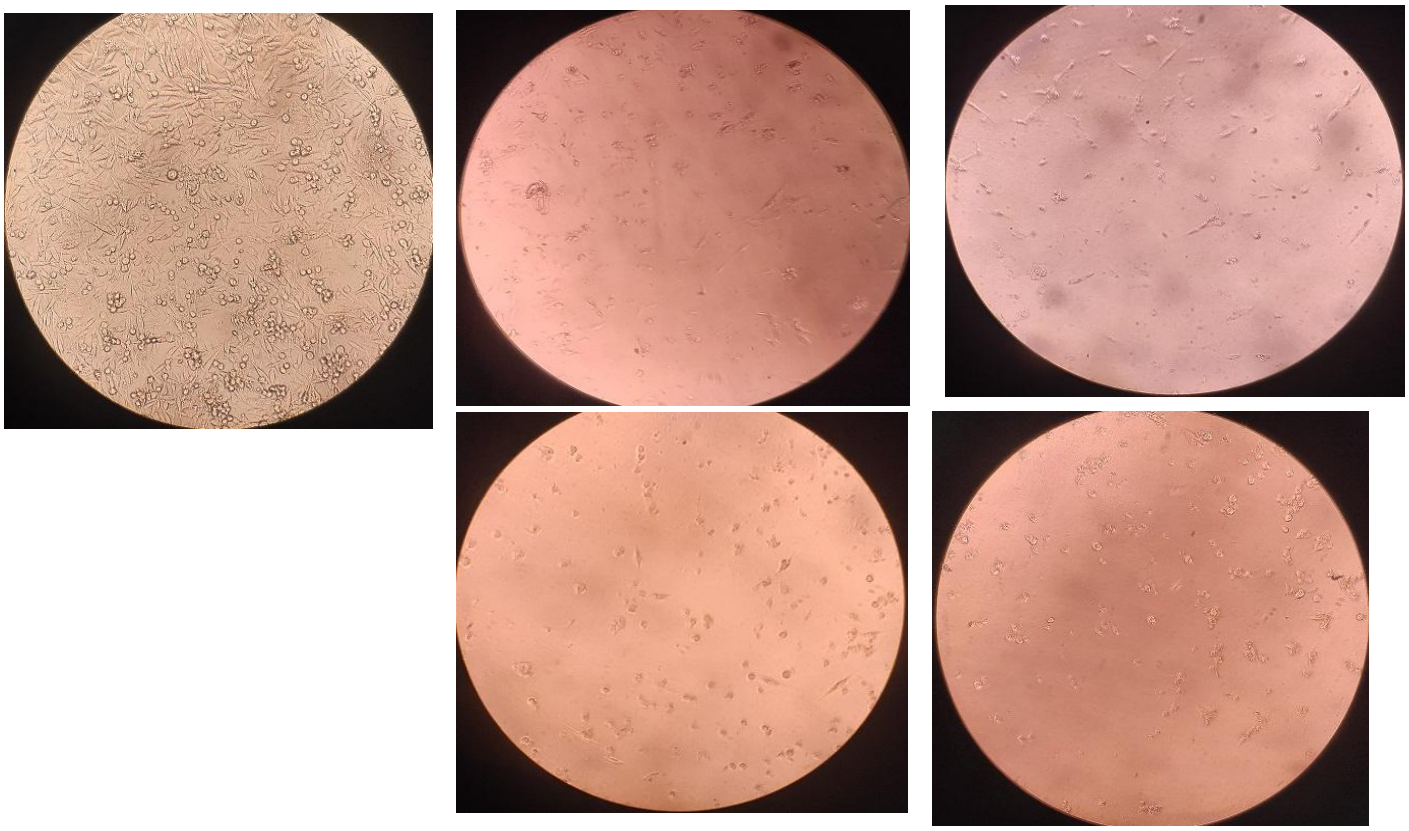

Fig 16. K562 cell line

DISCUSSION

The goal of the current work is to create polymeric nanoparticles. These were made using biodegradable polymers that the formulation was optimized and subsequently evaluated for its anticancer efficacy .loaded with dasatinib using the nanoprecipitation method. The formulation was optimized and subsequently evaluated for its anticancer efficacy. The formula was characterized for a number of parameters based on existing literature.

Estimation of Drug

The sample was tested for solubility in accordance with Dasatinib's profile; Dasatinib was discovered to be soluble in methanol and only slightly soluble in ethanol, DMF, and distilled water.

UV- Visible Spectrophotometric method

Dasatinib was quantified using a UV-Visible spectrophotometric method, with the absorption maximum (λmax) observed at 323 nm, as illustrated in Figure 01.

% Drug content

Displayed the drug content data for each formulation, along with the percentage of drug content for formulations F1, F2, F3, F4, F5, and F6 of polymeric nanoparticles. Drug content rises as polymer concentration rises.

Characterization of polymeric nanoparticles FT – IR studies

The FT – IR spectrum estimation was performed to verify for a pure sample of Dasatinib, NP1, NP3 & NP5 nanoparticles. the result is exhibited in Table: 02. The peak values which were present in the Dasatinib graph was approximately appeared in Formulations NP1, NP3, NP5.

Particle size analysis.

The procedures for determining particle size, PDI, and Zeta potential were explained. Dasatinib polymeric nanoparticles were discovered to have a drug entrapment efficiency ranging from 60.8% to 82.2%. This range indicates that an increase in polymer concentration led to an increase in drug entrapment. It demonstrates that improved trapping was discovered as the polymeric content rose. The outcome displayed in Table No. 03.

Transmission electron microscope.

The NP3 polymeric nanoparticles were characterized using High-Resolution Transmission Electron Microscopy (HR-TEM) along with Selected Area Electron Diffraction (SAED) analysis. The uneven form of the nanoparticles was observed Moreover; it was discovered that the particle portion was nonporous since light could only travel through it minimally. The fact that the particles resembled grains and lacked planes and patterns indicates that they are in an amorphous state.

The SAED section of a sample was calculated for the diffraction where the d spacing value of 0.2 was confirmed to have less intensity peaks; as a result, it was determined that NP4 was amorphous in nature. Using a scanning electron microscope.

NP4 nanoparticles were characterized using Field Emission Scanning Electron Microscopy (FE-SEM) coupled with Energy Dispersive X-ray Spectroscopy (EDX). The particles exhibited irregular morphology, smooth surface texture, and a nonporous structure.

Differential scanning calorimetry.

The DSC characterization has been explained. While the drug's peak, which corresponds to its melting point of 168°C, was found to be absent, the DSC thermograph of NP3 revealed endothermic peaks at 151.91°C and 237.13°C, indicating that the nature of the nanoparticle was found to be amorphous, involving uniform dispersion of drug in the matrix. The DSC thermograph of NP4 revealed polymers with a melting point closer to the glass transition temperature, and a peak at 168°C, which corresponds to the drug's melting point. These findings indicate that the nanoparticles are amorphous in nature and do not exhibit any interaction.

In vitro drug release studies

The polymeric nanoparticle drug release investigation was conducted utilizing the diffusion method, also known as the equilibrium dialysis membrane method. All formulations were evaluated in a pH 1.2 buffer for two hours, followed by testing in pH 7.4 phosphate buffer for the remainder of the study period. The obtained results are summarized in Table 04.The percentage of cumulative drug release at 4 hours was 30.29% for all formulations (NP1, NP2, NP3, NP4, NP5, and NP6). Within 48 hours, NP3 exhibited the maximum drug release in this formulation, while NP4 displayed the lowest drug release at the same time.

In vitro drug release kinetics

To confirm which model fit the data the best, a kinetic investigation was conducted. in accordance with § 4.4.10. Using KinetdS3 software, the release data were submitted to the Koresmeyer and Higuchi model. Table 05 displays the values of the computed regression coefficients. Peppa's model was determined to be the best.

In vivo release studies

The anti-cancer study was executed in MDA MB 231 & K562 cells. MTT assay was performed to evaluate the penetration capacity of optimized NP4 formulation against on MDA MB 231 and K562 cell lines. Pure drug Dasatinib and optimized formulation NP4 showed dose dependent death of MDA MB 231 & K562 cell lines. At low concentration i.e, 3.125 & 6.25 optimized formulation did not show much cell death as compare to pure drug.

As the concentration pure drug and NP4 was increased there was significant death of cells compare with pure drug against NP4 there was increased cell death for NP4 as compare to pure drug. This was due to the nanoform which effectively taken up by the MDA MB 231 & K562 cells as compare to pure drug. As results exhibited in table no 06 & 07.

Stability studies

Accelerated stability studies were carried out by storing the nanoparticle suspension or solution at room temperature and under refrigerated conditions (2–8 °C) for a period of three months. The findings are shown in Table 15. In the meantime, the in vitro drug release was found to be in the range of 59.99% to 76.23% at room temperature and 60.23% to 73.42% at 2°C, respectively. The percentage drug entrapment efficiency (DEE) ranged from 39.2% to 83% at room temperature, and from 39.5% to 82.33% under refrigerated conditions (2 °C).

CONCLUSION

- The dasatinib-loaded polymeric nanoparticle was effectively created with the use of Arabic gum, gum rosin, and locust bean gum as polymers in the Nanoprecipitation process.
- The prepared polymeric nanoparticles were characterized by various parameters and obtained significant results.
- To observe any potential interactions between the chosen medicine and polymer, FT-IR experiments were conducted. The results of the FT-IR analysis show that there was no drug-polymer interaction.
- All formulations showed high drug entrapment efficiency. Among the different formulation, NP4 and NP5 formulations were showed maximum drug entrapment efficiency.
- Particle size of prepared polymeric nanoparticles obtained in the range between 220.2 nm to 604.1 nm. Thus, particle sizes obtained were according to drug polymer ratio. NP4 has showed a least particle size.
- Surface morphology of prepared polymeric nanoparticle obtained from SEM & TEM has showed a smooth with irregular shape.
- It was discovered by DSC characterization that nanoparticles are naturally amorphous.
- The results of an in vitro drug release research demonstrated that the produced polymeric nanoparticles continued to release drugs for 48 hours. The release kinetics for different formulations has showed both peppas model and zero order kinetics, and thus it concludes that the mechanism was non-Fickian diffusion.
- From the results of in vivo Cell-line studies, Optimized NP4 formulation was effectively taken up by MDA MB 231 cell line and K562 cell line as compared to pure drug Dasatinib hence these was significant death of cells.
- Stability studies showed that there were no much changes in the parameters within 3 months.

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