



Treatment of non-small cell lung cancer

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ABSTRACT:the nanoparticle-based therapeutic system has excellent popularity due to its immense advantages over the conventional drug delivery system. The bypassing of biological barriers, enhancement of target specificity, reduction of serious side effects, and many more advantages having for nanoparticle-based drug delivery system. Polymeric nanoparticles such as poly (lactic acid), poly(lactic-co-glycolic) acid, gelatin, albumin, chitosan, and polycaprolactone have gained popularity in use due to their particle size, shape, biocompatibility, and controlled release profile.

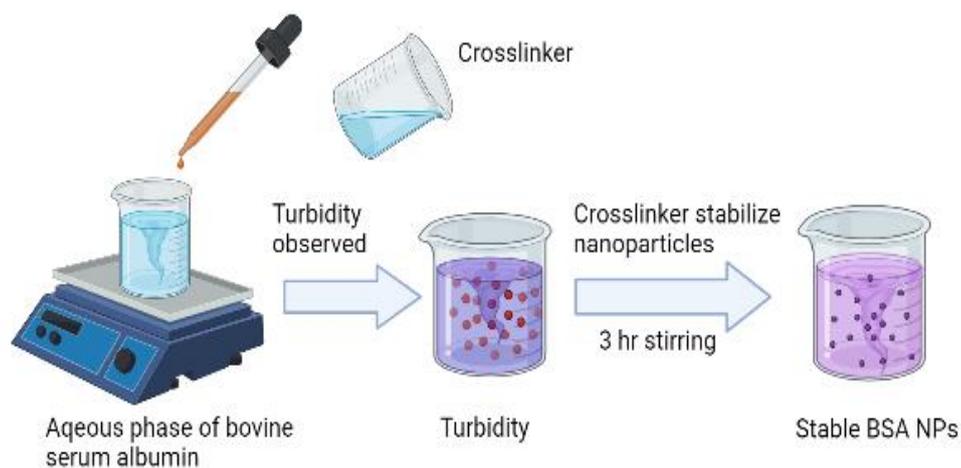
Keywords: Polymeric nanoparticles such as poly (lactic acid), poly(lactic-co-glycolic) acid, gelatin, albumin, chitosan, and polycaprolactone

Introduction: The tumor is a broad term for a wide group of diseases that can affect any part of the body and it is one of the deadliest diseases worldwide. Another term for cancer is cancer, neoplasms, etc. According to the WHO report 2021, nearly 10 million deaths cases were observed in 2020 due to cancer and out of which lung cancer mortality was found to be 1.80 million.

SCC is a type of NSCLC that acquires approximately 30% of lung cancers. This type of tumor is usually grown near the bronchi in the central part of the lung and its growing ability relatively slowly doubled

Objectives

- i. Analytical method development by using RP-HPLC
- ii. To formulate and optimize the dasatinib-loaded BSA nanoparticles by the desolvation method
- iii. Characterization of prepared dasatinib-loaded BSA NPs
- iv. To calculate encapsulation efficiency
- v. To study the *in-vitro* drug release study of dasatinib-loaded BSA NPs
- vi. Stability study



Desolvation method.

Material and instruments

Different materials and instruments used in the current experimentation were detailed respectively along with brief information (supplier name, model no., place, country, etc.)

Sr. No	Chemical	Uses	Manufacturer
1	BSA	Nanocarrier	HiMedia Laboratories Pvt. Ltd., Mumbai, India
2	Acetone	Desolvating agent	EMPLURA Merk, USA
3	EDC.	Crosslinker	Sigma-Aldrich Chemicals Co., MO, USA
4	Dasatinib	ActivePharmaceutical Ingredient	TCI, Tokyo, Japan

List of chemicals

Table 4.2List of Instruments

Sr No	Equipment	Model Number	Manufacturer
1	Weighing Balance	ME-204	M/s Mettler Toledo, USA
2	HPLC	1260	Agilent 1260 Infinity 2
3	pH meter	FP-20	M/s Mettler Toledo, USA
4	Differential Scanning Calorimetry	DSC-3 STAR	M/s Mettler Toledo, USA
5	Infrared Spectroscopy	Bruker Alpha 2	Bruker, USA
6	Zetasizer	Nano ZS	Malvern Panalytical Ltd. UK
7	Magnetic Stirrer	RT 10	IKA, Germany
8	Centrifuge	Eppendorf 5810 R	Merk KGaA, Darmstadt, Germany
9	SEM	Gemini SEM 500	Zeiss, Germany
10	TEM	2100F	JEOL, Tokyo Japan
11	Incubator shaker	-	REMI Sales and Engineering, Pvt. Ltd., Mumbai

Methodology

Analytical method development using reverse-phase high-pressure chromatography

Instrumentation

The quantification of the dasatinib was done using a reverse phase high pressure liquid chromatographic instrument (RP-HPLC) (Agilent 1260 Infinity 2) provided with a Luna C18 column (250 mm × 5mm; 5 µm particle size).

Chromatographic condition

Quantification of dasatinib was carried out by Luna C-18 column (250 mm × 5mm; 5 µm). ACN and phosphate buffer 7.0 pH were used as a mobile phase with an isocratic elution mode, an analytical method was developed with some modifications to the previously reported method

Preparation of primary standard solution

The standard solution was prepared by solubilizing 10mg of dasatinib in 10ml methanol into the volumetric flask. The prepared stock solution was sonicated for 15-20 min and stored in a refrigerator at 4°C.

Further stock solutions are prepared with a proper dilution of the primary stock solution with the mobile phase.

Linearity and construction of calibration curve

The linearity of the peak area response was analyzed by measuring six different concentrations in the range of 50ng/ml to 5mg/ml. The 10µl from each dilution was injected 3 times into the column. The drug elutes were quantified at 323 nm and the corresponding chromatogram was obtained.

Accuracy

The % recovery was determined by using three different control samples of dasatinib. The control samples of LBS, MBS, and HBS levels for dasatinib were 0.4, 0.5, and 0.6 respectively prepared in triplicate for each concentration % recovery, and % RSD was calculated.

Precision

Relative standard deviation termed obtained at intra-day and inter-day termed as precision. The different known concentrations were used for the quantification of inter-day and intra-day precision. (27)

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ of dasatinib were evaluated from the slope (S) of the standard calibration curve and y-intercept of regression equation taking as a standard deviation (σ) using the following equations (1) and (2) respectively.

$$LOD = \frac{3.3\sigma}{S} \dots\dots\dots (1)$$

$$LOQ = \frac{10\sigma}{S} \dots\dots\dots (2)$$

4.2.2 The preparation of dasatinib-loaded BSA NPs

Dasatinib-loaded BSA NPs were synthesized by the desolvation method. Briefly, BSA was dissolved into 4ml purified water and dasatinib was dissolved in the organic solvent (dimethylsulfoxide/dimethylformamide) in a separate beaker. Afterward both the solution mixed and kept for incubation at 200rpm for 1-3hr for interaction between polymer and drug. The desolvating agent (Acetone) was added to the incubated mixture dropwise with a constant flow rate and stirring speed at 1000

4.2.3 The optimization of formulation

The dasatinib-loaded BSA NPs optimized by varying concentrations of polymer: drug ratio and volume of a desolvating agent by considering the particle size, PDI, zeta potential, and encapsulation efficiency as dependent factors (28)The drug: polymer ratio directly affected the efficiency of encapsulation as well as particle size. The desolvating agent was selected on the basis of drug properties which affect the drug loading capacity(29).

4.2.4The characterization ofprepared dasatinib-loaded BSA NPs

4.2.4.1 The particle size, zeta potential, and particle size distribution

The particle size, zeta potential, and particle size distributionwere determined using the zeta sizer apparatus (Nano ZS Malvern Instruments Limited, Grovewood Road, Malvern, Worcestershire, UK)(30). Zetasizer works on the principle of dynamic Light Scattering in which the light backscatter at an angle of 173°. The prepared dasatinib-BSA nanoparticle sample was diluted with water in a ratio of 1:100.

4.2.4.2 Scanning electron microscopy

The surface morphology, as well as the shape of dasatinib-loaded BSA NPs, were evaluated with the help of Field emission scanning electron microscopy (GeminiSEM 500-8203017168) performed at electron high tension (EHT) of 4.00 kV. The images were captured by dispersing particle solution dropwise on silicon wafers and kept to dry, after that placed onto to carbon strip coated with gold under a vacuum for 15 min.

4.2.4.3 Transmission electron microscopy

Sample preparation for TEM was prepared by diluting the 10-20 µl of formulation in milli-Q water. Nanoparticle suspension of 15 µl was added to copper grids. The copper grids were mounted with samples and then allowed to dry by exposing them to dry filtered air. Finally, TEM (JEM 2100; JEOL Ltd., Tokyo, Japan) was used to take images with an accelerating voltage of 100 kV.

4.2.4.4 Differential Scanning Calorimetry (DSC)

DSC (DSC-3, M/s Mettler Toledo, Switzerland)wasused for the evaluation of the physical form of the prepared NP. Briefly, a sample equivalent to 1-3mg was put into the sample pan (silver) with tightly sealed. Samples were heated and scanned with an increasing temperature rate of 10°C/min over the range of 40°C to 400°C along with a 50ml/min flow rate of nitrogen gas. The analysis was done with an empty pan as a reference, and nitrogen purging was used to maintain the inert atmosphere.

4.2.4.5 Infrared Spectroscopy (IR)

The ATR spectrum of BSA was recorded on an FTIR spectrophotometer (FT-IR Spectrometer, BRUKER IFS-55, Switzerland) using the ATR method.ATR spectrum of dasatinibwascarried outfor the evaluation of the drug's structural characteristics before loading and after loading the dasatinib into the nanocarrier system.

4.2.5 Determination of dasatinib encapsulation efficiency

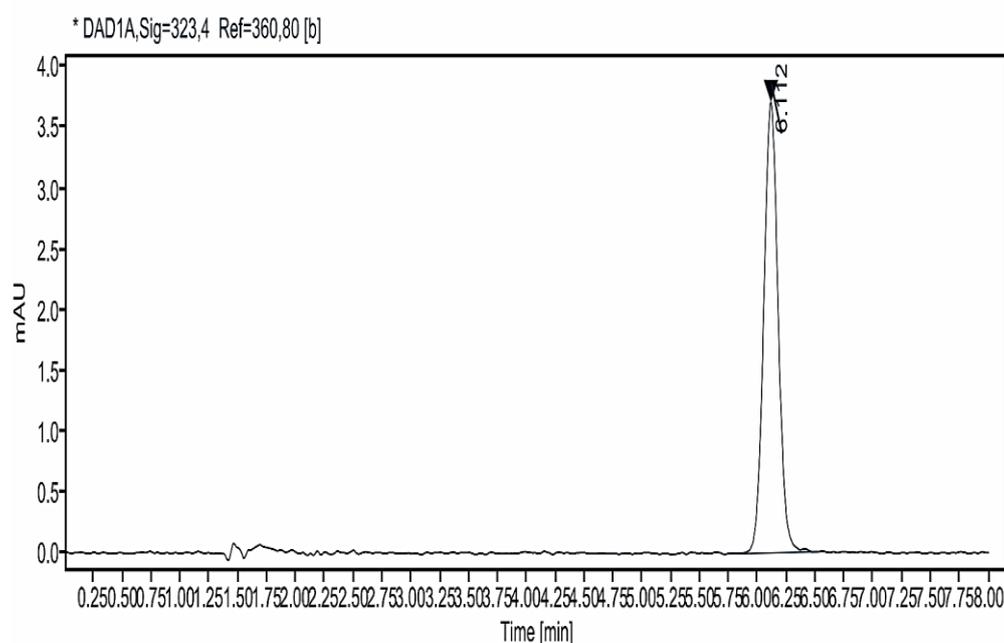
Dasatinib encapsulation efficiency (EE) means the determination of the amount of drug entrapped within the NPs. The quantification of encapsulation efficiency was done by the indirect method.

$$\% EE = \frac{\text{Intital quantity of drug added} - \text{drug in supernatent}}{\text{Intital quantity of drug added}} \times 100$$

4.2.6 In-vitro drug release studies of dasatinib-loaded albumin nanoparticles

In vitro drug release study of pure dasatinib and dasatinib-loaded BSA NPs was executed by employing the cellulose dialysis membrane of the molecular weight cut-off (MWC 8-12kDa). The dialysis membrane was activated by soaking overnight into the release medium (Buffer 7.2 pH) before its use. by dilution were made for quantification using a validated RP-HPLC method(31).

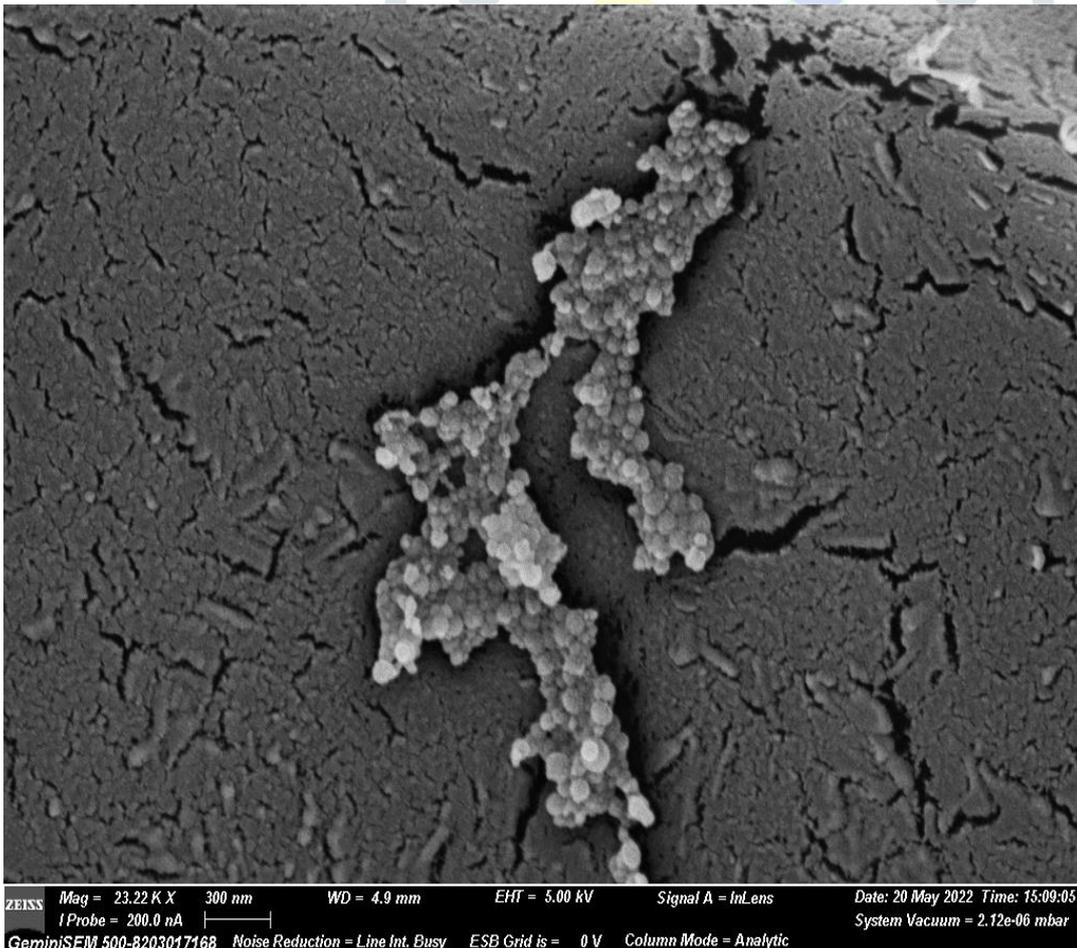
RESULT AND DISCUSSION



The RP-HPLC chromatogram of dasatinib



Particle size report of optimized (F10) formulation



Scanning electron microscope image of drug-loaded BSA NPs

Conclusion :In the present study, we have successfully formulated the dasatinib-loaded BSA NPs using the desolvation method. The selection of desolvating agent, crosslinker was based on the drug properties as well as by considering the compatibility of biological conditions. The optimized nanoparticles were characterized by SEM, and TEM which is clearly showing the formulated NPs are spherical which easily translocate through the respiratory tract as well as a trans-endocytosis process. DSC and FTIR also clarify the physical form and drug-polymer compatibility respectively. The dasatinib-loaded BSA NPs have a sustained release profile with first order kinetic which could be helpful for the reduction of side effect of dasatinib.

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