International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1583-1589 www.ijprajournal.com ISSN: 2249-7781

Treatment of non-small cell lung cancer

1.Sanjay G. Bhuktare, 2. Sourabh S. Jadhav, 3.Sandip P. Vajir, 4.Kalyan S. Deothankar

1. I/C Principal 2. Lecturer 3. Lecturer 4. Lecturer
Shri shivaji institute of pharmacy, basmat road parbhani.

Submitted: 15-05-2023 Accepted: 30-05-2023

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

I. 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 diseasesworldwide. 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

Desolvation method.

Objectives

i. Analytical method development by using RP-HPLC

ii. To formulate and optimize the dasatinib-loaded BSA nanoparticles by the desolvation method

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1583-1589 www.ijprajournal.com ISSN: 2249-7781

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

II. 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.)

List of chemicals

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

Table 4.2 List 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 PanalyticalLtd. UK
7	Magnetic Stirrer	RT 10	IKA, Germany
8	Centrifuge	Eppendorf 5810 R	MerkKGaA, Darmstadt, Germany
9	SEM	GeminiSEM 500	Zeiss, Germany
10	TEM	2100F	JEOL, Tokyo Japan
11	Incubator shaker	-	REMI Sales and Engineering, Pvt. Ltd., Mumbai

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1583-1589 www.ijprajournal.com ISSN: 2249-7781

III. METHODOLOGY

Analytical method development using reverse-phasehigh-pressure chromatography

Instrumentation

The quantification ofthe dasatinibwas 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 ofdasatinib was carried out by Luna C-18 column (250 mm × 5mm; 5 µm). ACN and phosphate buffer 7.0 pH were usedas amobile phase with an isocratic elution mode, an analytical method was developed with some modificationstothe previously reported method

Preparation of primary standard solution

The standard solution was prepared by solubilizing10mg of dasatinibin 10ml methanol into the volumetric flask. The prepared stock solution was sonicated for 15-20 minand stored in a refrigerator at 4^{o}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 measuringsix 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 werequantified at 323 nm and the corresponding chromatogram was obtained.

Accuracy

The % recovery was determined by usingthree different control samples of dasatinib. The control samples of LBS, MBS, and HBS levels for dasatinib were0.4,0.5, 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 LOQof 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.

$$\text{LOD} = \frac{3.3\sigma}{S} \quad \ldots\ldots\ldots (1)$$

$$\text{LOQ} = \frac{10\sigma}{S} \quad \ldots\ldots\ldots (2)$$

4.2.2 Thepreparation ofdasatinib-loaded BSA NPs

Dasatinib-loaded BSA NP sweresynthesized by the desolvation method. Briefly,BSAwas dissolvedinto 4mlpurified waterand dasatinibwasdissolved 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)wasadded to the incubated mixture dropwise with a constant flow rate and stirring speedat1000

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^{o}. 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

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1583-1589 www.ijprajournal.com ISSN: 2249-7781

(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 10ºC/min over the range of 40ºC to 400ºC 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.

4.2.5Determination of dasatinib encapsulation efficiency

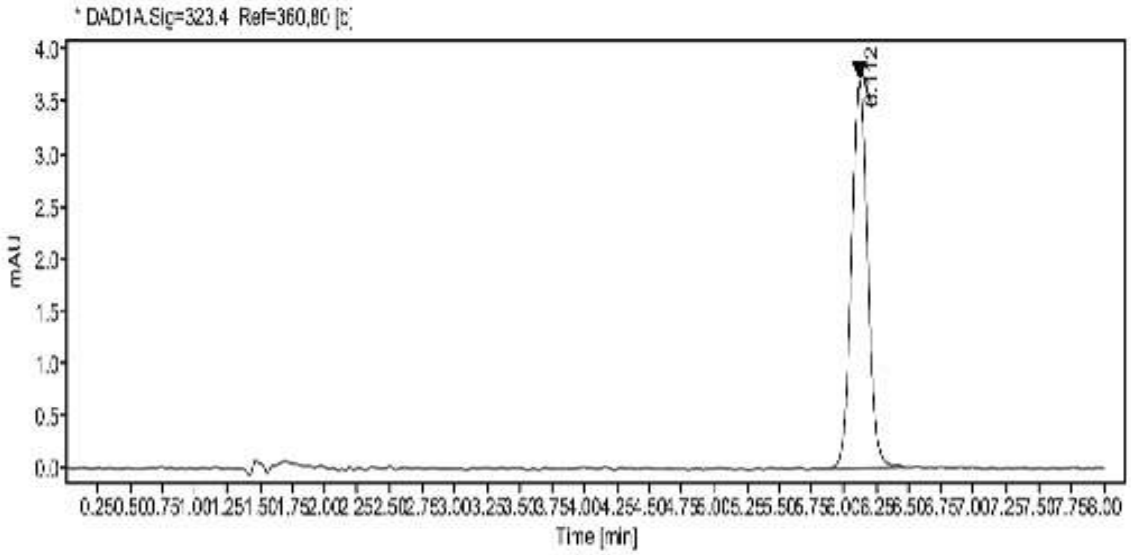
Dasatinib encapsulation efficiency (EE)means the determination of the amount of drug entrapped within the NPs. The quantification of encapsulation efficiency was done by the indirect method.

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

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

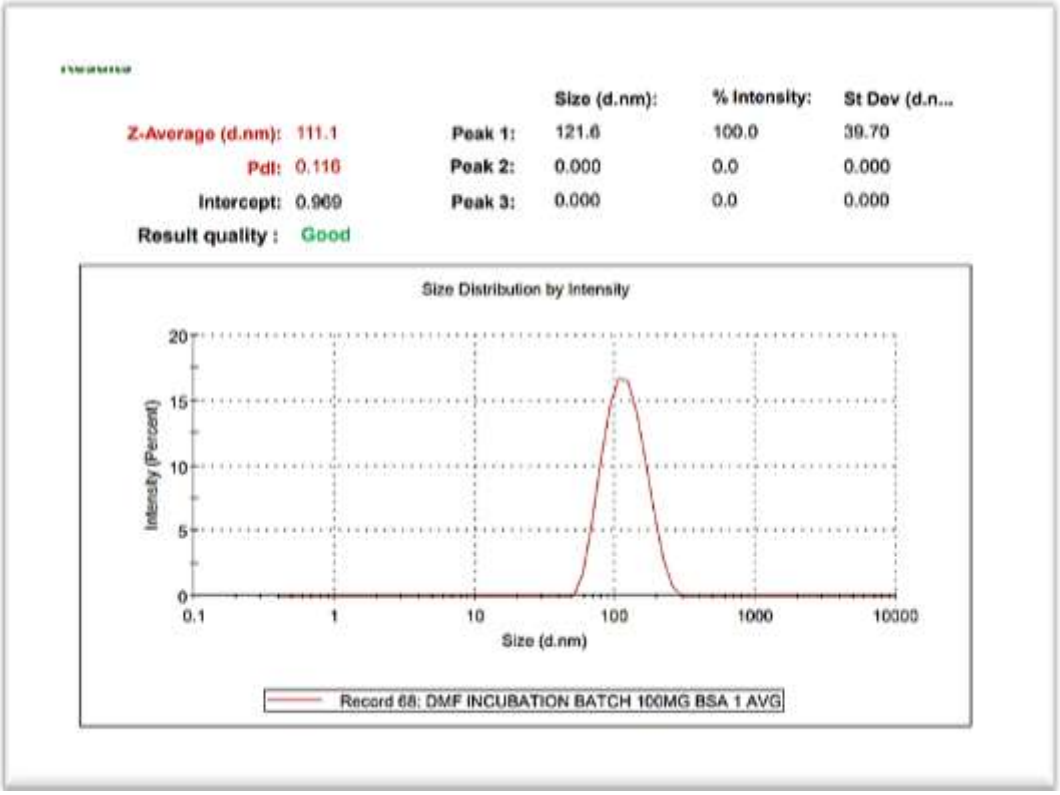
In vitrodrug release study of pure dasatinib and dasatinib-loaded BSA NPswas executed by employing the cellulose dialysis membrane of the molecular weight cut-off (MWC 8-12kDa).The dialysis membrane was activated by soaking overnight into the release medium (Buffer 7.2 pH) before itsuse.by dilution weremadefor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

IV. RESULT AND DISCUSSION

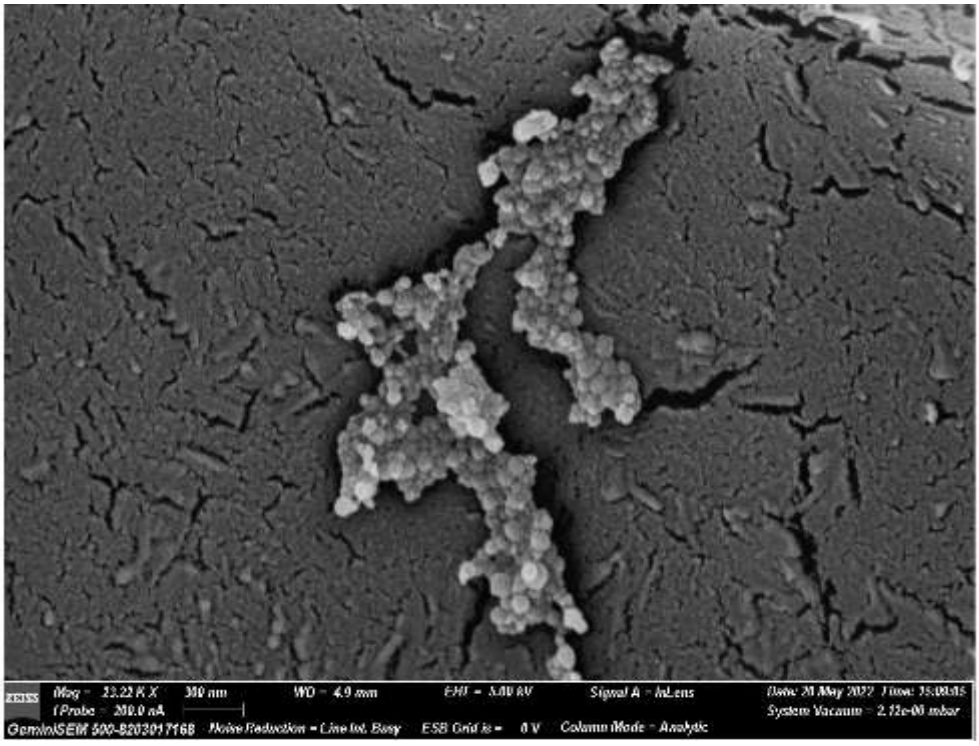


The RP-HPLC chromatogram ofdasatinib

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1583-1589 www.ijprajournal.com ISSN: 2249-7781



Particle size report of optimized (F10) formulation



Scanning electron microscope image of drug-loaded BSA NPs

International Journal of Pharmaceutical Research and Applications
Volume 8, Issue 3 May-June 2023, pp: 1583-1589 www.ijprajournal.com ISSN: 2249-7781

V. 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.

REFERENCE:

[1]. Cersosimo RJ. Lung cancer: A review [Internet]. Am J Health-Syst Pharm. Available from: https://academic.oup.com/ajhp/article-abstract/59/7/611/5157990

[2]. Types of Lung Cancer: Small Cell and Non-Small Cell Lung Cancer Types [Internet]. [cited 2022 Jan 12]. Available from: https://www.webmd.com/lung-cancer/lung-cancer-types

[3]. Lemjabbar-Alaoui H, Hassan OUI, Yang YW, Buchanan P. Lung cancer: Biology and treatment options. Vol. 1856, BiochimicaetBiophysicaActa - Reviews on Cancer. Elsevier B.V.; 2015. p. 189–210.

[4]. Minna JD, Roth JA, Gazdar AF. Focus on lung cancer. Vol. 1. 2002.

[5]. Ansari J, Palmer DH, Rea DW, Hussain SA. Anti-Cancer Agents in Medicinal Chemistry.Vol. 9. 2009.

[6]. Thomas A, Rajan A, Giaccone G. Tyrosine Kinase Inhibitors in Lung Cancer. Vol. 26, Hematology/Oncology Clinics of North America. 2012. p. 589–605.

[7]. Yamaoka T, Kusumoto S, Ando K, Ohba M, Ohmori T. Receptor tyrosine kinase-targeted cancer therapy. Vol. 19, International Journal of Molecular Sciences.MDPI AG; 2018.

[8]. Bahman F, Pittalà V, Haider M, Greish K. Enhanced anticancer activity of nanoformulation of dasatinib against triple-negative breast cancer. J Pers Med. 2021 Jun 1;11(6).

[9]. Zhao H, Xin Z, Liu G, Schaefer VG, Falls HD, Kaszubska W, et al. Discovery of tetralincarboxamide growth hormone secretagogue receptor antagonists via scaffold manipulation. J Med Chem. 2004 Dec 30;47(27):6655–7.

[10]. Obr A, Röselová P, Grebeňová D, Kuželová K. Real-time analysis of imatinib- and dasatinib-induced effects on chronic myelogenousleukemia cell interaction with fibronectin. PLoS One. 2014 Sep 8;9(9).

[11]. Zhang M, Tian J, Wang R, Song M, Zhao R, Chen H, et al. Dasatinib Inhibits Lung Cancer Cell Growth and Patient Derived Tumor Growth in Mice by Targeting LIMK1. Front Cell Dev Biol. 2020 Dec 4;8.

[12]. Su J, Zhou Y, Pan Z, Shi L, Yang J, Liao A, et al. Downregulation of LIMK1-ADF/cofilin by DADS inhibits the migration and invasion of colon cancer. Sci Rep. 2017 Mar 30;7.

[13]. Dong C, Li B, Li Z, Shetty S, Fu J. Dasatinib-loaded albumin nanoparticles possess diminished endothelial cell barrier disruption and retain potent anti-leukemia cell activity [Internet]. Available from: www.impactjournals.com/oncotarget

[14]. Phan C, Jutant EM, Tu L, Thuillet R, Seferian A, Montani D, et al. Dasatinib increases endothelial permeability leading to pleural effusion. European Respiratory Journal. 2018;51(1).

[15]. Babu A, Templeton AK, Munshi A, Ramesh R. Nanoparticle-based drug delivery for therapy of lung cancer: Progress and challenges. Vol. 2013, Journal of Nanomaterials. 2013.

[16]. Korashy HM, Rahman AFMM, Kassem MG. Dasatinib. In: Profiles of Drug Substances, Excipients and Related Methodology. Academic Press Inc.; 2014. p. 205–37.

[17]. Srivastava A, Prajapati A. Albumin and functionalized albumin nanoparticles: Production strategies, characterization, and target indications. Vol. 14, Asian Biomedicine.Sciendo; 2020. p. 217–42.

[18]. Elzoghby AO, Samy WM, Elgindy NA. Albumin-based nanoparticles as potential controlled release drug delivery systems. Vol. 157, Journal of Controlled Release. 2012. p. 168–82.

[19]. Joshi M, Nagarsenkar M, Prabhakar B. Albumin nanocarriers for pulmonary drug delivery: An attractive approach. Vol. 56, Journal of Drug Delivery Science and Technology.Editions de Sante; 2020.
[20]. John TA, Vogel SM, Minshall RD, Ridge K, Tiruppathi C, Malik A. Evidence for the role of alveolar epithelial gp60 in active transalveolar albumin transport in the rat lung. J Physiol. 2001;533(2):547–59.
[21]. Hoogenboezem EN, Duvall CL. Harnessing albumin as a carrier for cancer therapies. Vol. 130, Advanced Drug Delivery Reviews.Elsevier B.V.; 2018. p. 73–89.
[22]. Amighi F, Emam-Djomeh Z, Labbafi-Mazraeh-Shahi M. Effect of different cross-linking agents on the preparation of bovine serum albumin nanoparticles. Journal of the Iranian Chemical Society. 2020 May 1;17(5):1223–35.
[23]. Xu J, Huang Y, Zhao J, Wu L, Qi Q, Liu Y, et al. Cofilin: A Promising Protein Implicated in Cancer Metastasis and Apoptosis. Vol. 9, Frontiers in Cell and Developmental Biology.Frontiers Media S.A.; 2021.
[24]. Sozer SC, Egesoy TO, Basol M, Cakan-Akdogan G, Akdogan Y. A simple desolvation method for production of cationic albumin nanoparticles with improved drug loading and cell uptake. J Drug DelivSci Technol. 2020 Dec 1;60.
[25]. Ahmad J, Akhter S, Rizwanullah M, Amin S, Rahman M, Ahmad MZ, et al. Nanotechnology-based inhalation treatments for lung cancer: State of the art. Vol. 8, Nanotechnology, Science and Applications.Dove Medical Press Ltd; 2015. p. 55–66.
[26]. Ramachandra B, Naidu NVS. VALIDATION OF RP-HPLC METHOD FOR ESTIMATION OF DASATINIB IN BULK AND ITS PHARMACEUTICAL DOSAGE FORMS [Internet].International Journal of Pharmacy and Biological Sciences. Available from: www.ijpbs.comorwww.ijpbsonline.com
[27]. Chaudhari VS, Borkar RM, Murty US, Banerjee S. Analytical method development and validation of reverse-phase high-performance liquid chromatography (RP-HPLC) method for simultaneous quantifications of quercetin and piperine in dual-drug loaded nanostructured lipid carriers. J Pharm Biomed Anal. 2020 Jul 15;186.
[28]. Jahanban-Esfahlan A, Dastmalchi S, Davaran S. A simple improved desolvation method for the rapid preparation of albumin nanoparticles. Int J BiolMacromol. 2016 Oct 1;91:703–9.
[29]. Sozer SC, Egesoy TO, Basol M, Cakan-Akdogan G, Akdogan Y. A simple desolvation method for production of cationic albumin nanoparticles with improved drug loading and cell uptake. J Drug DelivSci Technol. 2020 Dec 1;60.
[30]. Zhang Y, Zeng X, Wang H, Fan R, Hu Y, Hu X, et al. Dasatinib self-assembled nanoparticles decorated with hyaluronic acid for targeted treatment of tumors to overcome multidrug resistance. Drug Deliv. 2021;28(1):670–9.
[31]. Li Q, Yang X, Zhang P, Mo F, Si P, Kang X, et al. Dasatinib loaded nanostructured lipid carriers for effective treatment of corneal neovascularization. Biomater Sci. 2021 Apr 7;9(7):2571–83.
[32]. Kandav G, Bhatt DC, Jindal DK, Singh SK. Formulation, Optimization, and Evaluation of Allopurinol-Loaded Bovine Serum Albumin Nanoparticles for Targeting Kidney in Management of Hyperuricemic Nephrolithiasis: Formulation, optimization, and evaluation of ABNPs for kidney targeting. AAPS PharmSciTech. 2020 Jul 1;21(5).