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Preparation, Characterization, and Evaluation of PLGA Nanobubbles for the delivery of Dasatinib

Bhukya Himaja, Research Scholar, Department of Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan, India,
Email: hima.aadrika@gmail.com
Dr. Disha, Research Guide, Department of Pharmacy, Shri JJT University, Jhunjhunu, Rajasthan, India
Dr. Suresh Mukkamalla, Research Co-Guide, Department of Pharmacy, Vijaya College of Pharmacy, Hyderabad, India

Abstract

Aim:
The goal of the current study is to create and improve PLGA nanobubbles that are loaded with the anticancer drug dasatinib.

Methods:
Modified Using a double water-in-oil-in-water (W/O/W) emulsion and the solvent-diffusion-evaporation method, create dasatinib -loaded PLGA nanobubbles. The point prediction approach provided by the Box Behnken design programme was recycled to determine the ideal PLGA nanobubble composition. The prepared nanobubbles were characterized by drug loading, entrapment efficiency, particle size, *in-vitro* studies, hemolytic studies, FTIR, DSC, and stability studies.

Results:
The optimized composition showed entrapment efficiency of 80.12 ± 4.11%, drug loading of 24.34 ± 2.12%, particle size of 69.86±3.44 nm with zetapotential of -35.64±1.86 and PDI 0.22±0.005. in-vitro studies exposed highest release of 99.58% with ultrasound for up to 24h. There was no evidence of a drug-polymer interaction, according to FTIR and DSC experiments. The TEM revealed homogeneous, spherical-shaped nanoparticles that ranged in size from 30 to 40 nm. Hemolytic activity revealed good safety profile with erythrocytes and stability studies showed Particle size and entrapment effectiveness did not alter significantly.

Conclusion
Results indicate that dasatinib loaded PLGA nanobubbles possibly useful in sustaining its discharge for a prolonged era for the cure of breast cancer.

Keywords: Dasatinib, chronic myeloid leukemia, Box-Behnken, Entrapment efficacy, In-vitro drug discharge

INTRODUCTION

A drug with several targets called dasatinib is used to treat chronic myeloid leukaemia. Other cancers include lungs, prostate, and ovarian cancer may be treated with dasatinib in the future [1]. Dasatinib is a white, crystalline powder that dissolves differently in aqueous solutions depending on the pH (from 18.4 mg/ml at pH 2.6 to 0.008 mg/ml at pH 6.0). At 24 °C, it dissolves poorly in aqueous solutions, with a solubility of around 8 mg/mL. Additionally, it showed significant affinity for binding to serum proteins (>90%), had an oral bioavailability range of 34% to 14%, and the main mode of elimination was oxidative metabolism via CYP3A4. Furthermore, the undesirable side effects of dasatinib, such as bleeding, gastrointestinal problems and its capacity to make endothelial permeability more likely, which can cause peripheral edoema and pleural effusion limit its clinical use [2]. Therefore, by enhancing dasatinib's aqueous solubility, ability to target cancer, and reducing its negative adverse consequences, nanocarriers could increase its therapeutic efficacy.

Recent developed nanoscale bubbles (nanobubbles) are promising in drug delivery [3]. The leaky endothelial tissue allows the nano-sized particles to more easily escape from the vasculature surrounding the tumour Because to the so-called enhanced permeability and retention (EPR) effect, some solid tumours tend to concentrate it.[4].

Biodegradable gas-filled nanobubbles ultrasound agents made of PLGA, commonly known as PLGA nanobubbles or PLGA NBs, have recently drawn a lot of attention since they can increase ultrasound signals as well as be employed as carriers for drugs and genes [5]. One of the most crucial steps in the creation of polymeric nanobubbles is the use of a two-emulsion technique for the process/formulation variables' optimization [6]. Box-Behnken design with

three levels was utilised.in the current study. to developed and optimized dasatinib loaded nanobubbles for drug delivery system via a double emulsion method [7]. The particle size, surface charge, and shape of dasatinib nanobubbles were used to characterise their physicochemical characteristics. The drug encapsulation efficacy and release behaviors of dasatinib loaded nanobubbles were also evaluated. Finally, we attempted to investigate the cellular uptake and in vitro anti-tumor efficacy of dasatinib loaded PLGA nanobubbles.

MATERIALS AND METHODS

We bought pure dasatinib from M.S.N. Labs in Hyderabad, India. Purchased was Poly (D, L-lactide-co-glycolide) 50:50, intrinsic viscosity 0.22 dl/g. Sigma Aldrich supplied Polyvinyl alcohol (PVA; mw 30,000–70,000) for Boehringer Ingelheim Pharma Gmbh & Co., Germany (St. Louis, MO, USA).

Formulation development

Synthesis of dasatinib loaded PLGA nanobubbles

A modified version of the previously discussedSolvent-diffusion-evaporation method using a double emulsion of water-in-oil-in-water (W/O/W) [8] was used to create PLGA nanobubbles that contained dasatinib.

Experimental design

A Box-Behnken response surface design with four components and three stages was completed using Stat-Ease Design Expert® software V8.0.1.to clarify the impact of formulation and process parameters. Figures 1 and 2

Data analysis

Statistics were applied to the collected results. Equation illustrates how a quadratic model with multiple regression analysis can be used to analyse each response parameter.

Where,
Y – Response parameter
β_0 – Intercept
$\beta_1 - \beta_4$ – Regression coefficients
β_{12}, β_{13}, β_{14}, β_{23} β_{24} and β_{34} – Interaction coefficients
β_{11}, β_{22}, β_{33} and β_{44} – Quadratic coefficients
X_1, X_2, X_3 and X_4 – Main influencing factors
X_1X_2 – Interactive effect
X_1^2, X_2^2, X_3^2 and X_4^2 – Quadratic effect
stages

Table 1. The dependent and independent variables are listed in the BBD, along through the stages and objectives for each

Independent variables			stages		
Variable		Units	Small	Middle	High
A	Amount of dasatinib	mg	160	240	320
B	Amount of PLGA	mg	250	375	500
C	Amount of PVA	% w/v	1	1.5	2
D	Stirring speed	Rpm	6000	8000	10000
Dependent variables			**Goal**		
Y1	Encapsulation efficiecny	%	Maximal		
Y2	Mean atom magnitude	nm	Minimal		
Y3	Zeta potential	mV	Minimize		

Table 2. Trial experiments' observed reactions in accordance with BBD

Expt	Amount of dasatinib (mg)	Amount of PLGA (mg)	Amount of PVA (% w/v)	thrilling speed (rpm)	Encapsulation efficiecny (%)	Mean molecule size (nm)	Zeta potential (mV)
1	160	250	1.5	7000	62.37	343.44	-16.16
2	320	250	1.5	7000	41.78	616.82	-28.64

3	160	500	1.5	7000	58.18	496.98	-20.42
4	320	500	1.5	7000	38.46	178.34	-31.48
5	240	375	1	6000	30.78	664.62	-20.24
6	240	375	2	6000	37.12	96.78	-29.54
7	240	375	1	8000	15.12	364.48	-38.56
8	240	375	2	8000	20.12	122.86	-18.64
9	160	375	1.5	6000	62.46	302.34	-14.42
10	320	375	1.5	6000	44.38	238.56	-25.14
11	160	375	1.5	8000	43.56	208.96	-16.82
12	320	375	1.5	8000	26.12	114.78	-29.92
13	240	250	1	7000	32.56	767.72	-31.64
14	240	500	1	7000	27.13	596.98	-34.12
15	240	250	2	7000	40.24	298.56	-26.68
16	240	500	2	7000	34.66	231.66	-28.88
17	160	375	1	7000	38.12	732.88	-23.66
18	320	375	1	7000	40.88	522.64	-35.28
19	160	375	2	7000	69.23	133.56	-17.86
20	320	375	2	7000	28.34	311.48	-31.28
21	240	250	1.5	6000	45.88	512.56	-23.06
22	240	500	1.5	6000	40.44	186.89	-23.56
23	240	250	1.5	8000	25.34	166.88	-24.12
24	240	500	1.5	8000	23.98	248.66	-30.66
25	240	375	1.5	7000	25.67	354.82	-12.34
26	240	375	1.5	7000	24.22	354.54	-14.26
27	240	375	1.5	7000	25.42	368.12	-13.48
28	240	375	1.5	7000	27.12	348.76	-13.42
29	240	375	1.5	7000	25.86	312.46	-13.12

Optimization

Under ideal circumstances, the nano formulation was created in three copies to confirm the efficacy of the optimization method.

Evaluation of dasatinib nanobubbles

Drug pay load, particle size, encapsulation efficiency, zeta potential and Poly dispersity index

Equation was used to determine the "percent drug pay load" and "percent drug encapsulation efficiency." [9]

Particle size, polydispersity index, and zeta potential Utilizing dynamic lighting scattering approach, they were evaluated.

Purpose of haemolytic action

In human blood, the PLGA nanobubbles' hemolytic activity was assessed.The percent hemolysis was calculated using following equation. [10] Where ABS_0 and ABS_{100} are the absorbance of the solution at 0 and 100 % hemolysis, respectively.

In-vitro drug release study

According to other reports [11, 12], the dialysis bag approach at 37 °C was used to investigate the kinetics of dasatinib's in vitro release from the nanobubbles both with and without ultrasound. As the donor phase, 3 ml of dasatinib nanobubbles aqueous solution (equal to 3 mg of dasatinib) were employed in place of 120 ml of pH 7.4 phosphate buffer at 0.01 M in a dialysate bag made of cellulose dialysis membrane by Spectrapore (receiving phase). By removing the release involves withdrawing 1 ml of the receiving phase at a predetermined period and replacing it with 1 ml of fresh phosphate buffer of dasatinib up to 24 hours was calculated. after using ultrasound (frequency 2.5 0.1 MHz, duration 1 min)), the release was

likewise observed. The medication discharge was monitored for 24 hours afterward the insonation of nanobubbles in the dialysis bag, as previously mentioned.

Nanobubbles of dasatinib that are stable in ultrasound

An ultrasound stimulus with an oscillation frequency of 2.5 0.2 MHz, an average acoustic pressure distribution value of 2.5 0.2 MPa, a nominal frequency of 50 Hz, and a nominal power of 30 W was applied to dasatinib-loaded nanobubbles.

Characterization of prepared dasatinib nanobubbles by FTIR, DSC, TEM and stability studies

The results of Fourier transformed infrared (FTIR) spectroscopy utilising the Tensor 27 FTIR Spectrophotometer in the range of 4000 to 600 cm1 and the potassium bromide disc technique were used to make the observations using transmission electron microscopy (TEM). A TAC-equipped Perkin Elmer DSC/7 differential scanning calorimeter (Perkin-Elmer, CT-USA) was used to perform differential scanning calorimetry (DSC). [13]

For one month, the steadiness of dasatinib nanobubbles 4 °C, 25 °C, and 40 °C, among other temperatures—was assessed. On the first, tenth, and thirty-first days, dasatinib-loaded nanobubbles' encapsulation effectiveness, average particle size, and PDI were assessed.

RESULTS AND DISCUSSION

In this work, Nanobubbles with PLGA shells have been created to transport dasatinib. The design's executive summary is as shown in figure 1. [14, 15]

Design Summary

Study Type	Response Surface	Runs	29
Design Type	Box-Behnken	Blocks	No Blocks
Design Model	Quadratic	Build Time (ms)	44.06

Factor	Name	Units	Type	Subtype	Minimum	Maximum	-1 Actual	+1 Actual	Mean	Std. Dev
A	Amount of dasatinib	mg	Numeric	Continuous	160.00	320.00	160.00	320.00	240.00	51.46
B	Amount of PLGA	mg	Numeric	Continuous	250.00	500.00	250.00	500.00	375.00	80.41
C	Amount of PVA	% w/v	Numeric	Continuous	1.00	2.00	1.00	2.00	1.50	0.32
D	Stirring speed	rpm	Numeric	Continuous	6000.00	8000.00	6000.00	8000.00	7000.00	643.27

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.	Ratio	Trans	Model
Y1	Encapsulation efficiency	%	29	Polynomial	15.12	69.23	36.3979	13.4695	4.5787	None	RQuadratic
Y2	Particle size	nm	29	Polynomial	96.78	767.72	351.66	189.221	7.93263	None	RQuadratic
Y3	Zeta potential	mV	29	Polynomial	-38.56	-12.34	-23.7034	7.65675	0.320021	None	RQuadratic

Figure 1. Summary of the Box-Behnken design

RSM optimization

Statistical analysis

A sequence of 29 experiments were executed as per a four-factor, three-level BBD. Mathematical equations were produced by means of multiple linear regression analysis for the stated variables and is presented in table 3. Figures (2, 3 and 4) displays the reasonably good agreement between predicted and actual results.

Table 3. For each of the three responses, regression equations are as follows:

Dependent Variable	Regression equation
Y1	26.07 -9.49 A – 2.11 B + 3.76 C – 8.90 D – 10.91 AC + 1.02 BD + 17.64 A^2 + 7.29 B^2
Y2	346.93 – 19.62 A – 63.87 B – 204.53 C – 64.59 D – 148.00 AB + 97.04 AC + 25.96 BD + 101.86 BD + 81.55 CD + 54.57 B^2 + 79.41 C^2 – 122.56 D^2
Y3	– 13.32 – 6.03 A – 1.56 B + 2.55 C – 1.89 D – 0.59 AD – 1.51 BD + 7.30 CD – 3.85 A^2 – 7.39 B^2 – 9.51 C^2 – 4.30 D^2

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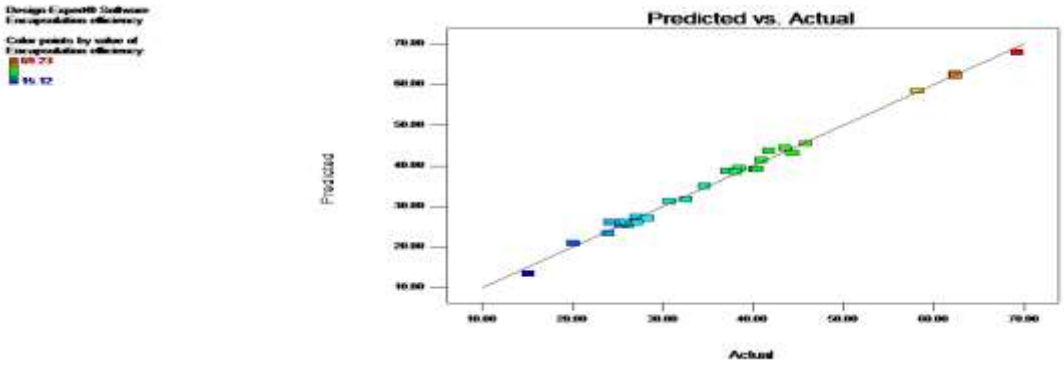

Figure 2: Contrast of the encapsulation efficiency's expected and real values

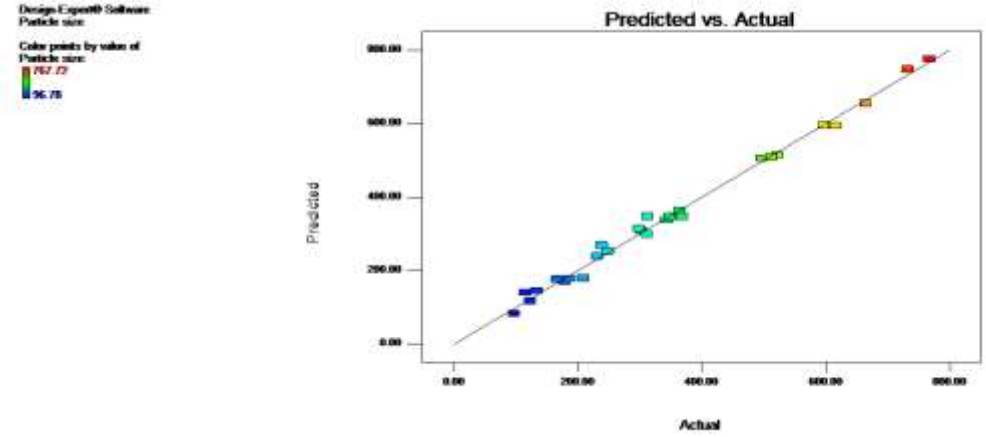

Figure 3: Comparison between predicted and actual values of particle size

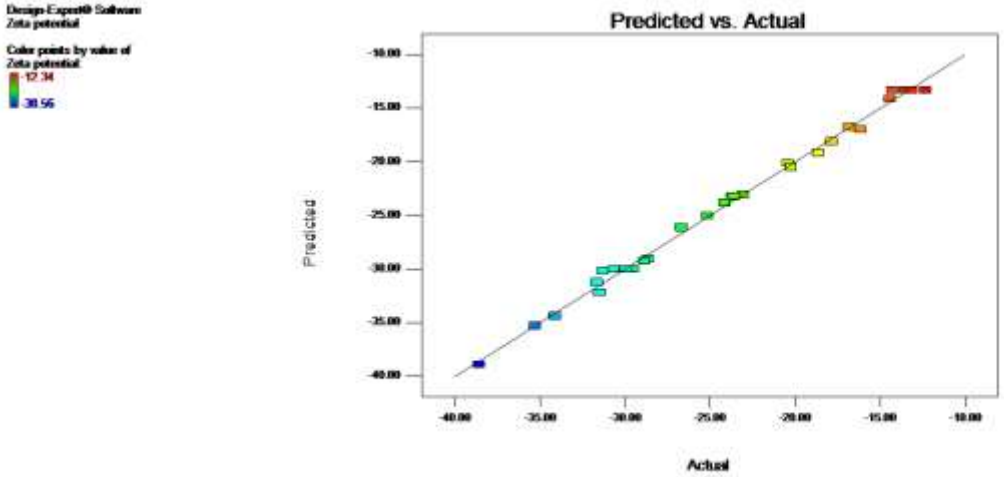

Figure 4: Contrast between predicted and actual values of zeta potential

Hemolytic activity

Up to the measured an amount of 10 mg/ml, The non-hemolytic nature of about PLGA nanobubbles' aqueous suspensions was found. With erythrocytes, drug-loaded nanobubbles likewise had a favourable safety profile.

Characterization

Table 5 lists the average particle size, Zeta potentials and polydispersity indices for the formulas of the nanobubbles are both taken into consideration. both before and after dasatinib loading. Particle size and The polydispersity index didn't change much and differ between drug-free and drug-loaded nanobubbles.

The surface shape and core-shell structure of nanobubbles between 60 and 70 nm were visible in TEM pictures (Figure 5). Dasatinib may be loaded into nanobubbles with a loading size of 24.34% and an encapsulation effectiveness of 80.12%.

Table 5. Nanobubbles physicochemical properties

	Blank nanobubbles	Dasatinib laden nanobubbles
Average particle size	72.54±2.62	65.86±3.44
Polydispersity index	0.23±0.005	0.12±0.005
Zeta potential	-33.36±4.22	-35.64±1.86
Encapsulation efficiency	-	80.12±4.11
Loading capacity	-	24.34±2.12

Figure 5. TEM image of dasatinib nanobubbles

Figure 6 shows a comparison of FTIR spectra of dasatinib, PLGA, PVA, blank nanobubbles and dasatinib loaded nanobubbles. The FTIR spectra of free drug contained some distinguishable peaks. at 3408.33, 3205.80, 2949.26, 2821.95, 1612.54, 1577.82, 1502.6, 1444.73, 1390.72, 1290.42, 1215.19, 1193.98, 1003.02, 862.21, 813.99, 773.48 and 590.24 cm^{-1}. The predictable peaks of PLGA polymer were observed at 2998, 2948, 1454, 1426 and 1397 cm^{-1}. FTIR spectra of PVA shows main typical peaks at 3280, 2917, 1690, 1425, 1324, 1081 and 839 cm^{-1}. The absence of characteristic peaks of drug in the FTIR spectra of dasatinib loaded nanobubbles indicates that the drug might be entrapped in the PLGA matrix.

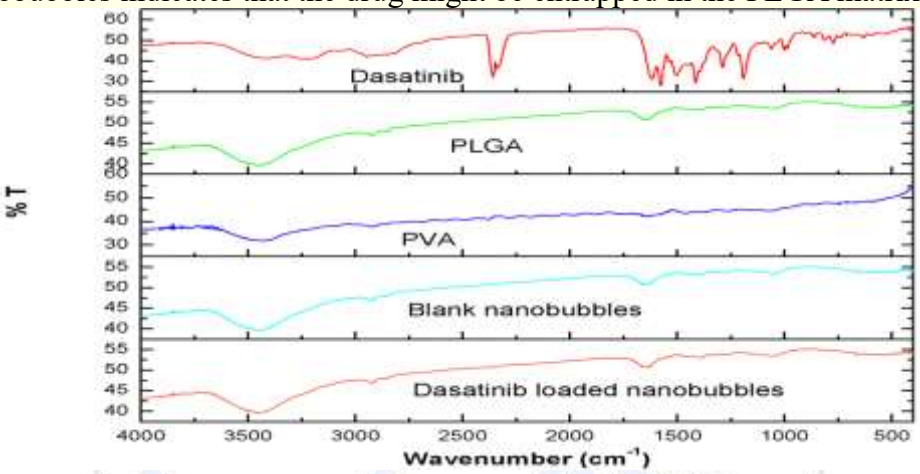

Figure 6. FTIR spectra of dasatinib, PLGA, PVA, blank nanobubbles and dasatinib loaded nanobubbles

Dasatinib's DSC curve exhibited an endothermic peak at the temperature that corresponds to its melting point: 291.06 degrees Celsius. The PLGA thermogram exhibited an endothermic peak at 58.5 degrees Celsius, which corresponded to the material's melting temperature.. The characteristic heatabsorbing peak at 192.65 °C is connected to the thermal degradation of the PVA. Similar melting transition properties of Blank nanobubbles and drug loaded nanobubbles show that the PLGA and PVA remained unaffected during encapsulation. (Figure 7)

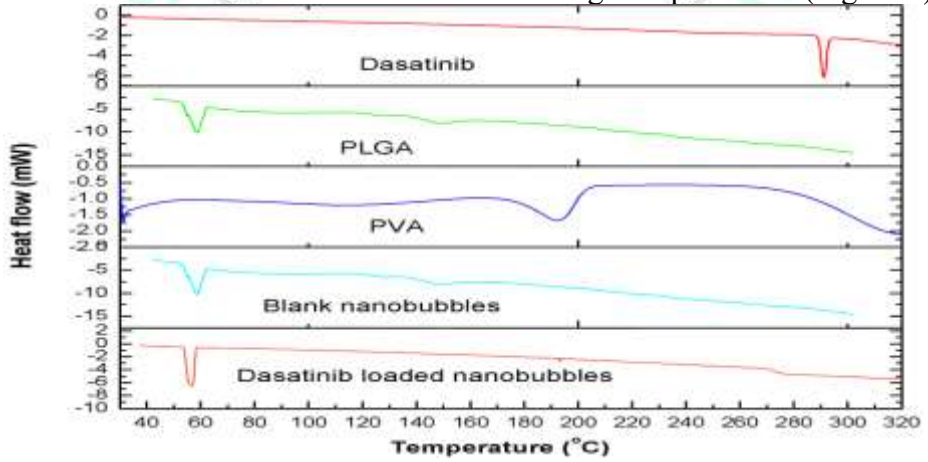

Figure 7. DSC thermogram of dasatinib, PLGA, PVA, blank nanobubbles and dasatinib loaded nanobubbles

Stability studies

No significant changes in encapsulation efficacy,PDI and particle size of dasatinib nanobubbles stored at different temperatures (Table 6)

Table 6: Encapsulation effectiveness, particle size, and PDI of dasatinib microbubbles deposit at divergent temperatures

Temperature (°C)	Time (days)	Encapsulation efficiency (%)	Particle size (nm)	PDI
4 ± 1 °C	0	80.12 ± 4.11	69.86±3.44	0.22±0.005
	15	78.92 ± 2.34	71.12±4.12	0.26±0.005
	30	78.68 ± 3.32	70.56±2.26	0.24±0.005
25 ± 2 °C	0	80.12 ± 4.11	69.86±3.44	0.22±0.005
	15	79.36 ± 3.67	70.12±4.56	0.24±0.005
	30	78.86 ± 2.38	71.98±3.32	0.24±0.005
40 ± 2 °C	0	80.12 ± 4.11	69.86±3.44	0.22±0.005
	15	76.54 ± 3.12	78.34±2.58	0.34±0.005
	30	60.34 ± 5.11	96.34±4.52	0.38±0.005
n = 3 ($p < 0.05$)				

CONCLUSION

For the administration of the anticancer medication dasatinib, PLGA-shelled and per fluropentane-filled nanobubbles were created in this work. Using response surface approach, the formulation's component parts were improved in terms of size distribution and particle size. Under ideal circumstances, nanobubbles showed homogenous particle size distribution. At all pH levels, the solubility of dasatinib nanobubbles is significantly higher than that of dasatinib solution. Dasatinib nanobubbles exhibit superior dissolving profiles and higher gastrointestinal stability than the suspension, according to an in vitro dissolution test, which significantly increases oral bioavailability. Furthermore, in vitro cytotoxicity tests showed that dasatinib nanobubbles significantly improved tumour cell growth inhibition. PLGA nanobubbles might be thought of as an intriguing tool in the creation of formulations that respond to ultrasound for targeted drug delivery.

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