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PREPARATION, OPTIMIZATION AND CHARACTERIZATION OF BENDAMUSTINE LOADED PLGA NANOPARTICLE

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Abstract:

The aim of this research work was to formulate and optimize bendamustine loaded polymeric nanoparticle by using factorial design. The emulsion solvent diffusion technique was employed successfully to get polymeric nanoparticle and the optimization was done by using 2^3 factorial design. In this research the effect of independent variable (Polymer concentration, surfactant concentration and sonication time) on dependent variable (particle size, entrapment efficiency) was elaboratedfor optimization of nanoparticle. The characterization of these nanoparticles was done by the different parameters such as interaction between the drug and excipients, mean particle size, morphology, zeta potential, % drug entrapment efficiency, % process yield, and in-vitro drug release behaviour. FTIR, TEM, zeta potential studies, and dialysis bag method were performed for this determination. The in vitro drug release data were analysed by using different kinetic models to know the release kinetics. The optimized nanoparticles were spherical in shape and showed particle size 103.5 ± 0.04 nm, PDI 0.307 ± 0.014, zeta potential -31.9 mV, % drugentrapment efficiency 78.13±4.16 and % process yield 74.51 ± 1.8%. The release kinetics studies revealed that drug release from the nanoparticles follow the Korsmeyer–Peppas model.

Keywords: Bendamustine, factorial design, in vitro drug release, kinetic model,polymeric nanoparticle

Introduction

Today nanomedicine is proving their efficacy in many diseases. These technologies are new but quickly increasing day by day. In this science materials the nanoscale range are utilized for the delivery of drugs on specific targets in a controlled way[1]. Nanotechnology has many advantagesfor the treatment of chronic diseases by their specificities and delivery on target of medicines. In disease contrast the major challenge is large sized drugs, there delivery and concentration at targeted site that includes the poor bioavailability, in vivo unsteadiness, and insufficient solubility that affects the absorption of drug in the body. The issue through targeted delivery not only reflects efficiency but adverse effects too. In these consequences recent drug delivery systems can be a good option for targeted delivery at specific sites[2, 3].

The recent situation is focused on the use of polymeric nanoparticles for drug deliveryas they have the ability to control the issues such as cell permeability, poor drug solubility. Now a days the use of biodegradable polymer is increasing for the preparation of nanoparticle.PLGA or poly (lactic-co-glycolic acid) is a Food and Drug Administration (FDA) approved copolymer which is used in the preparation of many nanocarrier systems, due to its biodegradability and biocompatibility. It belongs to the category of polyesters[4].

Cancer is one of the growing causes of death worldwide, in the past years, many research studies have focused on finding new therapies to reduce the side effects caused by conventional therapies. The Identification of specific genes involved in tumour genesis and progression of cancer is one of the important parameters to understanding the pathophysiology of cancers and finding therapeutic drug targets. Many research works have been carried to identify cancer biomarkers by using gene

expression profiles[5].Type of cancer of the blood and bone marrow.B-cell chronic lymphocytic leukaemia (CLL) develops from a type of white blood cell called B cells. It progresses slowly, usually affecting older adults.CLL may not cause any symptoms for years. When symptoms do occur, they may include swollen lymph nodes, fatigue and easy bruising.Treatment isn't always initially necessary, but may include chemotherapy[6].Bendamustine is an anticancer (Antineoplastic or cytotoxic) chemotherapeutic drug used in the treatment of chronic lymphocytic leukemia (CLL) and patients with indolent B-cell non-Hodgkin's lymphoma(NHL). Bendamustine belongs to a class of cancer chemotherapy drugs known as alkylating agents[7].Chemically, it is 5- [Bis (2-chloroethyl)-amino]-1-methyl-1H-benzimidazole-2-butanoic acid.It works by killing cancer cells or slowing their growth. It is a cancer medication that interferes with the growth of cancer cells and slows their growth and spread in the body.It is sparingly soluble in water and freely soluble in methanol.

Experimental design and optimization are tools that are used to systematically examine different types of problems.Optimization created on factorial design permits all factors to be varied simultaneously, therefore evaluating the effects of each variable at each level and stating the interrelationships between them[8]. The optimization study was designed to assess influence of process variables on characteristics parameters i.e. particle size and entrapment efficiency of nanoparticles.In order to choose the key variables through preliminary studies, one variable was changed at a time while keeping all others constant.

The objective of this study was to prepare and Bendamustine polymeric nanoparticle for intravenous administration using emulsion solvent diffusion technique. The formulation variables were optimized through 2^3 factorial design. The characterization of optimized formulation wasperformed by different parameters such as compatibility study of drug and excipients bysize ,shape,zeta potential, % drug entrapment efficiency, % process yield of nanoparticles. The selected optimized formulation was analysed for in vitro drug release, and the drug release data was fitted to different mathematical models such as zero order, first order, Higuchi model, Korsmeyer- Peppas model to determine drug release kinetics[9].

Material

The polymer PLGA was gifted fromEvonik Pharmaceuticals, Mumbai (India). Bendamustine isa kind of the alkylating agent which was received as gift sample fromDr. Reddy'sLaboratory Hyderabad. The polymer PVA was used as surfactant and dichloromethane was purchased fromSigma Aldrich, Mumbai, India. Dialysis membrane (Molecular weight cut off 10000-12000 Da) was purchased from Hi-media Laboratories, Mumbai, India.

Milli-Qquality purified water was used during the whole experiment and all other chemicalswere of HPLCgrade.

Method

The polymeric nanoparticle of Bendamustine was prepared by emulsion solvent diffusion technique. In this method desired amount of PLGA and accurately weighed quantity of drug was dissolved in 10 ml of dichloromethane. After that the obtained polymeric drug solution added to aqueous PVA solution (4%w/v) in a 100 ml beaker with continuous stirring[10]. The mixture was sonicated (Soniweld Probe Sonicator, Imeco Ultrasonics, India) for 5-7 minutes. The mixture was stirred for 4 hoursat 800 rpm with magnetic stirrer(Remi, India) until the emulsion was formed. 30 ml of deionized water was also added for complete diffusion of organic phase into water.The final Nanoparticles were separated by centrifugation technique

at10,000rpm for 30minutes.Afterward separation of the supernatant from precipitants was completed. This precipitant containing nanoparticles was freeze dried(Heto power dry LL 3000 Lyophilizer, Stuttgart, Germany)and evaluation studies were performed.

Optimization of BM loaded PLGA nanoparticle

PLGA nanoparticles prepared by emulsion solvent diffusion technique were optimized for minimum particle size and maximum entrapment efficiency using response surface quadratic model. Created on

the preliminary study, PLGA (polymer) and PVA (surfactant) were optimized by design expert software respectively[11]. Experimental factorial design-response surface methodology was used to study the effect of different variables on formulation properties like mean particle size (PS) (Y1) and entrapment efficiency (% EE) (Y2) of the prepared nanoparticle. The independent variables used for nanoparticles were:

a) Concentration of polymer (A) [0.1%-3%]
b) Concentration of surfactant (B) [1.0%-4%] and
c) Sonication time (C) [5-7 MIN]

Process and formulation parameter of BM-PLGA Nanoparticles

The prepared nanoparticles were optimized for selected variables on the basis of preliminary study using Box-Behnken design in which 8 batches were selected using 3 factors and 2 levels and data is generated for each batch. Amount of polymer (A), Amount of Surfactant (B) and Sonication time (C) called independent variables which would affect the dependent variable.These independent variables were represented by level −1, and +1 corresponding to the lowand high values respectively. Torepresent the effects of prearranged factors on the measured responses, 3D response surface graph was plotted. 3D response surface plots are helpful inthe determinationof relationship between independent variables and dependent variables. The optimization was done by Design expert version 10 (trial version) software. For this purpose, a formulation with the best desirability factor was selected and its batch data was examined. The process and formulation parameter and the coded values for independent and dependent variables are shown in table.1.1 and 1.2.

Table.1.1: Process and formulation parameters of PLGA Nanoparticle prepared by emulsion solvent Diffusion technique[12]

1) Preliminary studies for formation of nanoparticles		
Variable(s)	**Range**	**Selection parameter**
Conc. of PLGA (Polymer)	1.0–4.0 %w/v	Formation of Nanoparticle
Conc. of PVA (surfactant)	1.0-5.0 % w/v	
2) Selection and optimization of key variables		
Key variable	Range	Selection parameter
Conc. of PLGA (Polymer)	0.1-3.0% w/w	Particle size and Entrapment efficiency
Conc. Of PVA(Surfactant)	1.0-4.0 %	
Sonication time	5-7min	

Table.1.2: The coded values for independent and dependent variables used in experimental design:

Independent variables	**Levels**		**Dependent Variables**
	Low (-)	**High (+)**	
PolymerConcentration (A)	0.1	3	Particle size (Y1) and Entrapment Efficiency (Y2)
Surfactant Concentration (B)	1	4	
Sonication time (C)	5	7	

The chosen independent variables considerably influenced the observed responses for particle size and % EE. Polynomial equations representing the main effect and interaction factors were determined by Design-Expert software. The statistical validation of the polynomial equations was established by

ANOVA. Box-Behnken design engaged for the optimization of 8 batches of Nanoparticle has been shown along with the results in table.1.3.

Table 1.3: Independent variables all along with their coded level and actual levelof 8 batches of Bendamustine loaded PLGA Nanoparticle:

Nanoparticles Formulation	Independent variables (Coded values)			Independent variables (Actual Values)		
	A	B	C	A	B	C
NPF1	-1	1	-1	0.1	4	5
NPF2	1	1	-1	3	4	5
NPF3	-1	1	-1	0.1	1	5
NPF4	1	1	1	3	4	7
NPF5	-1	1	1	0.1	4	7
NPF6	1	-1	-1	3	1	5
NPF7	-1	-1	1	0.1	1	7
NPF8	1	-1	1	3	1	7
Dependent variables						**Constraints**
Y1 = Particle size (PS)						Minimize
Y2 = Entrapment efficiency (%EE)						Maximize

Characterization of preparednanop article:

To find the highest optimized formulation with minimum particle size and maximum entrapment efficiency, the 8 different batches of each Chitosan and PLGA nanoparticles were characterized for parameters written below:

Mean particle Size, PDI and Zeta potential:

Particle size distribution (mean diameter in nm),poly dispersity index and zeta potential of PLGA-based nanoparticles were calculated by dynamic light scattering using Malvern Zetasizer, ZS nano 90, Malvern Instruments, (USA). Measurements were done in triplicate manner at an angle of 90^0 maintained at 25^0C.Thesample was prepared with dispersing the nanoparticles insufficient amount of ultra purified water (pH 7) previous to the experiment. The particle size distributions of the nanoparticle were described as poly dispersity index (PDI), a measure of the distribution broadness of the particle size[13]. Zeta Potential is a valuable parameter to find out the physical stability of nanoparticles. ZP was calculated by using the Helmoltz–Smoluchowski equation:

$$\xi = EM \times \frac{4\pi\eta}{\varepsilon}$$ ……. Equa.1

Where

ξ denotes zeta potential in mV

A is Electrophoretic mobility

η is viscosity of dispersion medium

Ɛ is dielectric constant

Percentage yield, drug loading and entrapment efficiency:

The prepared nanoparticles(PLGA nanoparticles) were dried at room temperature, weighed and the percentage yield was calculated using equation:

Percentage yield % =Weight of nanoparticles obtained / weight of polymer+ weight of drug .....Equation 2

Percentage drug loading and entrapment efficiency:

The PLGA-loaded nanoparticles(equivalent to 20 mg drug) was mixed to 10.0 ml methanol, followed by centrifugation (High-Speed Refrigerated Centrifuge ,3K30, SIGMA, Germany) for 45 min at 12,500 rpm and then filtered using Millipore® membrane filter (0.2 µm). The filtrate was collected and properly diluted with methanol and measured spectrophotometrically (Systronic UV-Spectrophotometer, model 2201, Japan) at 329 nm for BM[14] . The entrapment efficiency percent (EE %) and Percentage drug loading (% DL) was calculated using the following formula.

$$\% \text{ Entrapment Efficiency} = \frac{\text{weight of drug in NP- weight of drug in supernatant}}{\text{X100 .....3}}$$

$$\% \text{ Drug loading} = \frac{\text{Weight of drug in nanoparticle}}{\text{Total weight of drug entrapped}} \quad \text{X100……..Equa.4}$$

$$\text{Total weight of nanoparticle}$$

Transmission electron microscopy (TEM)[15]:

The TEM analysis was done to calculate particle size and shape of polymeric nanoparticle. In this microscopy a drop of diluted sample was positioned on the surface of carbon coated copper grid and stained with negative stain using a drop of 2 % (w/w) aqueous solution of phosphotungstic acid for 30 seconds.After that staining, samples were dried at room temperature for 10 minutes to carry out investigation. TEM analysis of the samples was done.

Differential scanning calorimetry study (DSC)

For the determinationof physical state of the drug Bendamustine in the carrier system and the view of any interaction between the drug, and the polymer, differential scanning calorimetric analysis was done[16].DSC curves (known as thermogram) of pure drug BM with chitosan andPLGA, along with the lyophilized drug loaded nanocarriers were recorded on a scanning calorimeter(DSC Q20, TA Instruments-Waters LLC, USA). A slight amount of sample (nanoparticle) was placed in hermetically sealed aluminium pans and heated from 50–300 ˚C at a heating rate of 10˚C/min under a constant flow of dry nitrogen. DSC analysis of the samples was done from.

X-ray diffraction study (XRD)

XRD was executed to examine the nature (crystalline or amorphous) of prepared nanoparticle. X-ray powder diffraction test of pure drug BM with chitosan and PLGA, withthe lyophilized drug loaded nanocarriers were carried out using powder X-ray diffractometer (PANalytical 3 kW Xpert Powder, UK). Samples were placed in sample stage and scanned from 2^{θ} to $60^{\theta}$with an operating voltage of 40 kV and current 30mA. XRD analysis of the samples was done[17].

In vitro drug release

In vitro drug release study of BM loaded PLGA nanoparticles was calculated by using modified Franz diffusion cell using dialysis membrane(MW. Cutoff 10,000 Da).Dialysis membrane was reserved into double distilled water for 24 hours before using into modified Franz diffusion cell. After thatNanocarrier (equivalent to 2mg of pure drug) was located in the donor compartment and the receptor compartment was filled with 10 ml dissolution medium (pH 7.4 phosphate buffer for BM)

and the temperature was maintained at 37 ± 0.5^{0}C by continuous stirring at 100 rpm.After a regular time, intervals aliquots were withdrawn from the receptor compartment[18].All these aliquots were appropriately diluted with methanol and the amount of drug release was analysed by UV-visible spectroscopy at 329 nm for BM.

Drug release kinetics

To determine the drug release kinetics and mechanism of drug release from nanoparticle, drug release data were fitted to different kinetic models. Generally, one of the two conditions occurs. In Case I, where the drug transport mechanism from spherical matrices is by Fickian diffusion when $n \leq 0.43$, if $0.43 < n < 0.85$, it is anomalous (non-Fickian) transport and in Case II, for values of $n \geq 0.85$, zero order release kinetics is indicated[19]. The models studied were:

a) **Zero order rate kinetics[20]** – In thisstudy graph is plotted between the cumulative percent drug released with respect to time.
b) **First order rate kinetics** - Here the plot is between Log of Cumulative percent drug retained with respect to time.
c) **Higuchi's kinetics** – It is also known as the Higuchi's classical diffusion equation/ Higuchi matrix. In this kinetic performance the graph is plotted between the cumulative percent released with respect to time.
d) **Korsmeyer-Peppas exponential kinetics** – Here the graph is plotted between log of cumulative percent drug released with respect to time.

Result and discussion

Preparation and optimization of BM loaded PLGA nanoparticle

The chitosan nanoparticles were successfully prepared by emulsion solvent diffusion technique. The master formula was given in table.1.4.

Table.1.4:The formula for the preparation of PLGA nanoparticles

	Name of Ingredient	**Quantity**
1.	Polylactic glycolic acid (Polymer)	3mg/ml
2.	Polyvinyl alcohol (Surfactant)	2% w/v
3	Dichloromethane	10ml
4.	Water	50ml
Conditions		
Stirring speed	500 RPM	
Sonication time	5-7 minutes	
Temperature	Room temperature	

Optimization of PLGA nanoparticle

For the optimization processtotal 8 formulations of PLGA nanoparticle was prepared and optimized using 2^3 factorial design.The different concentration of polymer (0.5-3.0 % w/v of PLGA) and surfactant (1.0-2.0 % w/v) were selected for preliminary study for preparation of PLGA nanoparticles by Emulsion solvent diffusion method. Outcomes of preliminary studies within selected range of polymer and surfactant concentration demonstrated two kinds of phenomena i.e. solution, with low initial concentration of PLGA (0.5 % w/v) and surfactant (1.0% w/v), and high concentration of PLGA(3%w/v) and high concentration of surfactant (2% w/v) aggregates or precipitates were obtained. The particle size and entrapment efficiency of 8 formulations of NPs is shown in table.1.5.

Table1.5: Particle size and entrapment efficiency of 8 formulations of NPs

Nanoparticle Formulation no. (NSF)	Particle size (nm)	Entrapment efficiency (%)
NPF1	150.9±0.51	81.20±0.04
NPF2	145.2±0.17	80.09±0.07
NPF3	135.6±0.02	79.11±1.03
NPF4	128.2±1.05	76.20±2.31
NPF5	121.3±1.23	74.15±1.12
NPF6	111.2±0.22	70.10±1.03
NPF7	107.6±0.6	60.23± 3.12
NPF8	103.5±0.04	78.13±4.16

Effects on Particle size (Y1)

The particle size of 8 batches of PLGA nanoparticle ranged from103.5±0.04 nm to150.9±0.51 nm for 3 factor- 2 levels combinations. The effect of independent variables on particle size can be described by the following quadratic equation:

Y1 (particle size) = 115 + 2.165A + 0.740B – 0.672C + 0.94AB - 4.17AC– 6.22BC+ 11.30A^2 + 27.06B^2+ 16.12C^2 ····· Equation.5

Positive value(in equation no.5)before the factor in the regression equation indicates that the response increases with the factor and vice versa.The independent variable Awhich is polymer concentration had a noteworthy and positive effect on Y1 as denoted by the positive value in the quadratic equation.With the rise in concentration of polymer, the mean particle size quickly increased. It was clear that increase in polymer concentration may enhance the particle size of nanoparticle size because during the emulsification process increase in polymer concentration will increase the viscosity of organic phase which may led to formation of bigger size of nanodroplets.

The positive influence of surfactant concentration can be described on the basis that the main function of surfactant molecules is to provide stability to the emulsion nanodroplets and stop them from coalescing with each other. Thus, a minimum quantity of surfactant is required to get optimum range of nanoparticle.3D response plot representing the effect between ST-SC, PC–ST and SC-PC have been shown in figure 1.33,1.34 and 1.35 respectively; where ST is the sonication time, SC is concentration of surfactant and PC is concentration of polymer.

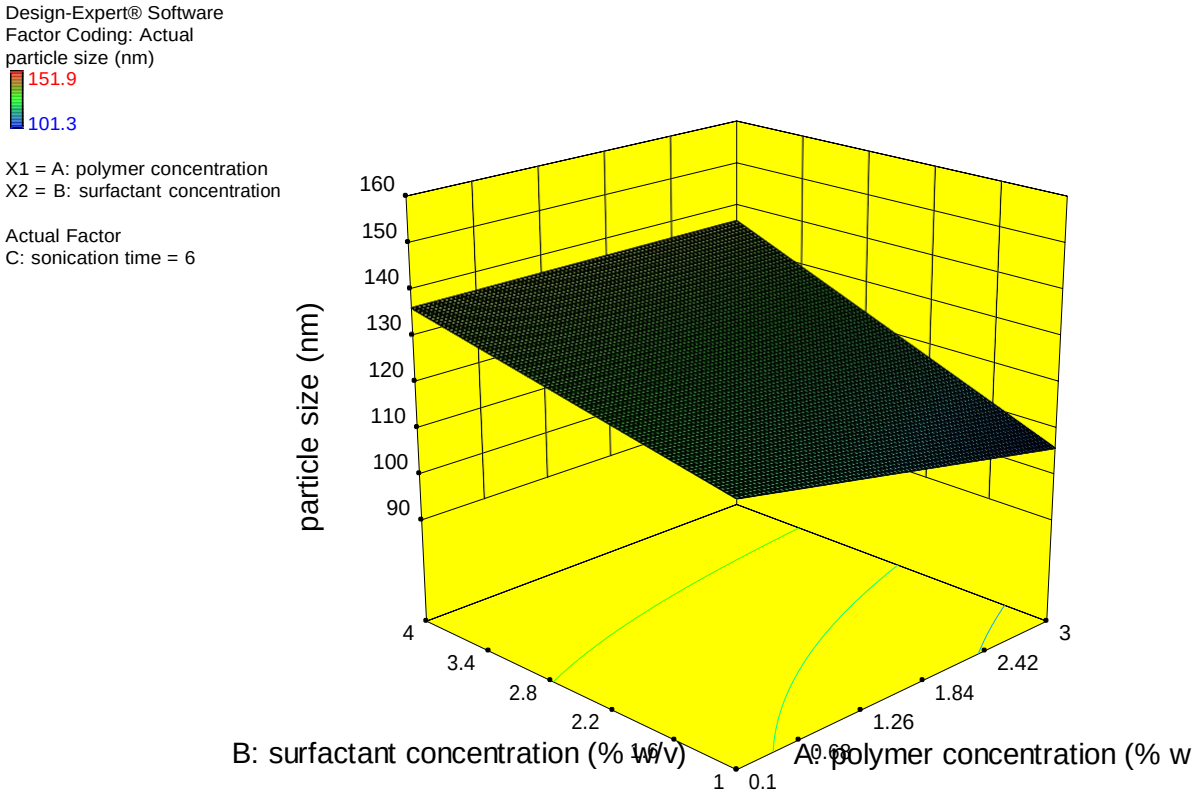


Figure 1.1: 3D surface plot between PC and SC (For BM-PLGA nanoparticle)

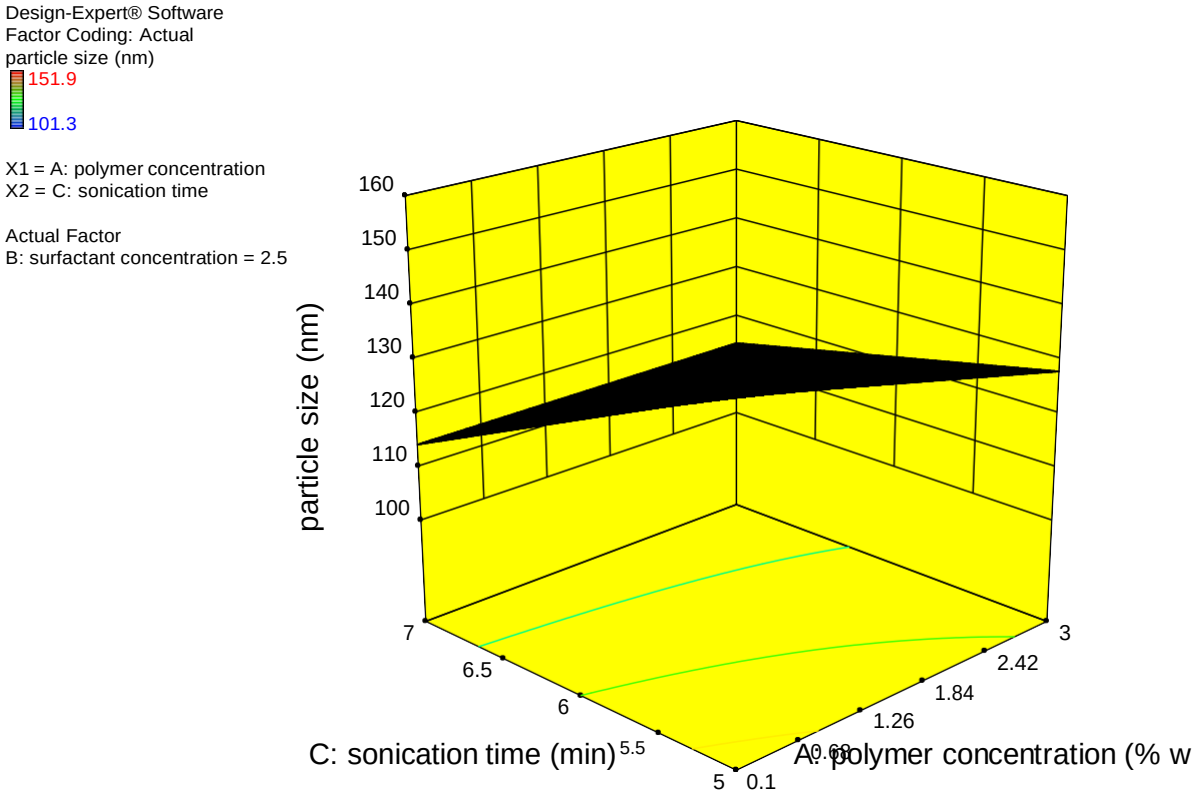


Figure 1.2: 3D surface plot between PC and ST

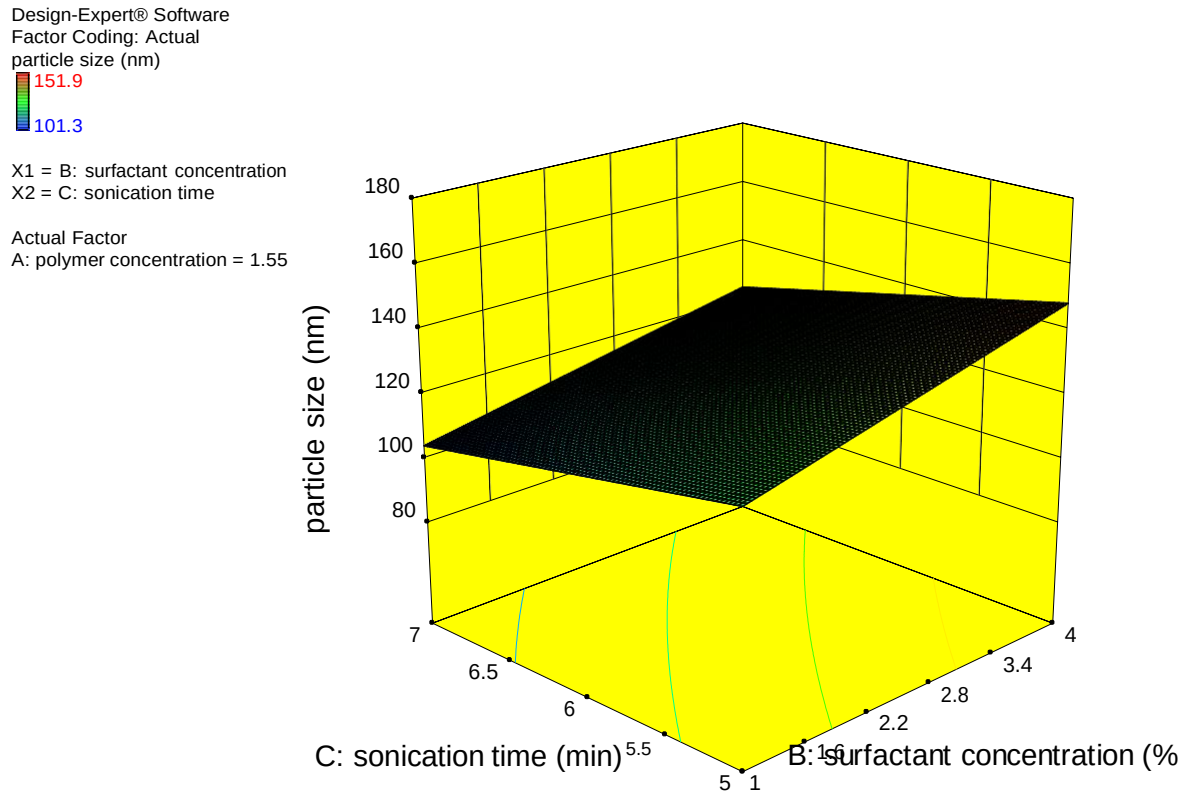


Figure 1.3: 3D surface plot between SC and ST

Effects on Entrapment efficiency (Y2)

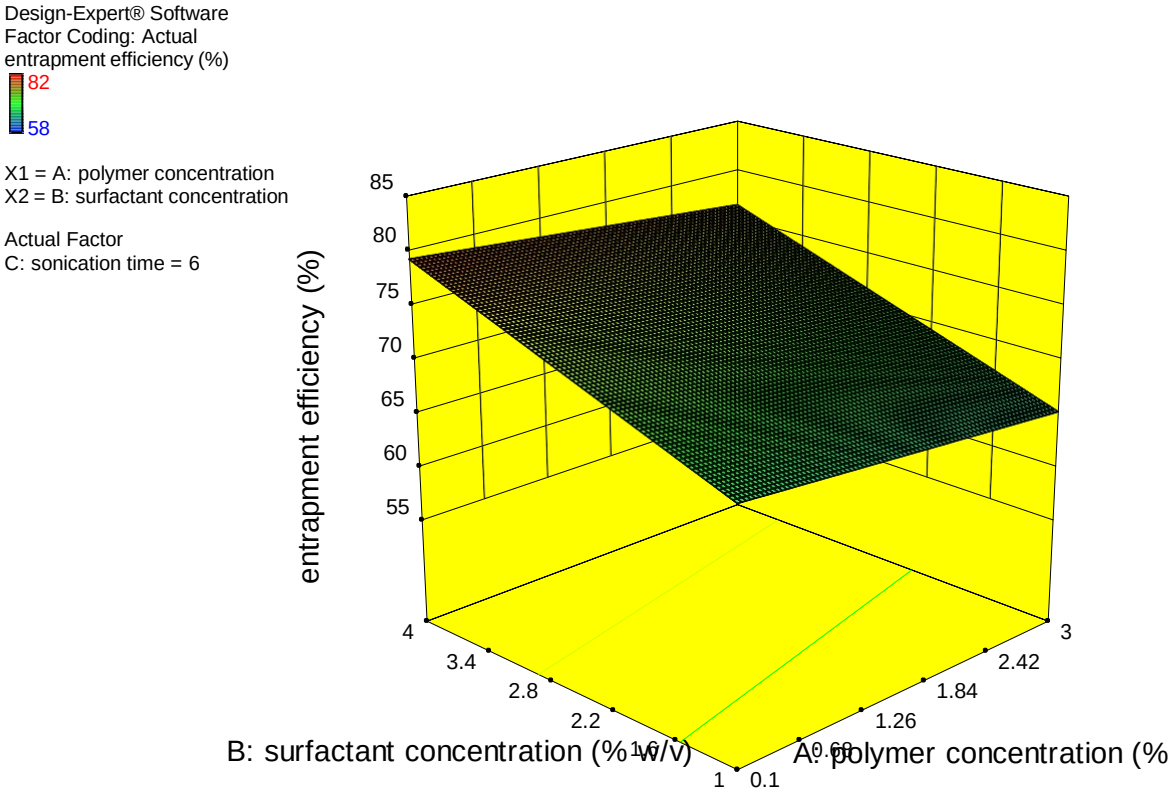
The entrapment efficiency of 8 batches of PLGA nanoparticle ranged from 58% to 82 % for numerous factors level combinations. The effect of various independent factors (variables) on entrapment efficiency can be described by the following quadratic equation:

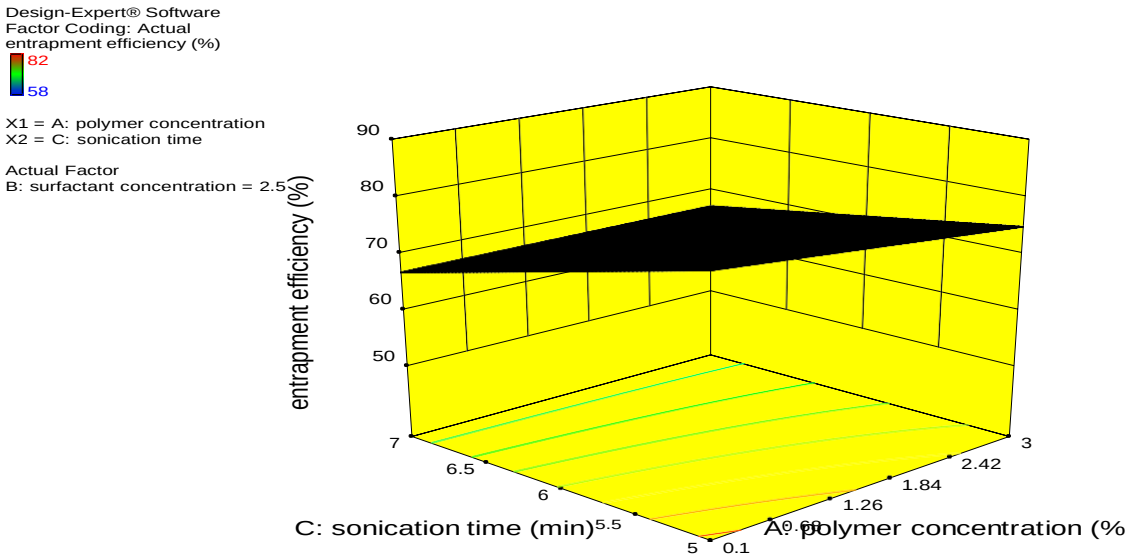
$\mathbf{Y_2}$ (EE%) = 75.67 + 3.14A-1.41B- 1.07C- 0.98AB+1.26AC- 1.35BC+ 0.70A^2- 4.28B^2- 3.50C^2..... Equation. 6

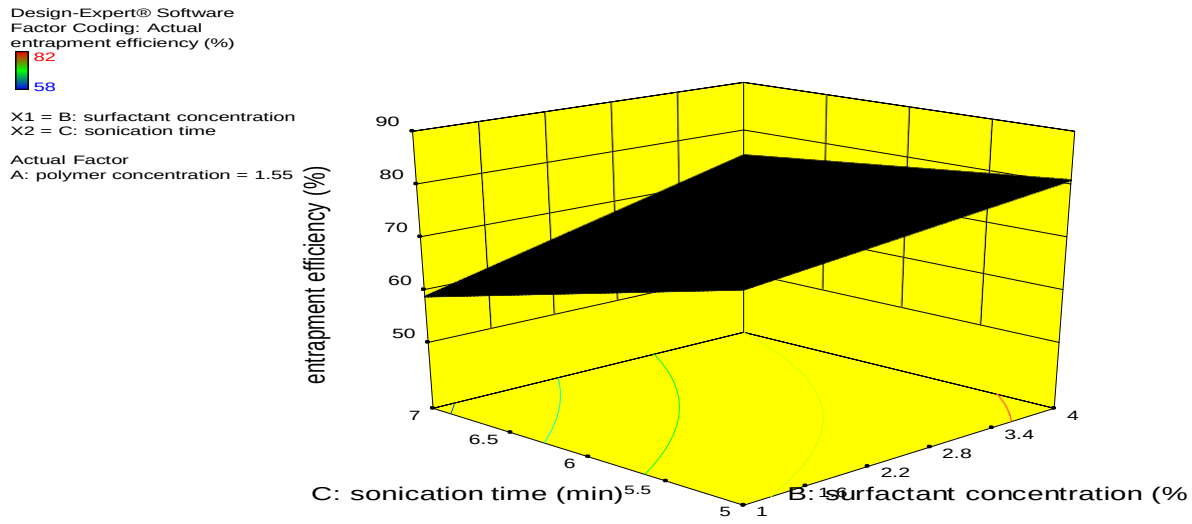
The positive sign of valuesbefore the factor in the above quadratic equation indicates a improvement of that particular response and vice versa. Positive sign before A indicates that the entrapment efficiency increases as polymer concentration raised,because increasing the polymer concentration increases the viscosity of the organic phase. It increases the resistance for the drug molecules to travel from the organic phase to the aqueous phase causing more drug molecules to be entrapped in the polymeric nanoparticles.

The negative sign (in equation no.6) beforeB indicated that entrapment efficiency decreases when surfactant concentration increased, because by increasing surfactant concentration (B), extra drug molecules can rapidly partition out into the aqueous phase throughout the emulsification process. Due to this less drug molecules are available in emulsion droplets to interact with PLGA molecules. The sonication time (C) has no significant effect on entrapment efficiency of drug molecule.

All the parameters were significant at $p \leq 0.05$. 3D response surface plot representing the effect between PC-SC, PC–ST and SC-ST have been shown in figure 1.36 to 1.38 for entrapment efficiency respectively.


Design-Expert® Software
Factor Coding: Actual
entrapment efficiency (%)
82
58
X1 = A: polymer concentration
X2 = B: surfactant concentration
Actual Factor
C: sonication time = 6
entrapment efficiency (%)
85
80
75
70
65
60
55
4
3.4
2.8
2.2
1.6
1
B: surfactant concentration (% w/v)
0.1
0.68
1.26
1.84
2.42
3
A: polymer concentration (%


Design-Expert® Software
Factor Coding: Actual
entrapment efficiency (%)
82
58
X1 = A: polymer concentration
X2 = C: sonication time
Actual Factor
B: surfactant concentration = 2.5
entrapment efficiency (%)
90
80
70
60
50
7
6.5
6
5.5
5
C: sonication time (min)
0.1
0.68
1.26
1.84
2.42
3
A: polymer concentration (%



According to the resultoptimized formulations NPF-8 obtained withparticle size 103.5 nm and entrapment efficiency 79 % withsonication time6 minutes, were found to be suitable.

The results of ANOVA model are summarized in table.1.6 for particle size and % drug entrapment efficiency. The value of determination coefficient and adjusting coefficient were > 90% which proves that the model is highly significant.

Table.1.6:Summary and results of analysis of variance for PS and EE (for BM-PLGA nanoparticle)

Response	**Sumof squares**	**Degreeof freedom**	**Mean square**	**F value**	**R^2**	**Adj. R^2**	**Perp. R^2**
Particle size	2321.46	7	687.38	10.60	0.9948	0.9845	0.9723
Entrapment efficiency	575.88	7	19.01	8.70	0.9807	0.9756	0.9678

Characterization of PLGA nanoparticles

The mean particle size of blank PLGA nanoparticle was found to be101.23 ± 0.04 nm and the size of optimized nanoparticle loaded with BM was calculated 103.50 ± 0.04 nm with the narrow distribution of poly dispersity index (PDI) i.e. 0.307.Zeta potentials of the blank nanoparticles was ranged from -29± 0.53 mV with a small increase up to -31.9±3.06 for drug loaded optimized nanoparticles.The size distribution and zeta potential of PLGA nanoparticles were shown in figure 1.7 and 1.8.

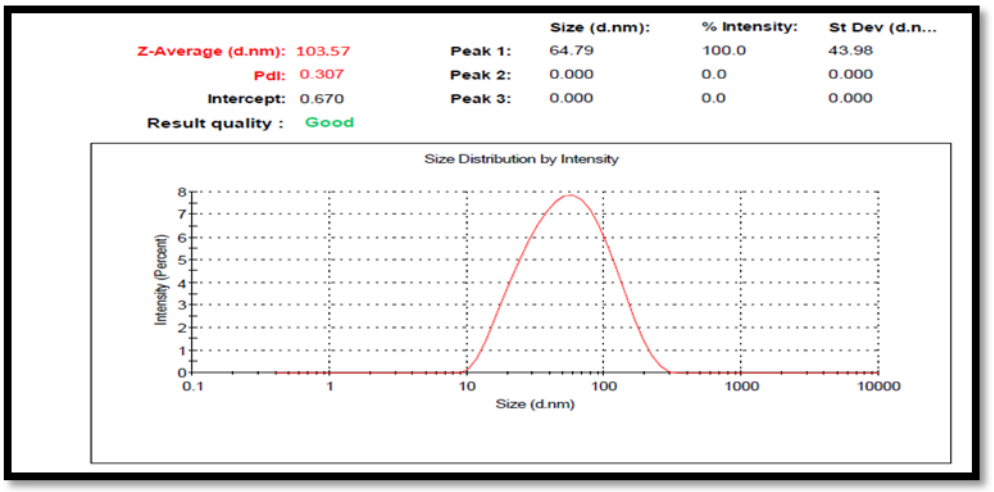


Figure 1.7: Mean particle size of PLGA BM nanoparticle

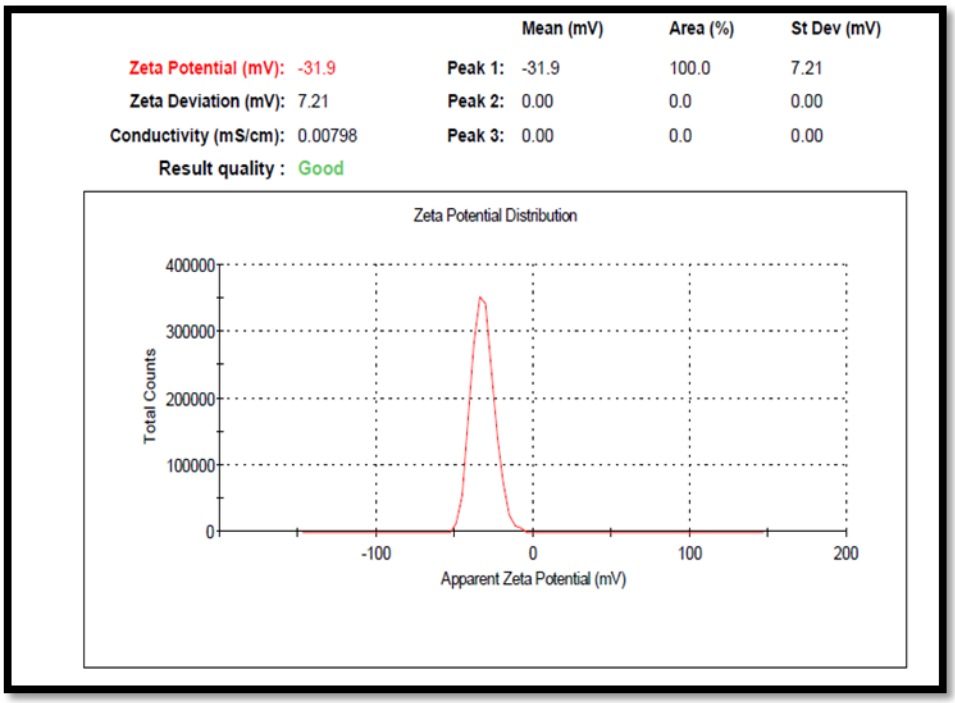


Figure1.8: Zeta potential of PLGA BM nanoparticles

Transmission electron microscopy

The transmission electron microscopy (TEM) was used to determine the morphology of the PLGA Bendamustine loaded polymeric nanoparticles. TEM scan shows the formation of spherical nanoparticle. The scan also discloses that the particles have a more or less uniform size distribution and low polydispersity index.The TEM images are represented in figure 1.9

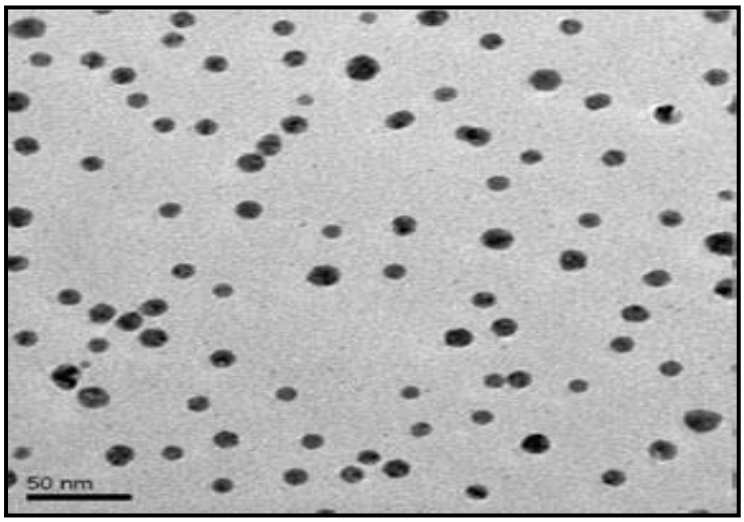


Figure 1.9: TEM image of BM loaded PLGA nanoparticle

Differential Scanning Calorimetry

The pure drug bendamustine displayed a sharp peak that resembles to melting at 155˚C, representing its crystalline nature. The broad peak is observed due to the thermal decomposition of the drug, with maximum temperatures around 400˚C.The PLGA polymer established a characteristic peak at 45.43ºC indicating towards glass transition temperature. Due to amorphous nature of PLGA no separate peak of its melting point was observed.The DSC curve of optimized nanoparticle did not show any crystalline drug material due to absence of sharp peak of bendamustine.

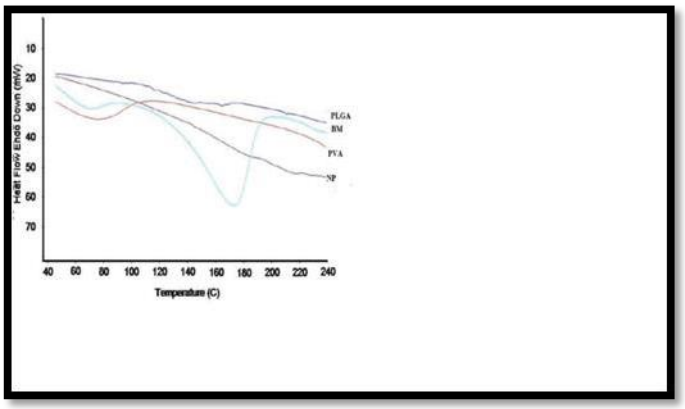


Figure.1.10: DSC thermogram of BM-PLGA nanoparticle

X ray Diffraction study

X-ray diffractogram of pure drug BM presented sharp diffraction peak at 2θ value of 3.3, 11.2, 12.0, 16.6, 7.8, 13.6, 15.4, 23.1 and 32.0. In PLGA nanoparticles deformed peak of BM was observed, indicating that the drug is mixed with PVA and does not exist in free form and comparative reduction in the diffraction intensities was observed in the nanoparticles.This may be because of the change in arrangement of crystals or reduction in the quality of crystals of BM and this result enhances the

conversion of crystalline nature of the drug into amorphous form helping in solubility enhancement.The x-ray diffractogram of pure drug BM, PLGA, PVA and nanoparticle is shown in figure 1.11.

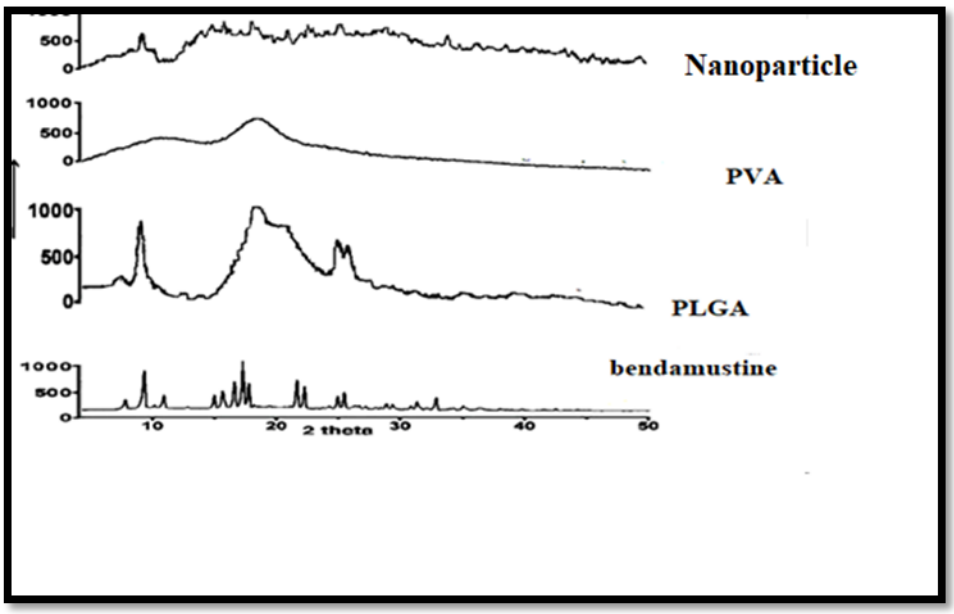


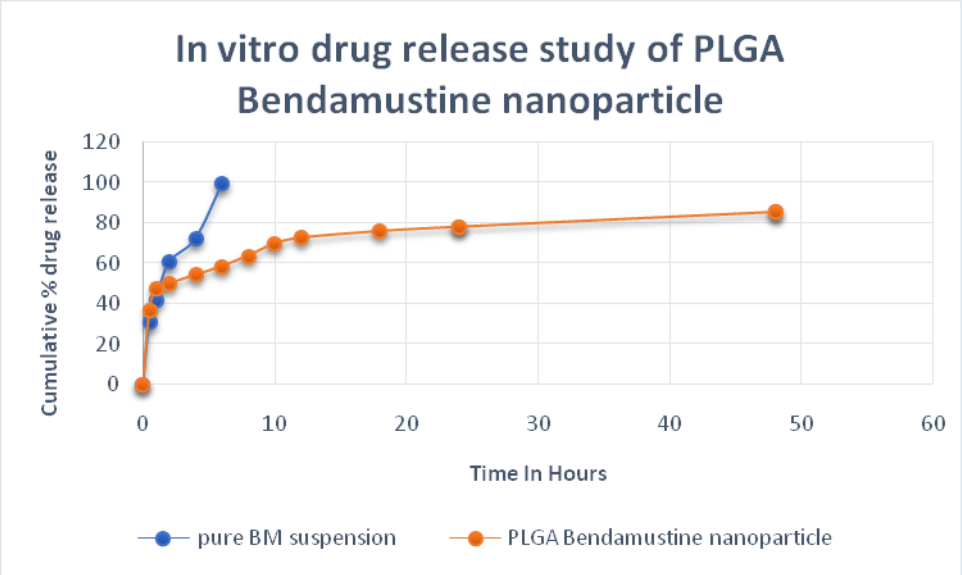
Figure 1.11: X-ray diffraction study of BM- PLGA nanoparticle

In vitro drug release study

The cumulative drug release of optimized PLGA nanoparticle is calculated for 24 hours of duration. The in-vitro release profile of the optimized nanoparticles is calculated in 7.4 pH phosphate buffer at 37^0C was shown in figure.1.44and compared with BM pure drug suspension. From the graph it is clear that pure drug suspension of BM released almost 99.32% of ± 0.40 of drug at the end of 6^{th} hours and optimized PLGA nanoparticle released 85.2 ± 0.24% of drug at its 48^{th} hour.The formulation exhibited a biphasic release profile with initial burst release, and followed by sustained release.

Table.1.7: cumulative drug release of optimized PLGA nanoparticle

Time In Hours	Cumulative % drug release of pure BM suspension	Cumulative % drug release (PLGA Bendamustine nanoparticle)
0	0	0
0.5	30.9 ±1.26	36.4 ± 1.13
1	41.8 ±0.71	47.5 ± 0.20
2	60.5 ± 0.13	49.9 ± 0.59
4	72.1± 0.31	54.2 ± 0.05
6	99.2± 0.40	58.4 ± 0.28
8	-	63.6 ± 0.18
10	-	69.7 ± 0.19
12	-	72.5 ± 0.04
18	-	75.8 ± 1.03
24	-	77.9 ± 0.02
48	-	85.2± 0.24



1.12:Drug release study of pure drug suspension of BM and BM loaded PLGA nanoparticle

Drug Releasee Kinetics

The coefficient of determination (R^2) of equation for release of BM from optimized PLGA nanoparticle in phosphate buffer was > 0.9 signifying first order release pattern.On the basis of best fitwith the highest correlationR^2value (0.98) and the magnitude ofthe release exponentn=0.67 indicates that the drug release mechanism is non-Fickian and follow the Korsmeyer-Peppas model.The results of drug release data closed to numerous drug release evaluation models are presented in table.1.8.

Optimized formulation no.	**Zero order**		**First order**		**Higuchi model**		**Korsmeyer-Peppas**		
	K	**R^2**	**K**	**R^2**	**K**	**R^2**	**K**	**N**	**R^2**
Lyophilized formulation of BM-CH	$0.0120x10^{-1}$	0.846	$1.3209x10^{-3}$	0.9012	1.902	0.960	$5.9492x10^{-3}$	0.674	0.978
BM suspension	$0.0189x10^{-1}$	0.826	$1.3101x10^{-3}$	0.9010	1.898	0.940	$5.9492x10^{-3}$	0.564	0.965

Conclusion

In this present work, Bendamustine loaded PLGA nanoparticles were successfully prepared by an emulsion solvent diffusion technique. Different preparation independent variables such as sonication time, polymer concentration, surfactant concentration was optimized by using factorial design. A 2^3 factorial design was applied to recognize the optimized formulation parameters for nanoparticle preparation with minimum particle size and high drug entrapment efficiency.

The morphology analysis confirmed that the nanoparticles were spherical with almost smooth surface. The preferred formulation (NPF.8) have particle size as 103.5±0.04nm, PDI as 0.368±0.014, zeta potential as -31.9 mV, drug entrapment efficiency 78.13 ± 4.16 %. In vitro release studies indicated non-Fickian or anomalous type of transport for the release of bendamustine from the nanoparticles.

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