

**Research Article****Conjugated nanoconstructs of Dacarbazine for the treatment of metastatic melanoma**

Harish Kumar Chandrawanshi\*, Neelesh Chaubey

College of Pharmacy, Sri Satya Sai University of Technology and Medical Sciences, SH-18, Bhopal-Indore Road, Sehore-466002, M. P., India

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**Abstract**

**Objective:** The prime objective of the study is to develop a dacarbazine loaded formulation with higher drug loading, improved systemic half-life and safety profile which can improve the patient compliance. **Material and Methods:** The Dacarbazine nanoparticles were processed by modified nanoprecipitation method. The processed nanoparticles are further described for particle size and zeta potential. TEM images of the prepared Dacarbazine loaded PLGA nanoparticles shows that they are smooth, spherical, discrete and uniform. The *in vitro* release of Dacarbazine and other studies were performed. **Results:** A raise in the percentage of the polymer an increase of the particle size was observed. Greater value of zeta potential more stable nano suspension and lower value points out the instability. TEM images of the Dacarbazine loaded PLA nanoparticles shows that they are smooth, spherical, discrete and uniform. The increase in concentration of the polymer in the organic phase caused the increase in drug content of the nanoparticles. The percentage drug entrapment efficiency was dependent on the polymer ratio, stirring speed and stirring rpm, entrapment efficiency of the PLA nanoparticles were found to be increased up to drug: polymer ratio of 1:3. An early quick release suggests that some quantity of drug was restricted on the outward of nanoparticles. **Conclusion:** The prepared nanoparticles exhibited high encapsulation efficiency, high drug content and moreover particle size is in nano range, zeta potential determination shows that they are stable. *In vitro* release other studies provided sustained release of the drug and stable formulation.

**Keywords:** Dacarbazine, PLGA, PLA, nanoformulation, melanoma

**Introduction**

Cancer is the major reason of fatality worldwide. The global cancer burden is estimated to have risen to 18.1 million new cases and 9.6 million deaths in 2019. Nowadays incidence of non-melanoma and melanoma skin cancers has mutually been increasing over the precedent couple of years. Among all types of cancer cases reported, the probability of occurrence of skin cancer is one in three according to Skin Cancer Foundation Statistics (SCF, 2017). According to WHO report 2019, approximately 3.5 million non-melanoma skin cancers and more than 140,000 melanoma skin cancers occur globally each year (WHO, 2019).

Melanoma is one of the most dangerous forms of skin cancer

which develops due to damage to skin cells triggering mutation/genetic defects responsible for rapid multiplication of skin cells to form malignant tumor (Nisha et al., 2013). The primary ways of preventing melanoma cancer is to avoid all UV/harmful light exposures but unfortunately increase in environment pollution enhances the vulnerability of cancer (Bharath and Turner, 2009).

Since long back nanotechnology in a part or as a whole is extensively applied to treat the life-threatening diseases like cancer. Currently, efforts are being made to target specifically cancer cells without injuring normal cells (Cristina Beiu et al., 2020). Nanotechnology has driven great advancement in drug delivery by improving safety, stability and targeting drug delivery to specific site (Serkan Yaman et al., 2020). Nanomedicine has gained enormous attention in last two decades after approval of nano drug – Doxil by USFDA. The nano drug doxil was developed for improving bioavailability and mean drug residence duration of drug systemically and subsequent discharge at the tumour location (Zhou et al., 2012). Subsequently, despite meticulous

**\*Address for Corresponding Author:**

Harish Kumar Chandrawanshi,  
College of Pharmacy, Sri Satya Sai University of Technology and  
Medical Sciences, SH-18, Bhopal-Indore Road, Sehore-466002, M.P.,  
India  
Email: hchandrawanshi@gmail.com

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screening and extremely low approval rates by the Food and Drug Administration (FDA), only a few drugs loaded nano-carriers including Abraxane and Marqibo have already been approved for cancer treatment (Eifler et al., 2011).

Dacarbazine is one of the active chemotherapeutic agents approved by Food and Drug Administration for the treatment of melanoma and Hodgkin's lymphoma. However, poor aqueous solubility, shorter half life and higher side effects trim down dacarbazine's use. Various nano-formulations of dacarbazine like nanoemulsions (Srikanth et al., 2011), cubosomes (Bei et al., 2010) and nano-structured lipid carrier (Musallam et al., 2015) are reported in literature. The prolonged shelf-life, enhanced therapeutic efficiency and reduced side effect were observed by these nanocarrier systems (Bei et al., 2010; Ding, 2011; Kakumanu, 2011). Henceforth, with regard to above discussed facts, the present study aims towards formulation and development of polymeric PLGA nanoparticles loaded with dacarbazine and conjugated nanoparticulate drug delivery system of dacarbazine along with the characterization of optimized formulation and toxicological, pharmacokinetic and pharmacodynamic evaluation of optimized formulations in skin cancer melanoma (Naves et al., 2017).

## Materials and methods

### Materials

Drug Dacarbazine was obtained from Celon Labs, Hyderabad, Telangana, PLA & PLGA were obtained from Chem Tech Pro, Vadodara. All the ingredients used were of analytical grade satisfying pharmacopoeia standards. Double distilled water was used for the performance of the experiments.

### Pre-formulation studies

This study data is essential to define the drug material and offer a frame work for the drug mixture with pharmaceutical excipients that can be used in the dosage form. Hence, identification and compatibility studies were carried out in preformulation studies of the selected sample of the drug.

### Identification of pure drug

Differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) studies were performed for the identification of drug and determined the drug-excipient interaction.

### Solubility studies

Solubility analysis was conducted by shake flask solubilization method to dissolve the drug, excipients and other ingredients used in the formulation of nanoconstructs. UV-Visible spectrophotometric method was adopted for the determination of amount of drugs (Prakash et al., 2008).

### Melting Point

The fine powder of Dacarbazine was filled in glass capillary tube

(one end was previously sealed) and kept in melting point apparatus. The melting points were observed and recorded.

### Compatibility studies

**Differential Scanning Calorimetry (DSC):** Differential scanning calorimeter was carried out by using DSC-60 Shimadzu, Japan. Samples were analyzed in a temperature ranging from 0–400 °C, at a heating rate of 5 °C/min under inert nitrogen atmosphere. The samples were prepared by pressing them in a DSC aluminum pans and subjected to analysis. This study was carried out physical interactions between the drug and polymers, if any.

**FT-IR Spectroscopy:** FTIR spectra were recorded by using Bruker (ALPHA-T) analyzer. Samples are pressed into a disc after mixing with KB, and scanned with IR beam from 400 to 4000  $\text{cm}^{-1}$ . Spectral graph of the pure drug, pure polymer and formulated nanoparticles were obtained. Confirmation of the identity of the raw materials and determination of the chemical interactions between the drug and excipients, if any, was obtained from this study.

**Determination of  $\lambda_{max}$ :** Most of the drugs absorbs the light in the ultra violet wavelength (200-400 nm), since they have aromatic groups or contains double bonds. Dacarbazine (100 mg) was dissolved in 100 ml of phosphate buffer solution (pH 7.4) (stock solution). This solution was suitably diluted to obtain 20  $\mu\text{g/ml}$ . The prepared sample was scanned by using UV/Visible spectrophotometer between 200-400 nm. The maximum obtained value was considered as  $\lambda_{max}$  for the pure drug.

### Preparation of Dacarbazine loaded PLGA nanoparticles

Dacarbazine nanoparticles were prepared with modified Nano precipitation method (Bilati, 2007). Briefly in modified nano precipitation method specified amount of Dacarbazine and PLGA was dissolved in 5 ml of acetone. 10 ml of 1% PVA in phosphate buffer 9 was prepared. Added both the solutions and kept for continuous magnetic stirring for 2 hrs to evaporate the organic solvent. The NP suspension is then centrifuged at 3,000 rpm for a time duration of 15 min using high-speed cooling centrifuge (Remi, C4). Sediment was discarded and the supernatant was preserved.

### Preparation of Dacarbazine loaded PLA nanoparticles

Dacarbazine loaded nanoparticles were prepared by the solvent evaporation or nanoprecipitation method (Archana et al., 2012). In this method, acetone is as a solvent for dissolving PLGA and Dacarbazine, and homogenization was carried out at 19,000 rpm for a time period of 5 min. This solution was added drop by drop to previously prepared aqueous polyvinyl

alcohol (PVA) solution with a concentration of 1% w/v. It was continuously homogenized for 20 min. A rotary vacuum evaporator (B-480 Buchi, Switzerland) was used to remove the organic solvent present in the above solution. The final preparation was adjusted to 10 ml, with deionized water.

### Optimization studies

The Optimization Studies for the development of polymeric nanoparticles using modified nanoprecipitation method, nanoprecipitation method and emulsion and solvent evaporation method by altering the polymeric concentrations so as to achieve lesser particle size with higher entrapment of drug.

The stirring speed and the time of stirring were identified as the primary parameters that affect the property of nanoparticles. Various experimental trials were carried out to identify the ideal *viz.* concentration of the polymers used, speed of rotation and time of rotation (Table 1 and 2).

### Characterization of Dacarbazine loaded PLGA Nanoparticles

#### Particle size

The particle size of the prepared nanoparticles was measured by laser diffraction in a mastersizer particle size analyzer.

#### Zeta potential

The Polydispersity index and Zeta potential of the prepared nanoparticles was determined by using a zetasizer. The results of these studies are shown as mean  $\pm$  standard error.

#### Surface morphology

*Scanning electron microscopy (SEM):* The uniformity in the

particle shape and morphology of the prepared nanoparticles were studied using a scanning electron microscope. The nanoparticles were dispersed in water and then drop coated on aluminum stub with a double-sided carbon tape. A gold sputter coating was made over the sample with unit at 10 Pa vacuum for 10 S (SC7620, Japan). The image was captured at desired magnification with a typical acceleration potential used was 30 kV.

*Transmission Electron microscopy (TEM):* The surface morphology of the particles was studied using transmission electron microscopy set at 200 kV by placing an air-dried nanoparticle suspension on copper microscopy grids.

#### Drug content

Drug content was determined by taking 1 ml of the Dacarbazine loaded PLGA, PLA nanosuspension. To the prepared nanosuspension samples (1 ml), 1ml of aqueous potassium di hydrogen phosphate was added and the mixture was centrifuged at  $13000 \times g$  at  $15^\circ C$ . The clear supernatant was removed and analyzed spectrophotometric method and drug content was calculated. Each batch of sample was analyzed in triplicate and the results were measured as mean  $\pm$  SD.

#### Drug loading and Encapsulation/Entrapment efficiency

Percent drug encapsulation efficiency and drug loading capacity were determined spectrophotometrically. The drug loaded nanoparticles were centrifuged at  $13000 \times g$  and the supernatant was assayed for the amount of unbound drug by spectrophotometer (Bilati et al., 2007).

**Table 1.** Various formulations of Dacarbazine loaded PLGA nanoparticles

S. No.	Ingredients	Dacarbazine	PLGA	1% PVA	Acetone
1	DPGN-1	100mg	100mg	50ml	20ml
2	DPGN-2	100mg	200mg	50ml	20ml
3	DPGN-3	100mg	300mg	50ml	20ml
4	DPGN-4	100mg	400 mg	50ml	20ml

**Abbreviations:** DPGN = Dacarbazine loaded PLGA Nanoparticles, PLGA = Poly lactic glycolic acid

**Table 2.** Various formulations of Dacarbazine loaded PLA Nanoparticles

S. No.	Ingredients	Dacarbazine	PLA	1% PVA	Acetone
1	DPLN-1	100mg	100mg	50ml	20ml
2	DPLN-2	100mg	200mg	50ml	20ml
3	DPLN-3	100mg	300mg	50ml	20ml
4	DPLN-4	100mg	400 mg	50ml	20ml

**Abbreviations:** DPLN = Dacarbazine loaded PLA Nanoparticles, PVA = Poly vinyl alcohol

### **In vitro drug release studies**

The *in vitro* release profiles of the prepared nanosuspensions were studied by diffusion across an artificial membrane to determine the permeation/release rate of drug from optimized formulations. Suitable compendia method of dissolution used for determining the release behavior and kinetics of drug release from formulation.

Nanosuspension with known concentration of Dacarbazine was taken in double opened diffusion tube with semipermeable membrane tied at one end (donor compartment). Specified volume of buffer was placed in a 250 ml beaker (receptor compartment) and the nanosuspension loaded diffusion tube was dipped in the buffer solution. The buffer in the receptor compartment was constantly agitated using a magnetic stirrer which was maintained at 37°C. Equivalent volume of the fresh media was added after withdrawal of each sample from the receptor compartment for estimation of released drug. The experiment was carried out in triplicate and the values were reported as mean value  $\pm$  standard deviation (Shrinidh et al., 2010).

### **Stability studies**

Stability is used to define as the extent to which the product remains within specified limits throughout its period of storage and use. The chemical and physical stability of drug was determined according to ICH guidelines under accelerated storage environment ( $40 \pm 2^\circ\text{C}/75 \pm 5\% \text{RH}$ ) and long term storage environment ( $25 \pm 2^\circ\text{C}/60 \pm 5\% \text{RH}$ ) (ICH guidelines).

## **Results and discussion**

### **Solubility of pure Dacarbazine**

The solubility of drug was determined by for various solvents

like water, ethanol, acetone chloroform etc serial solubilization method, drug found slightly soluble in almost all solvents.

### **Melting point**

The melting point of sample of Dacarbazine was observed and was recorded 214-215°C.

### **Compatibility Studies**

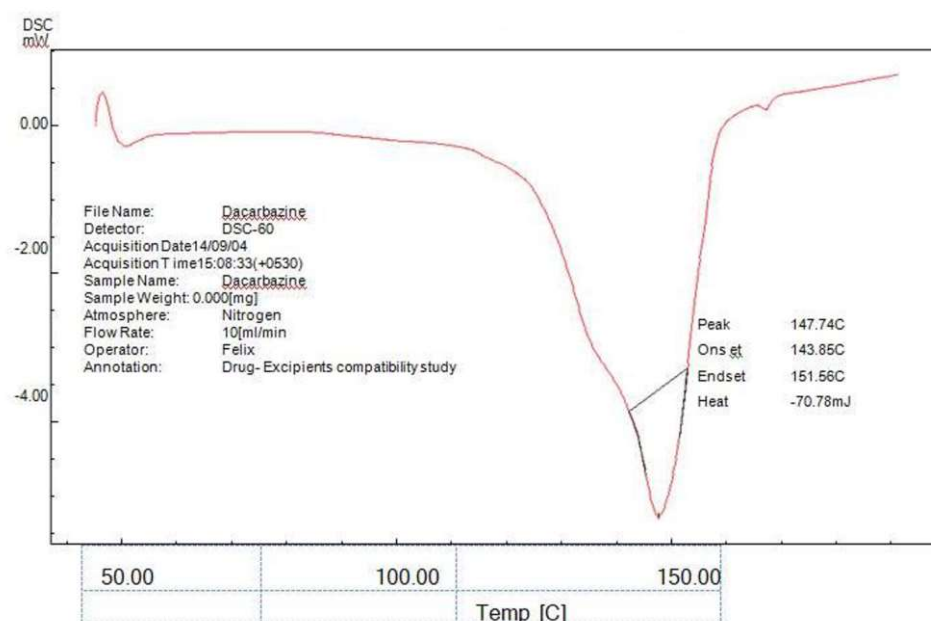
#### **Differential Scanning Calorimetric Analysis (DSC)**

The DSC Thermal analysis is suitable for characterizing relations among multiple constituents of the solid ingredients. DSC was used to evaluate changes in thermodynamic effects that occur when solid supplied heat energy. Variations can be observed in the method of melting, desolvation, recrystallization and solid phase alterations indicated by endothermic or exothermic peaks of thermogram. DSC thermogram showed solid endothermic peak of Dacarbazine at 148.1°C. The DSC curve reveals that there is no significant interaction in the endothermic peak of the drug, polymer and surfactant in the physical combination. The Thermograms of Dacarbazine, PLGA and PVA were shown in figure 1.

#### **FT-IR Spectroscopy**

The FT-IR spectroscopy study was performed out individually to determine the compatibility among Dacarbazine and the excipient PLGA, PVA used for the preparation of nanoparticles. The FT-IR study was performed for Dacarbazine, PLGA and physical blend of Dacarbazine.

On comparing the peaks in FT-IR spectrums, it can be



**Figure 1.** DSC Thermogram of Dacarbazine

interpreted that the characteristic peaks of Dacarbazine were not affected in the formulations. It confirms that there was no significant chemical incompatibility between Dacarbazine and the excipients used for this formulation.

The FT-IR spectrum of pure Dacarbazine, polymer, and the prepared nanoparticles were studied to detect any compatibility issues. The peaks in The IR spectrum of Dacarbazine were compared with that of the prepared nanoparticles (Figure 2).

### Preparation and Characterization of PLGA nanoparticles containing Dacarbazine

The Dacarbazine nanoparticle was prepared by modified nanoprecipitation method. In this method, the polymer, Dacarbazine, and the stabilizer are dissolved in organic solvent acetone. The above solution is poured in magnetic stirring into an aqueous having stabilizer, which led to the formation of nanoparticles; by rapid diffusion. The usefulness of this method is limited to water miscible solvents, in which the diffusion rate is enough to yield spontaneous emulsification.

Sufficient numbers of experiments/trials were performed to determine the suitable conditions for incorporation of the into the PLGA nanoparticles. An effective entrapment was attained by dissolving the Dacarbazine in the PLGA solution, followed by the addition of PVA. The prepared nanoparticles were discrete and free flowing. The optimization studies were carried out by the process and product optimization and the

characterization studies were carried out for the selected formulations.

### Optimization studies

Optimization studies were carried out by varying in polymeric concentration, speed of rotation and time of rotation.

### Characterization of Dacarbazine loaded PLGA nanoparticles

#### Particle size

The particle size analyses were carried out for all the batches of nanoparticles prepared for the optimization. The results of the particle size were revealed in the table 3.

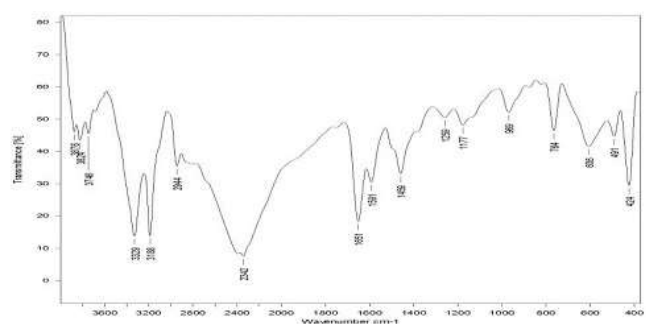
#### Zeta potential

The surface charge of the nanoparticles was assessed by evaluating the zeta potential of the nanoparticles by Malvern zetasizer. The results were shown in table 3.

#### Surface morphology

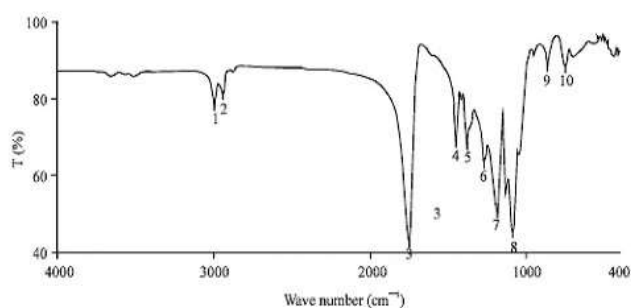
##### Transmission Electron Microscopy (TEM)

TEM provides the particulars about inner composition such a morphology, crystallization, strain or even magnetic areas. TEM images of the prepared Dacarbazine loaded PLGA nanoparticles shows that they are smooth, spherical, discrete and uniform. No drug crystals were spotted in the images.

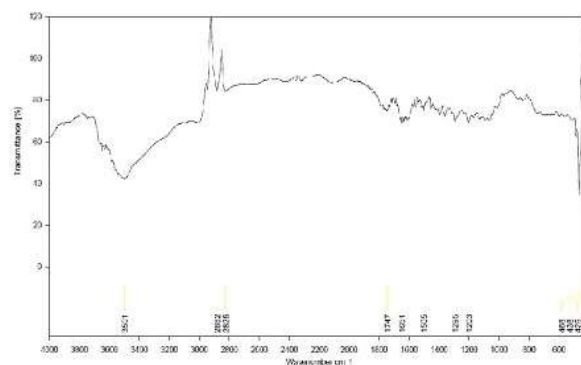


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Sample Form: Instrument type and / or accessory  
Sample Scans: 10

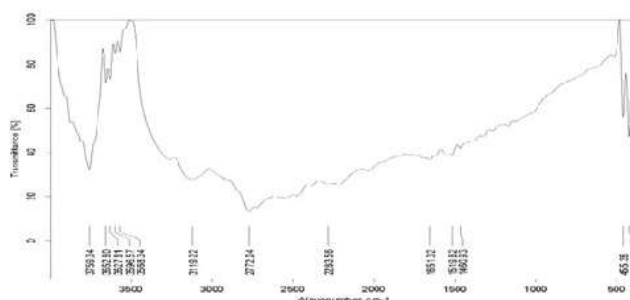
FTIR Spectrum of Dacarbazine



FT-IR spectrum of PLGA



FTIR Spectrum of PVA



FTIR Spectrum of Dacarbazine and polymers

Figure 2. FTIR of drug and polymers

**Table 3.** Particle size and Zeta potential of the loaded PLGA nanoparticles

Sl. No	Code	Particle size (nm)	Zeta potential (mV)
1	DPGN-1	273±1.7	-27±1.51
2	DPGN-2	266±1.4	-29±1.8
3	DPGN-3	246±2.4	-27±1.4
4	DPGN-4	289±3.2	-25±1.6

### Drug content and Entrapment efficiency

The Drug content of the prepared Dacarbazine loaded PLGA nanoparticles were evaluated after making suitable dilutions using the established analytical method. The drug content of DPGN-3 was found to be 0.955µg/ml. The rise in concentration of polymer in the organic phase produced an increase in drug content of the nanoparticles.

The encapsulation efficiency of Dacarbazine in the PLGA nanoparticles was 68.50 to 83.15% which is quite satisfactory. However, the percentage of entrapment efficiency of the drug was reliant on the polymer ratio, stirring speed and stirring rpm. The nanoparticles with DPGN-3 shows average percentage of entrapment efficiency of 83.15%, formulation with DPGN-1, DPGN-2, DPGN-4, shows 68.50%, 74.72%, and 79.00% respectively. The entrapment efficiency of The PLGA nanoparticles were originates to be increased up to drug: polymer ratio of 1:3. This may be due to increased adsorption of the Dacarbazine on the surface of the polymeric matrices. However, a further increase of polymeric concentration had not indicated increase in entrapment efficiency.

### In vitro drug release studies

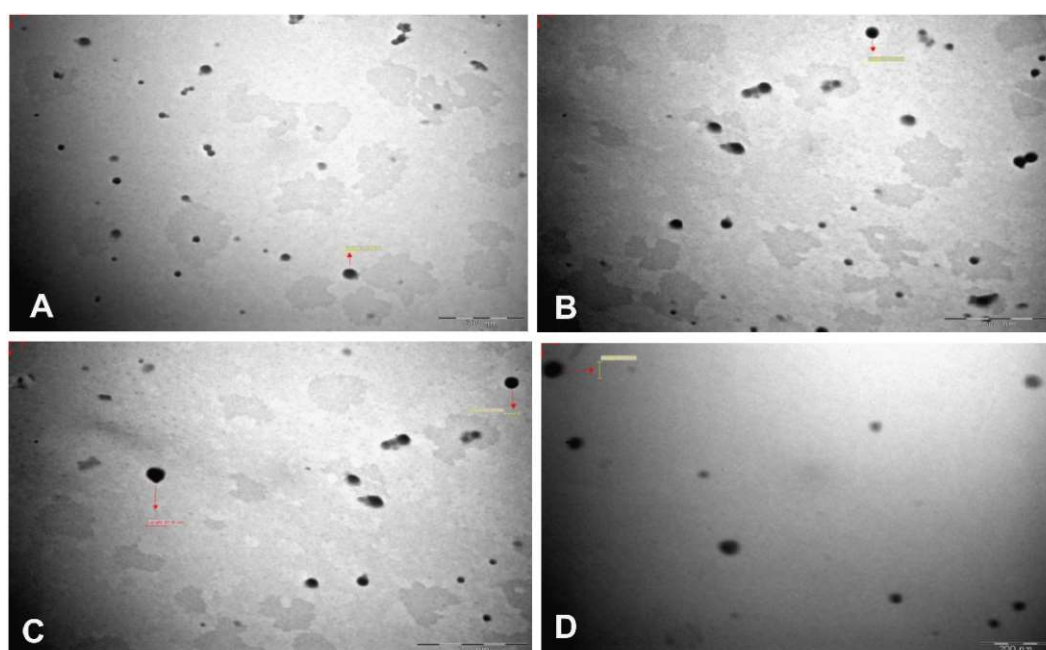
The *in vitro* release of Dacarbazine loaded PLGA nanoparticles

showed prolonged and sustained release of Dacarbazine. The results of *in vitro* diffusion studies of optimized nanoparticles were shown in the table 4. It was obvious that *in vitro* release of Dacarbazine exhibited rapid initial burst release and then followed by the sustained release up to 24 hrs. An initial quick release suggests that specific quantity of drug was confined to the surface of nanoparticles. DPGN-3 shows maximum release of drug when compared to other batches of nanoparticles.

The comparison of the results of optimized Dacarbazine PLGA nanoparticles, created on the particle size, zeta potential, drug content, entrapment efficiency and *in vitro* release studies has been shown in table 5. The formulation DPGN-3 indicated the least particle size, higher zeta potential, drug content, entrapment efficiency and sustained drug release. Hence among the different trials of Dacarbazine loaded PLGA nanoparticles, DPGN-3 has been identified to carry out further studies.

### Stability studies

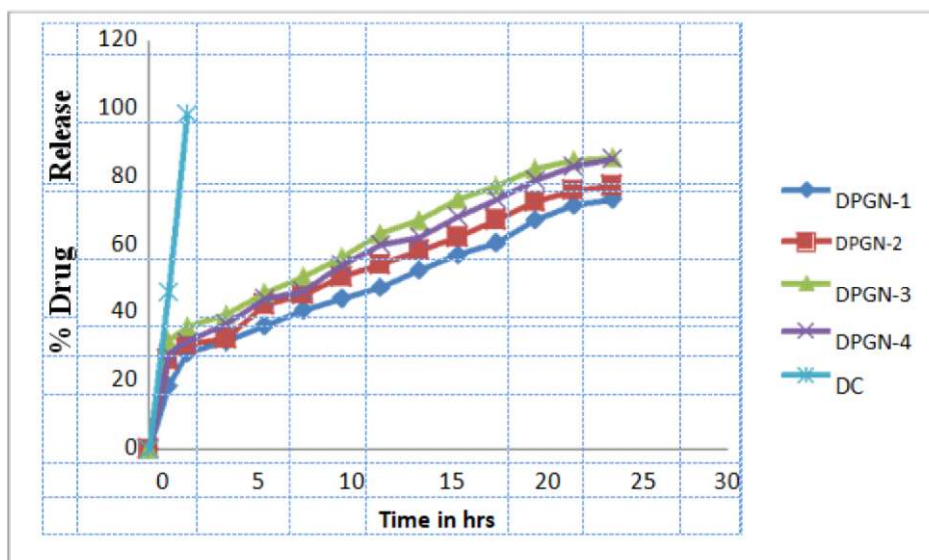
Stability studies were carried out for DPGN-3. The prepared Dacarbazine loaded PLGA formulations were



**Figure 3.** TEM micrographs of (A) DPGN-1 (B) DPGN-2 (C) DPGN-3 and (D) DPGN-4 nanoformulation

**Table 4.** Characterization studies report of optimized nanoformulation

Code	Particle size (nm)	Zeta potential (mV)	Drug content ( $\mu\text{g/ml}$ )	Entrapment efficiency (%)	<i>In vitro</i> drug release (%) (24 hrs)
DPGN-3	246 $\pm$ 2.4	-27 $\pm$ 1.4	0.955 $\pm$ 1.3	83.15 $\pm$ 1.4	86.67 $\pm$ 2.4

**Figure 4.** *In vitro* drug release profiles of Dacarbazine loaded PLGA nanoformulations in Phosphate buffer pH .4

stored at the following conditions *i.e.*, 5  $\pm$  3  $^{\circ}\text{C}$ , 30 $\pm$ 2  $^{\circ}\text{C}$ , 65%  $\pm$  5% RH (long term stability), 40 $\pm$ 2  $^{\circ}\text{C}$ , 75%  $\pm$  5% (accelerated stability). Every three months the drug content, *in vitro* release studies were determined for the nano-formulation subjected for long term stability studies.

The drug content of DPGN-3 stored at 5 $\pm$ 3 $^{\circ}\text{C}$  for a period of 12 months exhibited a slight fall in the drug content when compared to the initial drug content of the nano-formulation after storing the sample for 12 months.

The optimized nanoformulation DPGN-3 at 40 $\pm$ 2 $^{\circ}\text{C}$ , 75 $\pm$ 5% RH after 0, 6, & 12 months indicated substantial decrease in cumulative drug release when related to the initial cumulative drug release of the same nanoformulation.

On comparing the drug content after storing the optimized nanoformulation DPGN-3 at 5 $\pm$ 3 $^{\circ}\text{C}$ , 30  $\pm$  2 $^{\circ}\text{C}$ , 65  $\pm$  5% RH, when compared to previous data of the same formulation there was a minor decrease in the drug content after 12 months of the storage.

Every three months the *in vitro* drug release almost remains the same for the optimized nano-formulation DPGN-3 stored at 5 $\pm$ 3 $^{\circ}\text{C}$ , 30  $\pm$  2 $^{\circ}\text{C}$ , 65  $\pm$  5% RH up to 12 months.

However, The Dacarbazine nano formulation, which was subjected for accelerated stability studies on 40  $\pm$  2 $^{\circ}\text{C}$ , 75 $\pm$ 5% RH, shows a major decrease in drug content and *in vitro* drug release. It may be due to storage at high temperature which leads the degradation of Dacarbazine loaded PLGA nanoparticles. Hence, there is a major

decrease in the drug content and cumulative percentage of *in vitro* drug release. Therefore, from the stability studies it was observed that the prepared Dacarbazine loaded PLGA nano-formulation DPGN-3 will be stable at 5 $\pm$ 3 $^{\circ}\text{C}$ , 30  $\pm$  2 $^{\circ}\text{C}$ , 65  $\pm$  5% RH for a period of 12 months.

#### Compatibility Studies

##### Differential Scanning Calorimetric Analysis (DSC)

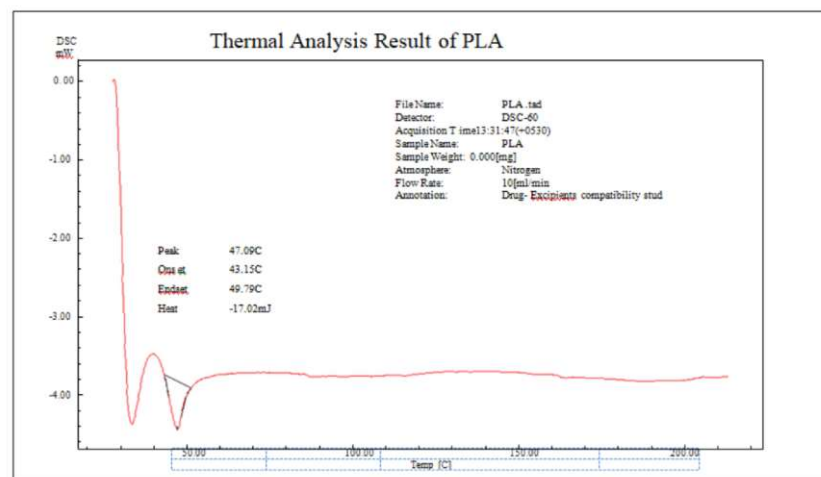
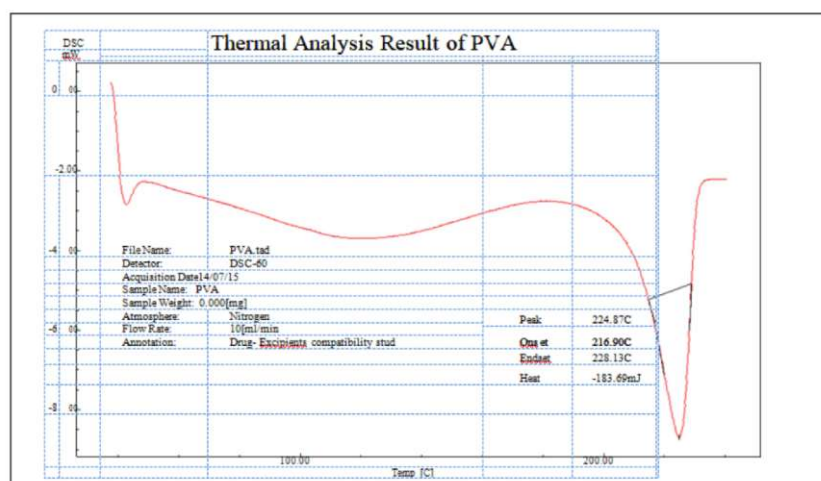
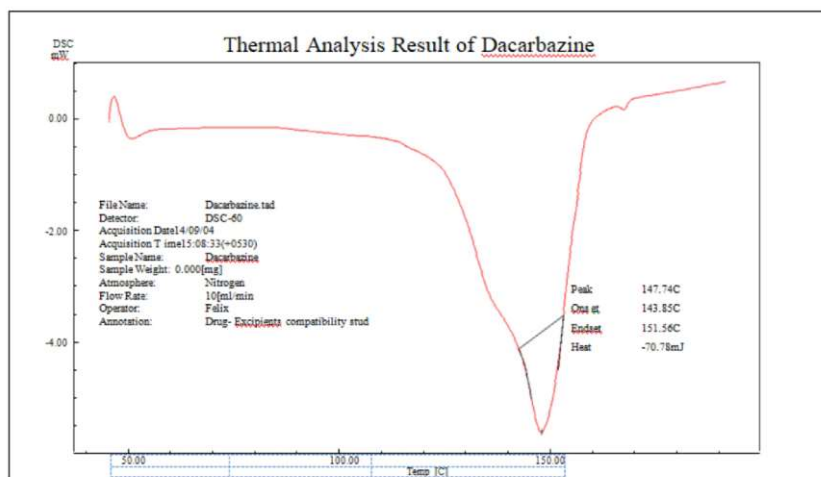
Thermal analysis was used to assess fluctuations in Thermodynamic properties that happen when the material supplied heat energy. Variations that can be detected in the method of melting, desolvation, recrystallization and solid phase changes indicated by endothermic or exothermic peaks at Thermogram. DSC Thermogram showed solid endothermic peak of Dacarbazine at 148.4 $^{\circ}\text{C}$ . DSC Thermogram of physical mixture shows three endothermic peaks at 223.14  $^{\circ}\text{C}$  respectively. The DSC curve reveals that there is no major interface in the endothermic peak of the drug, polymer and surfactant in the physical mixture. The Thermograms of Dacarbazine, PLA and PVA were shown in figure 5.

##### Preparation of PLA nanoparticles

PLA nanoparticles loaded with Dacarbazine were processed by the nanoprecipitation method. To optimize the product parameters and process parameters 04 typical formulations were designed and studies were carried out for Dacarbazine loaded PLA nanoparticles. The prepared nanoparticles were discrete and free flowing.

**Table 5.** Particle size and zeta potential of Dacarbazine loaded PLA nanoparticles

Sl. No	Code	Particle size (nm)	Zeta potential (mV)
1	DPLN-1	219±1.2	-20±2.1
2	DPLN -2	232±2.3	-22.6±1.5
3	DPLN -3	245±1.4	-22.4±1.8
4	DPLN -4	251±3.1	-21.3±1.2

**Figure 5.** DSC Thermogram of Dacarbazine, PLA and PVA

## Characterization of Dacarbazine loaded PLA nanoparticles

### Particle size

The literature review that the size of particles is extremely reliant on the preparation method adopted and conditions employed. Though the previous reports suggests that a rise in the concentration of the polymer a gain of the particle size. The Dacarbazine loaded PLA nanoformulations were optimized by product and process parameters. The mean particle size of nanoformulations were carried out which was tabulated in table 6. However, the particle size also changes with polymer viscosity and stirring speed of rotation of the stirrer.

### Zeta potential

The surface charges of the nanoparticles were assessed by evaluating the zeta potential of the nanoparticle by Malvern zeta sizer.

### Surface morphology

#### Transmission Electron Microscopy (TEM)

The particularly internal composition such morphology, crystallization, stress or even magnetic fields were studied. TEM images of PLA nanoparticles containing Dacarbazine were found to be smooth, spherical and uniform.

### Drug content

The samples were examined after making suitable dilutions using the well well-known analytical procedure. The drug content of PLA nanoparticles was found to be 0.855, 0.983, 1.205 and 0.857 $\mu$ g/ml for DPLN-1, DPLN-2, DPLN-3 and DPLN-4 respectively. The raise in concentration of polymer in organic phase produced an increase in drug content of the nanoparticles.

## Entrapment efficiency

Drug Entrapment efficiency shows important role in preparation of a drug delivery method particularly for expensive drugs and directly associated to the Therapeutic properties of the system. The encapsulation efficiency of Dacarbazine in the PLA nanoparticles was within the range of 62.53 to 72.32% which is quite satisfactory. The percentage entrapment efficiency of the optimized Dacarbazine loaded PLA nanoparticles were resolved by the technique described.

However, the percentage of entrapment efficiency of the drug was reliant on the polymer ratio, stirring speed and stirring rpm. The nanoparticles with DPLN-3 show average percentage of entrapment efficiency of 74.53%, formulation with DPLN-1, DPLN-2, and DPLN-4 show 62.53%, 67.25% and 73.23% respectively. The entrapment efficiency for PLA nanoparticles were found to be increased up to drug: polymer ratio of 1:3. This may be due to increased adsorption of the dacarbazine on the surface of the polymeric matrices. However, a further increase of polymeric concentration had not indicated increase in entrapment efficiency.

### In vitro drug release

The *in vitro* release of Dacarbazine loaded PLA nanoparticles showed prolonged and sustained release of Dacarbazine. The results of *in vitro* diffusion studies of optimized nanoparticles were shown in the table 6. It was obvious that *in vitro* release of Dacarbazine exhibited quick initial burst release and then followed by the sustained release up to 24 hrs. An initial quick release

**Table 6.** *In vitro* Drug Release profile of optimized Dacarbazine loaded PLA formulations in phosphate buffer pH 7.4.

Time (hrs)	DPLN-1	DPLN-2	DPLN-3	DPLN-4	Dacarbazine
0	0	0	0	0	0
1	19.37 $\pm$ 1.3	26.24 $\pm$ 1.5	29.31 $\pm$ 1.3	27.35 $\pm$ 1.2	49.8 $\pm$ 1.5
2	29.18 $\pm$ 1.2	33.48 $\pm$ 2.4	35.38 $\pm$ 1.6	34.13 $\pm$ 1.3	99.01 $\pm$ 2.1
4	31.53 $\pm$ 3.2	35.25 $\pm$ 2.3	40.34 $\pm$ 1.7	38.85 $\pm$ 2.4	--
6	37.57 $\pm$ 3.4	43.73 $\pm$ 1.5	47.13 $\pm$ 2.2	45.67 $\pm$ 1.7	--
8	41.16 $\pm$ 3.5	47.42 $\pm$ 2.1	52.27 $\pm$ 1.5	47.57 $\pm$ 1.7	--
10	45.71 $\pm$ 3.5	50.83 $\pm$ 1.4	57.23 $\pm$ 2.4	55.31 $\pm$ 1.4	--
12	47.52 $\pm$ 1.9	53.23 $\pm$ 1.4	64.89 $\pm$ 1.3	61.64 $\pm$ 1.8	--
14	52.32 $\pm$ 2.2	59.12 $\pm$ 3.5	69.39 $\pm$ 1.4	63.57 $\pm$ 2.3	--
16	58.87 $\pm$ 2.5	63.39 $\pm$ 3.1	74.93 $\pm$ 2.1	69.13 $\pm$ 2.7	--
18	60.75 $\pm$ 1.7	67.52 $\pm$ 1.5	78.75 $\pm$ 3.1	74.82 $\pm$ 3.4	--
20	68.52 $\pm$ 1.6	73.64 $\pm$ 1.7	84.53 $\pm$ 2.4	80.96 $\pm$ 2.1	--
22	73.75 $\pm$ 2.0	76.72 $\pm$ 2.5	87.95 $\pm$ 3.0	84.75 $\pm$ 2.7	--
24	75.39 $\pm$ 3.2	78.73 $\pm$ 2.4	92.59 $\pm$ 1.8	90.15 $\pm$ 1.5	--

**Table 7.** Characterization studies report of optimized nanoformulation

Code	Particle size (nm)	Zeta potential (mV)	Drug content ( $\mu\text{g/ml}$ )	Entrapment efficiency (%)	<i>In vitro</i> drug release (%) (24 hrs)
DPLN-3	245 $\pm$ 1.4	-22.4 $\pm$ 1.8	1.25 $\pm$ 1.6	92.59 $\pm$ 1.3	92.59 $\pm$ 1.8%

recommends that some quantity of drug was confined on the surface of nanoparticles. DPLN-3 shows maximum release of drug when compared to other batches of nanoparticles.

The comparison of the results of optimized The PLA nanoparticles, based on size of particle, zeta potential, drug content, entrap efficiency and *in vitro* release study, the formulation DPLN-3 was indicated the least particle size, higher zeta potential, drug content, entrapment efficiency Then drug release. Hence among the different trials of Dacarbazine loaded PLA nanoparticles. DPLN-3 has been identified to carry out further studies.

#### Stability studies

Stability studies were performed for DPLN-3. The prepared dacarbazine loaded PLA formulations were stored at the following conditions i.e., 30 $\pm$ 2 °C, 65%  $\pm$  5% RH (long term stability), 40 $\pm$ 2°C, 75%  $\pm$  5% RH (accelerated stability). Every three month the drug content, *in vitro* release studies were carried out for the nanoformulation subjected for long term stability studies.

The drug content of DPLN-3 stored at 5 $\pm$ 3°C for a period of 12 month showed a slight decrease in the drug content when compared to the initial drug content of the nanoformulation after storing the sample for 12 month.

The optimized nanoformulation DPLN-3 at 40 $\pm$ 2°C, 75 $\pm$ 5% RH after 0, 6, & 12 months showed important decrease in cumulative drug release when associated to the initial cumulative drug release of the same nanoformulation. On comparing the drug content after storing the optimized nanoformulation DPLN-3 at 5 $\pm$ 3 °C, 30  $\pm$  2 °C, 65  $\pm$  5% RH, when compared to previous data of the same formulation there was a slight decrease in the drug content after 12 months of the storing.

Every three months the *in vitro* drug release almost remains the same for the optimized nanoformulation DPLN-3 stored at 5 $\pm$ 3°C, 30 $\pm$ 2°C, 65  $\pm$  5% RH up to 12 months.

However, the nanoformulation, which was subjected for accelerated stability studies at 40  $\pm$ 2°C, 75 $\pm$ 5% RH, shows a major decrease in drug content and *in vitro* drug release. It may be due to storage at high temperature which leads the degradation of the loaded PLA nanoparticles. Hence, there is a major decrease in the drug content and cumulative percentage of *in vitro* drug release. Therefore, from the stability studies it was

witnessed that the prepared the loaded PLA nanoformulation DPLN-3 will be stable at 5 $\pm$ 3°C, 30  $\pm$  2°C, 65  $\pm$  5% RH for a period of 12 months.

#### Conclusion

Dacarbazine loaded polymeric nanoparticles were designed, optimized and prepared by emulsion polymerization method. Two widely accepted and physiologically safe polymers PLGA and PLA were used to for the development of nanoformulations. The physiochemical characterization and other performances of the formulations were evaluated and found suitable for treatment of melanoma disease. In this study, Dacarbazine hydrochloride is selected as a model drug. The prepared formulations were found to be stable at room temperature for period of 1 year. The nanoformulations are intended for sustained release of the drug for a period of 24 hours and this may reduce the dosing frequency, thereby minimizing the side effects. Thus, the nanoparticles of Dacarbazine can potentially improve the therapeutic activity of Dacarbazine, with simultaneous reduction in decrease of free drug in general systemic circulation. This will considerably reduce the adverse drug reactions of Dacarbazine, it should be noted that the predominant adverse drug reactions over the therapeutic activity is the major reason for to withdraw in the market. Hence the nanoparticulate system of Dacarbazine indicates the possibilities to bring back the drug to the market. However more patient studies are suggested.

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