JOURNAL OF CRITICAL REVIEWS

ISSN- 2394-5125 VOL 08, ISSUE 02, 2021

PREPARATION AND CHARACTERIZATION OF PEG-ALBUMIN-CISPLATIN (PAC) NANOPARTICLES INTENDED TO TREAT BREAST CANCER

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Running title: Improvement of bioavailability of PEGylated-albumin nanoparticles for Breast cancer.

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ABSTRACT

The present research aimed to prepare serum stable long-circulating PEGylatedCisplatin-BSA nanoparticles for breast cancer and was prepared by desolvation technique. Prepared nanoparticles were characterized for drug entrapment efficiency (EE), particle size, scanning electron microscope (SEM), zeta potential, Fourier transform infrared spectroscopy (FTIR), and differential scanning calorimetry (DSC). *In-vitro* release studies were performed in phosphate buffer saline pH 7.4 at 37˚±0.5˚C for 21 days. The in-vivo animal studies were performed for drug release studies for 28 days, and a cell viability assay was performed. The entrapment efficiency was about 80.5%,the mean particle size of obtained nanoparticles was 2.55-27.5 µm, and it was spherical with a smooth surface. The zeta potential is found to be -28 mV, and the nanoparticles are found to be stable. FT-IR studies conducted drug-excipient interaction studies,

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demonstrating that the drug was not changed during the fabrication process. The DSC results also showed no significant shift in the endothermic peaks confirming the drug's stability in the formulations. Polymeric peaks revealed that the drug is in an amorphous state in the formulations. The Cisplatin-BSA nanoparticles exhibit a most interesting release profile with a small initial burst followed by a slower and controlled release with improved bioavailability of 3-fold than pure drug.

Keywords: Cisplatin, albumin, long-circulating polymeric nanoparticles, PEGylation, desolvation, breast cancer.

INTRODUCTION

Novel/Controlled drug delivery technology represents one of the broader areas of science that involves a multidisciplinary scientific approach, contributing to human health care and pharmaceutical development[1]. Drug targeting is a selective drug release at specific physiological sites, cells, tissues, organs, or in which the pharmacological effect is required. The concept of drug targeting is used to improve the therapeutic index and efficacy of drugs by increasing their localization to specific organs, tissues, or cells and decreasing their potential toxic, side, and adverse effects at normal, sensitive sites. In targeted cancer therapy, conventional chemotherapeutic agents lack intrinsic target specificity. They are rationally modified to focus and redirect their cytotoxicity to tumor cells[2]. The development of a drug delivery system faces several challenges reaching the target site, which is often far away from the administration site (drug targeting), remaining at the target site to deliver the drug, preferably in a time-controlled manner, limiting the drug's adverse effects and ensuring biocompatibility. The efficacy of many drugs is often limited by their potential to reach the site of therapeutic action.

In most cases (conventional dosage forms), only a small amount of administered dose reaches the target site. At the same time,most of the drug distributes throughout the rest of the body by its physicochemical and biochemical properties. Therefore, it is challenging to develop a drug delivery system that optimizes a drug's pharmaceutical action while reducing its toxic side effects in vivois.

Chemotherapy has become an integral component of cancer treatment for most cancers. Conventional chemotherapeutic agents exhibit poor specificity in reaching tumor tissue and are often restricted by dose-limiting toxicity. Developing controlled release and targeted drug

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delivery may provide a more efficient and less harmful solution to overcome the limitations found in conventional chemotherapy[3]. Serious side effects often limit the efficacy of cancer chemotherapy because of the toxicity of anticancer drugs to both tumor and normal cells. Cancer is one of the most challenging diseases, characterized by developing mutated cells that divide uncontrollably.Its pathogenesis may result from geneticderegulation or mutations that result from acute or chronic exposure to xenobiotics orenvironmental pollutants. Cancer cells can spread to different organs in a process calledmetastasis. The conventional therapeutic strategies used in cancer treatment are chemotherapy, surgery,and radiation therapy.These therapies are used routinelybased on the disease's pathological stages and clinical signs. Despite the advancesin treatment protocols, the patient'slong-term survival is low, and there is a high incidenceof adverse effects of chemotherapy. Often, drugs used in chemotherapy have poor watersolubility. Hydrophobic drugs have reduced biocompatibility and must beadministered in higher dosages to achieve therapeutic concentrations[4]. Also, low hydrosolubilitytranslatesintoreduceddrugavailabilityandhighsystemtoxicity.Besides,chemotherapy drugs generally have little specificity and cause significant damage tohealthy tissues and, consequently, cause adverse reactions.

In addition to the problemsrelatedtotheanticancerdrugsaddressed,early cancer detection isalsoachallenge.Inthis sense, the combination of target-oriented drug delivery systems and controlled release can bethealternativestoovercome some limitationsofconventionalchemotherapy,andnanotechnologymightbethesolution.

Nanotechnology includes a broad multidisciplinary field that has evolved rapidlyoverthepastdecade[5].Theword "nanoparticle" comesfromtheGreekword "Nanos," whichmeans "dwarf" particles.Theprefix "nano" means "onebillion."According to the American Society for Testing and Materials (ASTM) E2456-06 standard, ananoparticleisaparticlewhosesizeisbetween1and100nm.However,thisdefinitionhas not been strictly followed by all authors. Thus, some publications report that particleslargerthan100nmarenanoparticles. Recently, nanotechnology has evolved rapidly and applied in the mostdiverse domains, including in the medical field.Nanoparticles can be used in severalareas, such as in tissue engineering, pharmaceutical, aerospace, and microelectronicsindustries, in the production, processing, protection, and packaging of food, among others[6].

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Oraland injectablesarethemostusedroutesinadministeringdrugsthroughconventionalpreparations,such assolutions,emulsions,suspensions,andsolidpharmaceutical forms (tablets, capsules, etc.).However, in some cases, these preparations can have limitations, namely, reduced efficacy due to the difficulty of the drug reaching exclusively its specific site of action, by circulating throughouttheorganismandaffectingbothunhealthy andhealthy cells,possibly causingserious adverseeffects[7]. Currently,nanotechnologyhasallowedextraordinaryprogressinthetransportandrelease of drugs in specific target locations in living organisms and in a specificintended time (drug release time control).The development of nanosystems tunedfor the release of a drug at a specific target location often reduces some of theadverse effects and toxicity of the carried drug. Several nanocarriers are beinginvestigated, including organic (liposomes, dendrimers, micelles, among others) andinorganicnanoparticles,suchasmagneticnanoparticles,silver nanoparticles,goldnanoparticles,quantumdots,etc.Inadditiontoorganicandinorganic nanoparticles,therearenanoparticlesofahybridnature,suchasnanoparticleswithaninorganiccore surroundedbyorganicmaterial. A targeted drug delivery system must control the drug's fate in thebody, protecting the cells and tissues that are not the target of the therapy[8]. These drugs'nanocarriers are endowed with optimized and well-defined physical, chemical, and biologicalpropertiestoimprove their cellular uptake orofthedrugaboutlarger molecular structures. Additionally, the possibility of controlling the size, surfacecharge,andsurfacechemistryofthenanoparticlesactingascarriers,aswellasthereleaseofpre-loaded drugs at a specific site, allows overcoming other limitations of conventionaltherapies, namely, the need for higher dosages, low bioavailability, and chemical instabilityof the administered drug. Suppose the nanocarriers are designed and produced to accumulate on the target successfully. In that case,therewillbelowersystemicadverseeffectsandbettertherapeuticefficacy.Nowadays,theproducti onofnanoparticlestailoredwithpre-definedphysical-chemicalpropertiesallowsforadapting thedrug-deliverynanoparticleto specific cancer and different anticancer drugs since each type of cancer hasuniquebiologicalexpressions. Each nanocarrier transports more than one molecule of a drug, thus increasing the concentration of the drug successfully delivered to the target tumor whilesimultaneouslywithoutbringingconsequencestohealthytissues.Transporting different

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anticancer drugs per nanoparticle is possible, leading to a synergistic anticancer effect. Thisallows for reducing the concentration of each drug, avoiding toxicity and the developmentof resistance of the tumor to the chemotherapy. However, the nanoparticles have limited drug loadingcapacity. Thenanoparticledrugdeliveringsystemscanbeadministeredinseveralways,including oral, nasal, parenteral, and intraocular, among others, but systemic administration isthe typical method used[9]. It is possible to choose one route over another, to optimize patientcomplianceorreducemanufacturingcosts.Despitetheseveraladvantagesmentioned,thenano particleshavesomelimitationsthatmustbeovercomebeforebeingusedroutinelyintheclinicorcom mercialized,especiallyfortargeteddeliveryofcancertherapeutics.To overcomesomeofthesedifficulties,activecellularnanodeliverystrategiesallowhigheraffinityto specific target ligands, increasing the probability of cellular uptake. However, evenactivetargetingstronglydependsonpassivediffusionthroughouttheorganismuntilthetarget tumor tissues. In addition, the passage of nanosystems carrying drugs throughthe blood-brain barrier still constitutes a generalized challenge. Also, generally, thenanotherapeutic agents still have many limitations, including, among others, low bioavailability and the consequent use of high doses to compensate for the low amount of nanocarriersreaching the target successfully, which consequently causes serious adverse effects. Moreinformation about this topic, including a review of past clinical trials with the nanocarriersfor cancer therapeutics, can be encountered in the work of Rosenblum et al. For thesereasons, the development of strategies that minimize the described limitations becomesrelevant, andhence,morestudies inthisfieldareimperative.The need for intravenous (IV) formulations and the advantage of enlarging surface contact with an external medium to control release kinetics have encouraged the development of nanoparticles. Despite several advancements, thedrugtransport at high concentrations to solidtumors seems still to be a challenge; nanoparticles have been widely attempted for delivering cancer agents to tumors. Sanjeeb K shaoo*et al.,* 2011 prepared polymeric nanoparticles and used as carriers for systemic and targeteddrug delivery systems.Cisplatin is synthetic lyophilisedpowder formula$[Pt(NH_3)_2Cl_2]$molecular weight 301.1 g/mol melting point is270 °C[10].Cisplatin (platinol), cis-diammiedichloroplatinum(II) (CDDP) is an antineoplastic in the class of alkylating agent, Platinum based chemotherapy agent used to treat various types of carcinoma, lymphomas, sarcomas, germ cell tumors, advanced

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ovarian cancer,advanced bladder cancer, advanced testicular cancer, small cell lung cancer, it was the first member of its class which includes carboplatin and oxaliplatin. Cisplatin is a chemotherapy medication used to treat a number of cancers. Bioavailability of Cisplatin is 100% with intravenous injection and belongs to BCS class-IV.These include testicular cancer, ovarian cancer, cervical cancer, breast cancer, bladder cancer, head and neck cancer, esophageal cancer, lung cancer, mesothelioma, brain tumors and neuroblastoma. It is given by injection into a vein. CDDP acts by cross linking guanine bases in DNA makes the strand unable to coil and separates the strands, alkyl groups in turn inhibits their correct utilization by base pairing and causes a miscoding of DNA, attachment of alkyl groups to DNA bases, results in repair of DNA fragments by replacing the alkylated bases, prevents RNA transcription and DNA synthesis and from the affected DNA, 2) formation of cross-links between atoms in the DNA prevents DNA synthesis or transcription.most activein the resting phase of the cell drugs are cell cycle non-specific. Cisplatin been used in the treatmentosteogenic sarcoma or neuroblastoma of doses of 90 mg/m^2 intravenously once every 3 weeks or 30 mg/m^2 intravenously once weekly. Re-current brain tumors of doses of 60 mg/m^2 intravenously daily for two consecutive days every 3 to 4 weeks have been used. It has not been clearly determined whether dosage shouldroutinely be reduced in patients with renal impairment. Some dosages that have been tried are 75% of the usual dose when creatinine clearances of 10 to 50 mL/min are present.Similarly, creatinine clearances of less than 10 mL/min should be 50% of usual dose[11]. Clinical use of this drug is marred with emergence of intrinsic and acquired resistance and severe side effects such as acute nephrotoxicity and chronic neurotoxicity. These side effectslimit the dose administered to patients

To overcome these problems Nanoparticle Albumin-bound (NAB) technology which has been developed by Abraxisbiosicences is a novel patented technology. Albumin is versataileprotein carrier for drug delivery, it is non toxic, bio-compatible, non immunogenic and bio degradable. It is ideal material to achieve safe, solvent free, efficient and targeted drug delivery the first Formulation entered the market and is Abraxane. K. Madhavi and Jithanetal.,2011 prepared polymeric based polymeric based nanoparticles using bovine- serum albumin. In this study, we particularly focused on pegylated albumin nanoparticles. PEG is used to impart the *in-vivo* longevity to drug carriers, PEGylation reduces the protein binding (opsonization) stealth nanoparticles circulates longer time in the blood it leads to more

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accumulation in the tumour , interacts more with target and enhances tumour targeting. Mittsching B etal, 2011 administered albumin bound (nab) paclitaxel nanoparticles as intravenous infusion[12].

In this study, we further aimed at improvement of the Formulation by conjugating pegylation to nab technology. The main objective of this study was to prepare and evaluate serum stable long circulating PEG-Albumin-Cisplatin nanoparticles intended to be administered in breast cancer, improving the therapeutic index of the drug and decreasing intrinsic and acquired resistance severe chronic neurotoxicity, electrolyte disturbances, myelosuppression[13].

MATERIALS AND METHODS

Cisplatin was purchased from Natco pharmaceuticals Ltd. Hyderabad. Bovine serum albumin [fraction v] specification-Albumin min 98.5% was purchased from Qualikems fine chem. Pvt. Ltd, Mumbai. Glutaraldehyde was purchased from SDFCL, Hyderabad. Methanol was purchased from SDFCL. Methanol HPLC grade was purchased from SDFCL, Hyderabad. Ethyl acetate was purchased from Qualikems Fine chem. All the other chemicals were of analytical grade. Magnetic stirrer, cyclomixer, ultracentrifuge and micro centrifuge bought from REMI Equipments Pvt. Ltd. were used. HPLC from waters used in the analysis of drug levels in the plasma and the tissues. A Shimadzu UV- Visible spectrophotometer was used in the analysis of samples from *in-vitro* drug release and drug content assays. A bath sonicator from Fischer brand was used.

METHOD

Desolvation technique:

Cisplatin nanoparticles were prepared by using desolvation technique. Nanoparticles of Cisplatin were prepared by using biodegradable bovine serum albumin as the polymer. This experiment was conducted in dark condition as the drug degrades in the presence of light.

Polymeric solution:

BSA was taken between50mg and 200mg and was dissolved in 2ml of purified distilled water.

Drug solution:

Cisplatin dissolved in 8ml of ethanol.Drug solution is added dropwise to the above polymeric solution under magnetic stirring (500rpm) which results in the formation of opalescent suspension at room temperature.After desolvation, 0.11ml of 8% gluteraldehyde in water (V/V)

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was added to the suspension.This cross linking process of the colloidal suspension was performed over a time period of 24 hrs.The suspension then obtained was subjected to 5 cycles of differential centrifugation (12,000*g,8 min).The pellets then obtained by centrifuged to original volume with distilled water.Each redispersion step was performed in a bath sonicator over 5 min[14].

PEGylation of Cisplatin-bovine serum albumin nanoparticle:

Prepared BSA-cisplatin nanoparticles were stirred with PEG (6000, 4000 and 1500) respectively for at least 5 min in ultra sonicator.The resultant solution was taken in a RBF and lyophilized under freeze dryer.It takes approximately 2hrs for freezing and 1hr for drying under vacuum in freeze dryer.The resultant dried particles obtained were collected as PEGylated BSA Cisplatinnanoparticle for further use. The formation of nanoparticles using this technique was confirmed by a scanning electron microscope and a zetasizer.In formulation F_2, F_3, and F_4PEG-6000, PEG-4000, and PEG-1500 are used respectively for the PEGylation of the BSA-cisplatin nanoparticles. PEGlyation of the BSA-cisplatin nanoparticles is done to prepare serum stable long circulating nanoparticles in the blood[14].

Drug-excipient compatibility studies:

FTIR: Fourier Transform Infrared (FTIR) spectroscopy:

FT-IR is used for the drug-excipient compatibility samples of about 5mg was mixed thoroughly with100mg KBr IR powder and compacted under vacuum at a pressure of about 12psi, for 3min. The resultant disc was mounted in a suitable holder in Perkin Elmer IR spectrophotometer and the IR spectrum was recorded from 4000cm^{-1} to 400cm^{-1} in a scan time of 12min. these studies were done for 1) Bovine serum albumin 2) PEG-6000 3)PEG-4000 4) PEG-1500 5) Pure drug (Cisplatin) 6) Cisplatin-BSA nanoparticles 7) Cisplatin-BSA+PEG6000 nanoparticles 8) Cisplatin-BSA+PEG-4000 nanoparticles 9) Cisplatin-BSA+1500 nanoparticles.

Characterization of PEG-Albumin-Cisplatin Nanoparticles[15]:

Particle size, Zeta Potential and surface morphology:

In order to examine the particle surface morphology and shape, scanning electron microscopy (SEM) was used. A Cisplatin nanoparticles solution was spread over a slab and dried under vacuum. All the samples such as 1) Cisplatin-BSA nanoparticles, 2) Cisplatin-BSA+ PEG-6000 nanoparticles, 3) Cisplatin-BSA+PEG-4000 4) Cisplatin-BSA+PEG-1500 nanoparticles

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were shadowed in a cathodic evaporator with gold layer 20nm thick photograph were taken using a JSM-5200 SEM (Tokyo, Japan) operated at 20Kv.The electrophoretic mobility and zeta potential were measured using a zeta potentiometer (Malvern Zetasizer). To determine the zeta potential, nanoparticles sample were diluted with KCl (0.1 mm) and placed in the electrophoretic cell where an electric field of 15.2 V/cm was applied. Each sample was analyzed in triplicate.

Drug entrapment efficiency:

Drug entrapment efficiency was determined by the dialysis method. Drug entrapment efficiency was calculated for the samples cisplatin-BSA nanoparticles, cisplatin-BSA+PEG-6000 nanoparticles, cisplatin-BSA+PEG-4000 nanoparticles, cisplatin-BSA+PEG1500 nanoparticles Take a beaker containing 100ml of phosphate buffer of pH 7.4and keep them on the magnetic stirrer for stirring.10mg of samples was weighed and taken in a broken test tube containing 10ml of phosphate buffer of pH7.4.Bottom end of the test tube was packed with the diffusion membrane.Place the test tube inside the beaker and adjust it such that the test tube is submerged in the beaker containing phosphate buffer of pH 7.4.Now collect the samples 10 ml from the beaker for every 1hr till 10 hrs and replace it with the fresh medium of buffer.

$$\text{Entrapment efficiency \%} = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the nanoparticles}} \times 100\ \%$$

In-vitro drug release studies:

In-vitro drug release experiments were conducted for cisplatin drug. The release medium used was phosphate buffer of pH 7.4 which contains ascorbic acid and butylatedhydroxytouleneat a concentration of 1% and 0.1% respectively to prevent degradation. About 10mg of each sample cisplatin-BSA nanoparticles, cisplatin-BSA+PEG-6000 nanoparticles, Cisplatin BSA+PEG-4000 nanoparticles, cisplatin-BSA+PEG-1500 nanoparticles were weighed and redispersed in 100ml of release medium (phosphate buffer pH 7.4)The entire beaker was kept at 37±0.5^{o}C under stirring at 100rpm.At the time intervals of [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 24, 48 till 21 days] the samples 10ml was withdrawn and was replaced with the fresh medium of buffer. The samples withdrawn were filtered by using 0.2µ sterile filter. The amount of drug in the release medium was determined by UV-Visible spectrophotometer at 225 nm. The cumulative amount of drug release over the time period was plotted. The data was fitted to various models that indicate the type of drug release. To determine the enhancement in

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the solubility with the Formulation, solubility studies with Cisplatin and with the formulations were conducted[16].

Differential scanning calorimetry (DSC):

Differential scanning calorimetry (DSC) is a thermo analytical method used to study thermal transitions involving energy or heat capacity changes. Thermal properties of the powder samples were investigated with differential scanning colorimetry. Approximately 10mg of samples was analyzed in an open aluminum pan and heated at scanning rate of 10^{o}c/min between 0^{o}C and 400^{o}C. Magnesia was used as the standard reference materials. The thermograms of Cisplatin were obtained and for the samples cisplatin-BSA-nanoparticles, cisplatin-BSA+PEG-6000 nanoparticles, cisplatin-BSA+PEG-4000 nanoparticles, cisplatin-BSA+PEG-1500 nanoparticles.

In-vivo animal studies:

Animal experiments were conducted with the institutional animal ethics committee of Geetanjali college of pharmacy, Hyderabad (IAEC No.1684/PO/a/12/CPCSEA). The study was conducted using 12 wistar rats divided into 3 groups (n=4), each mice was injected via lateral vein , native Cisplatin is injected for group-1, cisplatin nanoparticle is injected for group-2 , pegylatedCisplatin nanoparticles is injected for group-3, blood sample was collected from retro orbital plexus at different time intervals. Serum was separated, the concentration of Cisplatin was determined by HPLC analysis (maiti k et al.,int j Pharma 2007; 330(1-2),155-63).

Cell viability assay:

The cell viability assay with pureCisplatin and optimized Pegylated-BSA-Cispaltin nanoparticles was carried out inMDA-MB-231 cell lines following the protocolpreviously described[4]. The method is described indetail as below.MDAMB231 and BT 549 cell lines were grown asadherent in DMEM medium supplemented with 10%fetal bovine serum, 100 µg / ml penicillin, 200 µg/ml, streptomycin, 2 mM L-glutamine, and culture wasmaintained in a humidified atmosphere with 5% CO_2.

Preparation of samples for cytotoxicity:

Formulations and blank nanoparticles were dispersed/dissolved in sterile PBS, PEG and ethanol to desiredconcentrations for the treatment as follows.

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Cytotoxicity evaluation:

Cytotoxicity of formulations was determined byMTT assay based on mitochondrial reduction ofyellow MTT tetrazolium dye to a highly colored blueformazan product. $1x10^4$ Cells (counted by Trypan blue exclusion dye method)) in 96- well plateswere incubated with formulations with series ofconcentrations for 48 h at 37° in DMEM with 10%FBS medium. Then the above media was replacedwith 90µl of fresh serum free media and 10 µl ofMTT reagent (5 mg/ml) and plates were incubated at 37° for 4 h, there after the above media was replacedwith 200 µl of DMSO and incubated. The absorbancewas measured at 570 nm on a spectrophotometer(spectra max, Molecular devices).

Statistical analysis:

All experiments were done more than four times andthe data were expressed as mean ± standard deviationand Tukey's *post-hoc*test was done to analyzesignificance of difference between different groupsusing the statistical analysis software package SPSS (version 16.0, IBM, USA).

RESULTS AND DISCUSSIONS:

Preformulation studies for Cisplatin have been performed to know the drugs physicochemical properties so as to design it to a suitable formulation. Cisplatin is a class-IV drug classified under BCS classification with low solubility and high permeability. The physicochemical properties were described. Poor solubility leads to poor dissolution, therefore to enhance the dissolution of the drug, different techniques have been employed such as particle size reduction by forming nanoparticles which is a novel technique. SEM was used to determine the particle size of samples such as 1) F4 cisplatin nanoparticles, 2) Cisplatin-BSA+PEG-4000 nanoparticles, 3) Cisplatin-BSA+PEG-1500 nanoparticles from figure. 1,2,3, it was concluded that the average particle size for all formulations was found to be in nano range of 150nm to 400nm. Surface morphology and shape were visualized. The particles were appeared as spheres. From this study it has been concluded that there is a size reduction of particle which results in enhancing dissolution rate of the Cisplatin.

The results of percentage drug entrapment efficiency are shown in the table. 2.From the results shown in the table, itcan beinferredthat thereis a proper distribution ofCisplatinin thenanoparticles. Thepercentageentrapmentefficiencywasfoundtobe65.5% to85.5%. A maximum of 85.5 % drug entrapment efficiency was obtained in thecisplatin-BSA nanoparticle. Release of

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the drug from the is about 13.5%, 16.5%, 17.5%, 25.6 and 14.5% in different formulations F_1,F_2, F_3, F_4 F_5formulations with-in 2hrs was observed as given in table. 3. The sustained release activity of the drug was due to the slow release of drug entrapped inside the PEGylated polymeric matrix. Drug compatibility studies were performed by fourier transform infrared spectroscopy (FT-IR) has been performed to know the drug excipient compatibility and to check the presence of drug and polymer in the nanoparticle formulations. The chemical interaction between the drug and the polymer can be observed by the change in the infrared profile of nanoparticles of Cisplatin and can be interpreted by careful study of spectra from figure 7, 8, 9 and 10.FT-IR is done for1) Cisplatin-Pure drug 2) FTIR Spectra of Cisplatin-Bovine Albumin-PEG-4500 3) FTIR Spectra of Cisplatin-Bovine Albumin-PEG-5000 4) FTIR Spectra of Cisplatin-Bovine Albumin-PEG-6000. FT-IR spectra obtained for Cisplatin shows characteristic peaks at 3274cm^{-1}(NH_3 group, stretching vibrations are seen at 3188 cm^{-1}). The spectra of Cisplatin loaded polymer blend were not characteristic different from the spectra of the Cisplatin. The peaks appearing at 3250cm^{-1}, 3170cm^{-1}, 2876cm^{-1}, 1103cm^{-1}, 2850cm^{-1}, 1271cm^{-1}, 1111.03cm^{-1}, 2860cm^{-1},557$cm^{-1}$for cisplatin and their polymers were also appearing in cisplatin-loaded polymer blend, indicating the chemical stability of cisplatin in the blend. The blends retained the integrity of drug and as a reason these polymers were selected for further studies. FT-IR studies demonstrated that the drug was not changed in the Formulation during the fabrication process.

DSC is done for the stability test for samples such as pure drug-cisplatin, physical mixture and optimized Formulation of nanoparticle F4. DSC thermograms obtained for the pure drug (60^0C) and for the Formulation showed no significant shift in the endothermic peaks in physical mixture (70^0C) and optimized nanoparticle F4 (40^0C)confirming the stability of the drug in the formulations and only polymer peak was observed, which revealed that drug is in amorphous state in the formulations as shown in figure from figure 11, 12 and 13.

The % drug release was determined for 21 days and all the formulations are compared. The plots of cumulative percentage drug release v/s time for all the five formulations (F_1, F_2, F_3, F_4, F_5) were drawn and represented graphically as shown in the table. 4 and figure.4 and F4 formulation showed highest drug release, which was selected as optimized Formulation. Plasma drug concentrations from the animal studies concluded there is huge drug release by BSA-PEG cisplatin nanoparticle compared to BSA nanoparticle and native drug cisplatinand showed an increase in drug release was observed 4 fold times than the native Cisplatin.

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The anti-proliferation efficacy of free cisplatin and pegylated cisplatin BSA nanoparticles on breast cancer cell line (MDA-MB-231) were determined at different concentrations 0, 20,40,60,80,100,120 mcM for about 72 hours. The results showed that PEGylatedCisplatin-BSA nanoparticles were more antiproliferative than that free Cisplatin(figure6). blank nanoparticles did not show any cytotoxic effects .this suggests that improved Formulation against breast cancer has been developed in the form of PEGylatedCisplatin-BSA nanoparticles.

CONCLUSION

In conclusion, serum stable long-circulating PEGylatedCisplatin-BSA nanoparticles prepared were prepared physicochemical characteristics were studied. Our investigation suggests that albumin nanoparticles may act as a useful and safe carrier for Cisplatin with 3 folds increase in drug release.The various uses of albumin as a drug carrier that have emerged in the past 10 years are of considerable interest and range from extending the half-life of therapeutically active proteins and peptides to drug targeting in oncology. The development and market approval of the cisplatin albumin nanoparticle, Cisplatin, can be viewed as a landmark, not just for albumin-based drug delivery technology but also fornanomedicine.

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Table: 1 Preparation of serum stable long-circulating polymeric nanoparticles of cisplatinnanoparticles

FORMULATION	F_1	F_2	F_3	F_4	F_5
Cisplatin	100mg	100mg	100mg	100mg	100mg
BSA	200mg	200mg	200mg	200mg	100mg
PEG(6000)	__	20mg	___	___	___
PEG(4000)	___	__	20mg	____	____
PEG(1500)	___	__	___	20mg	____
Ethanol	8ml	8ml	8ml	8ml	8ml
Distilled water	2ml	2ml	2ml	2ml	2ml
Glutaraldehyde(8%v/v)	0.11ml	0.11ml	0.11ml	0.11ml	0.11ml

Table 2: Drug entrapment efficiency of cisplatinnanoparticle formulations

FORMULATIONS	**% DRUG ENCAPSULATED**
F_1	**65.5**
F_2	**72.3**

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F_3	**80.5**
F_4	**85.5**
F5	**68.3**

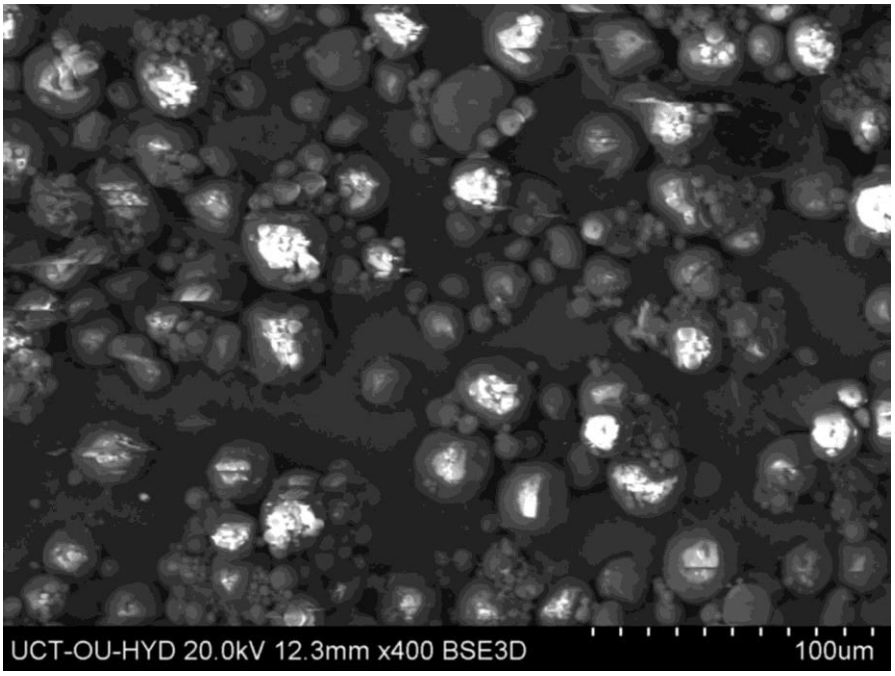

Figure: 1 SEM pictogram of optimized cisplatin nanoparticles F4 for surface morphology

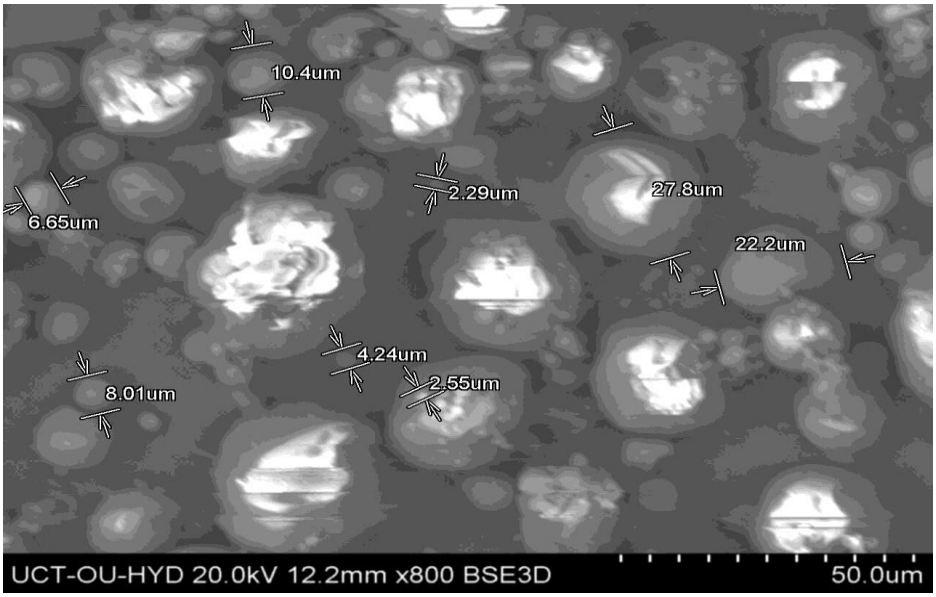

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Figure: 2 SEM pictogram for optimized Formulation of F4 cisplatin nanoparticles for particle size

Table: 3 In-vitro drug release studies of Cisplatin from various formulations F1 to F5

Time in days	F1	F2	F3	F4	F5
0	0	0	0	0	0
0.5	13.5	16.5	17.5	25.6	14.5
1	17.2	23.2	23.2	51.5	18.2
2	19.5	28.2	27.2	54.2	19.2
3	22.3	34.7	30.2	62.5	21.2
4	24.2	38.2	32.2	68.9	25.2
5	31.2	41.5	38.5	72.5	31.5
6	36.2	45.5	44.5	74.2	35.5
7	39.2	48.2	52.2	78.9	38.2
8	42.2	51.5	57.5	84.5	43.5
9	47.2	54.6	62.6	88.2	48.6
10	50.6	58.2	73.2	92.5	51.2
11	52.6	61.6	79.6	93.5	55.6
12	58.6	66.2	82.2	94.6	59.2
15	66.2	71.2	87.2	95.5	65.2
18	71.2	79.2	89.2	96.2	72.2
21	79.2	84.6	92.6	98.2	77.6

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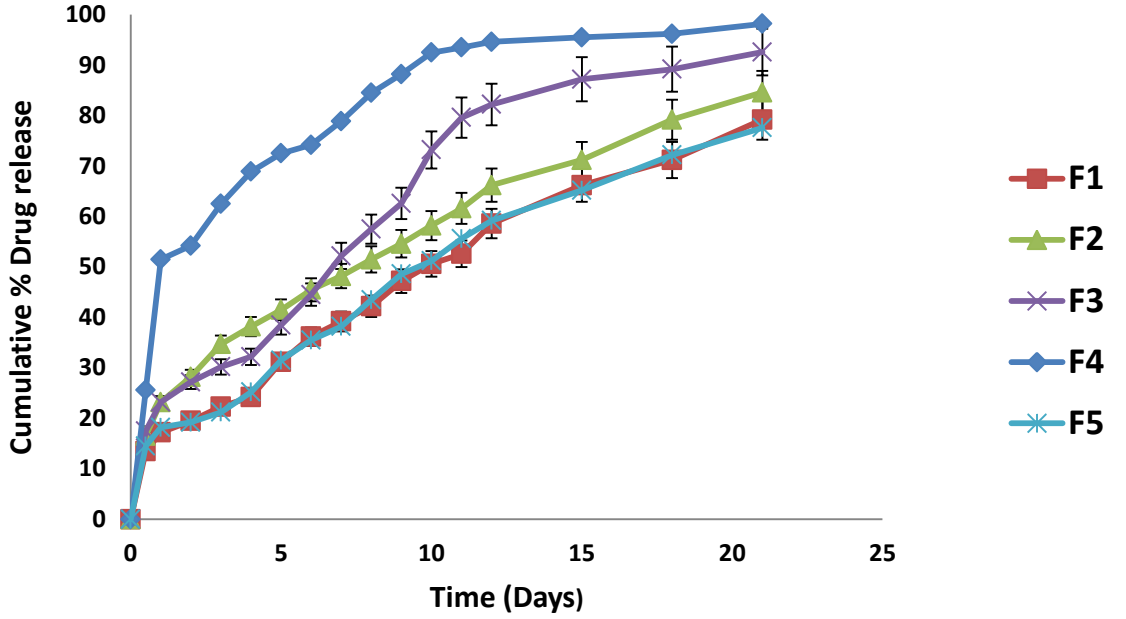

Figure: 3 In-vitro drug release studies of Cisplatin from various formulations F1 to F5

Table: 4 Plasma drug concentration of Cisplatin with various formulations F1 to F5 and native drug cisplatin

Time in hr	F5-BSA Nanoparticles	F4-PEG+BSA Nanoparticles	Native Cisplatin
0	0	0	0
1	214.5	308.5	25.3
2	295.6	445.6	45.7
4	332.3	523.7	150.5
6	397.4	613.4	210.8
8	423.8	678.9	125.5
10	500.8	728.5	54.7
12	515.2	805.8	23.3
24	401.6	887.6	12.7
48	365.6	910.6	5.5

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96	267.2	976.9	1.5
144	189.2	354.6	0
192	124.5	323.5	
240	109.3	301.9	
288	89.5	277.9	
336	77.6	210.5	
384	39.7	110.6	
432	5.5	25.6	
504	0.7	8.9	

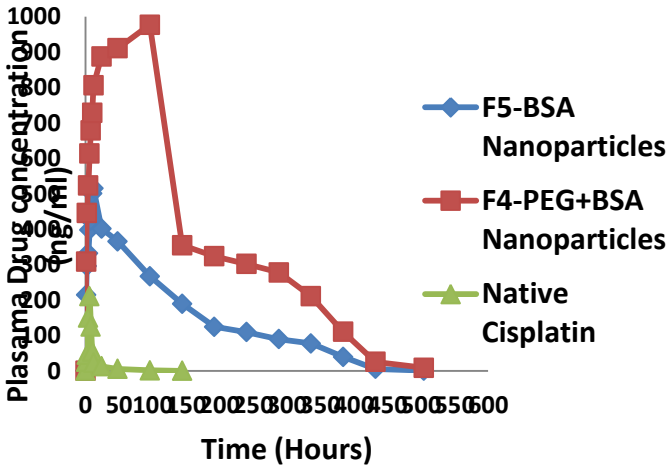

Figure: 5 Plasma drug concentration of Cisplatin with various formulations F1 to F5 and native drug cisplatin

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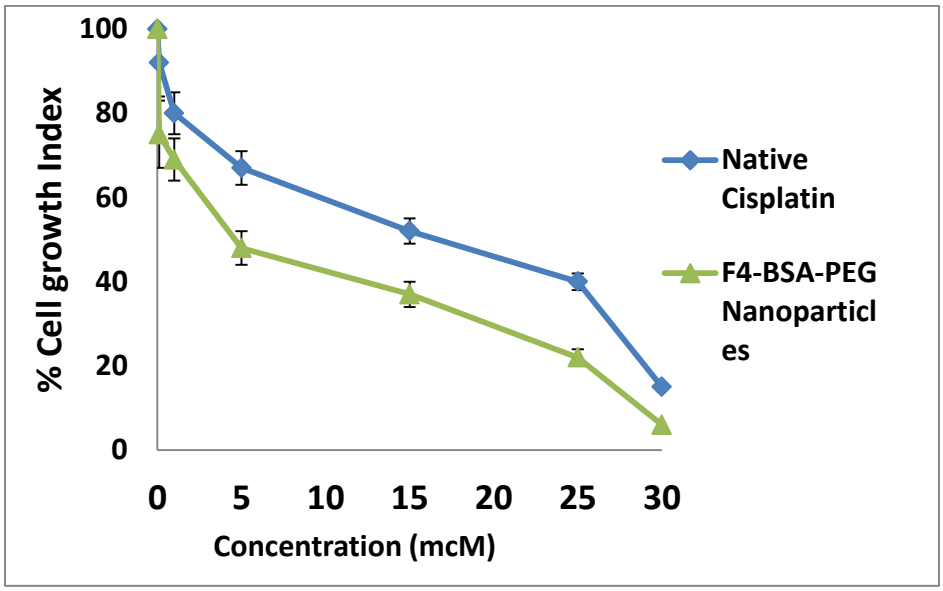

Figure: 6 Cell viability assay with various samples of native cisplatin and F4-BSA-PEG nanoparticles

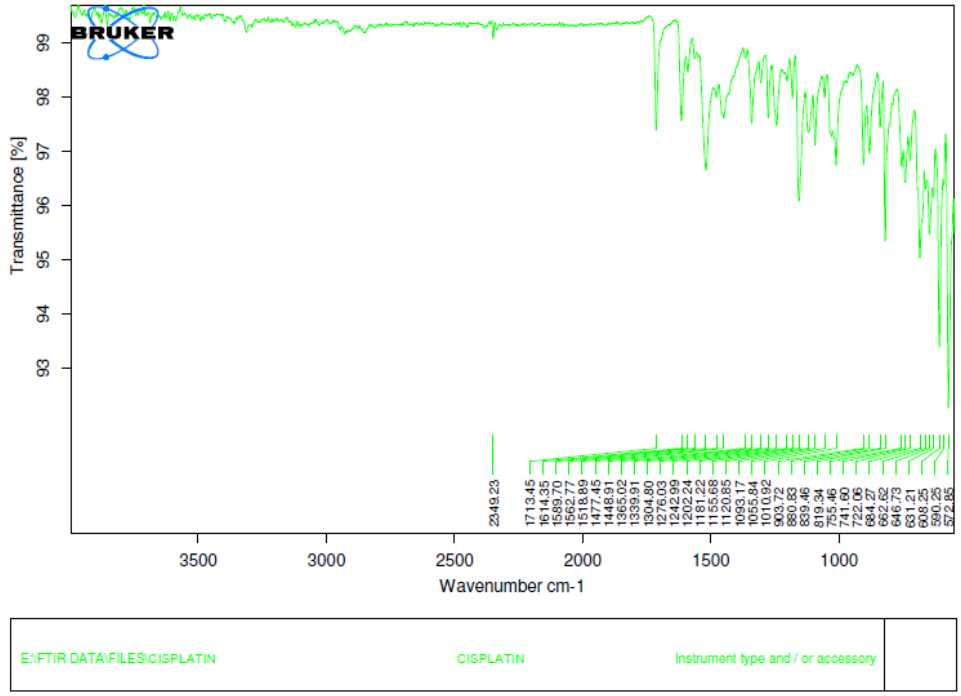

Figure: 7FTIR Spectra of Cisplatin-Pure drug

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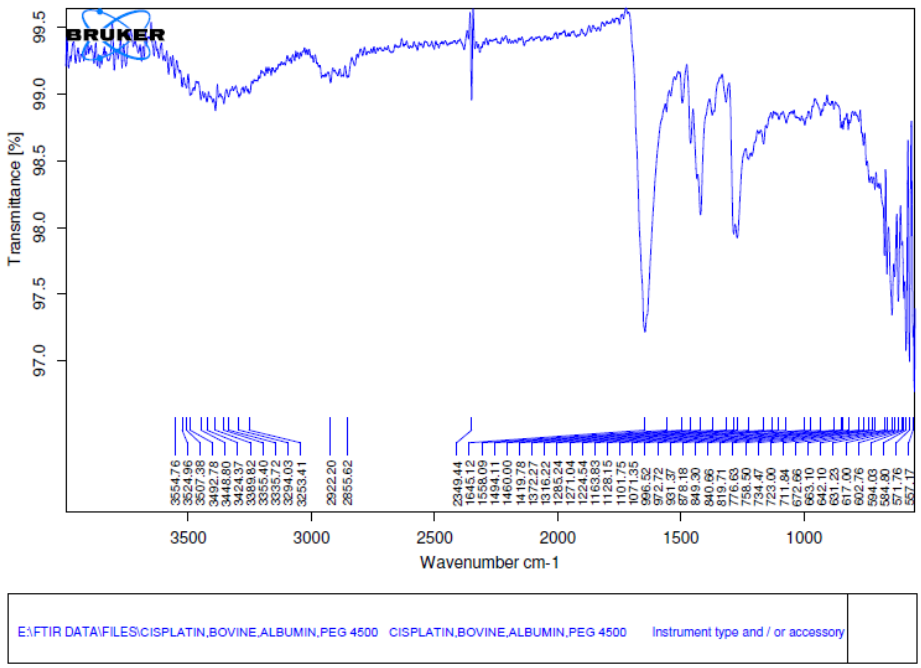

Figure: 8FTIR Spectra of Cisplatin-Bovine Albumin-PEG-4500

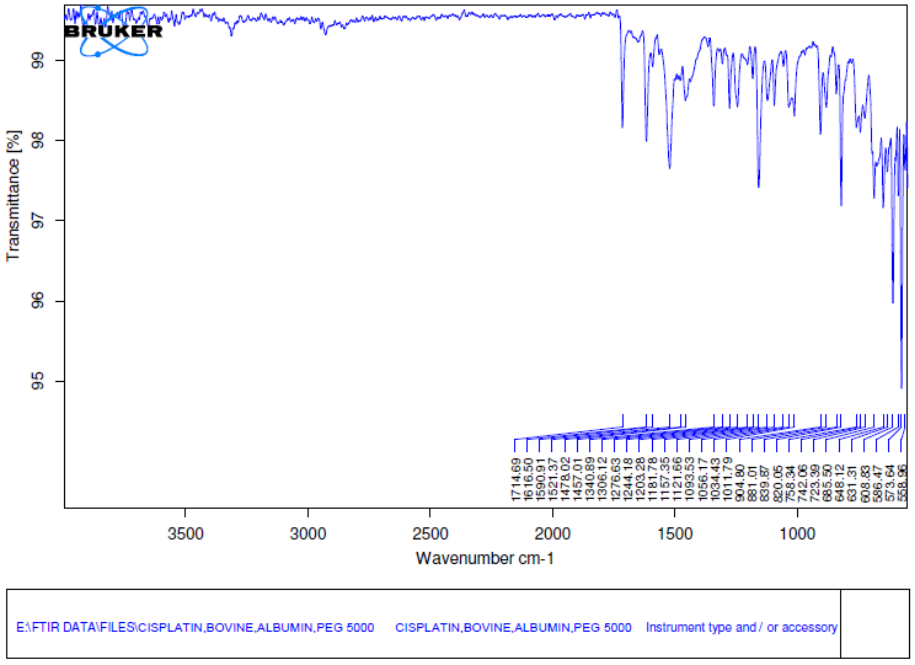

Figure: 9FTIR Spectra of Cisplatin-Bovine Albumin-PEG-5000

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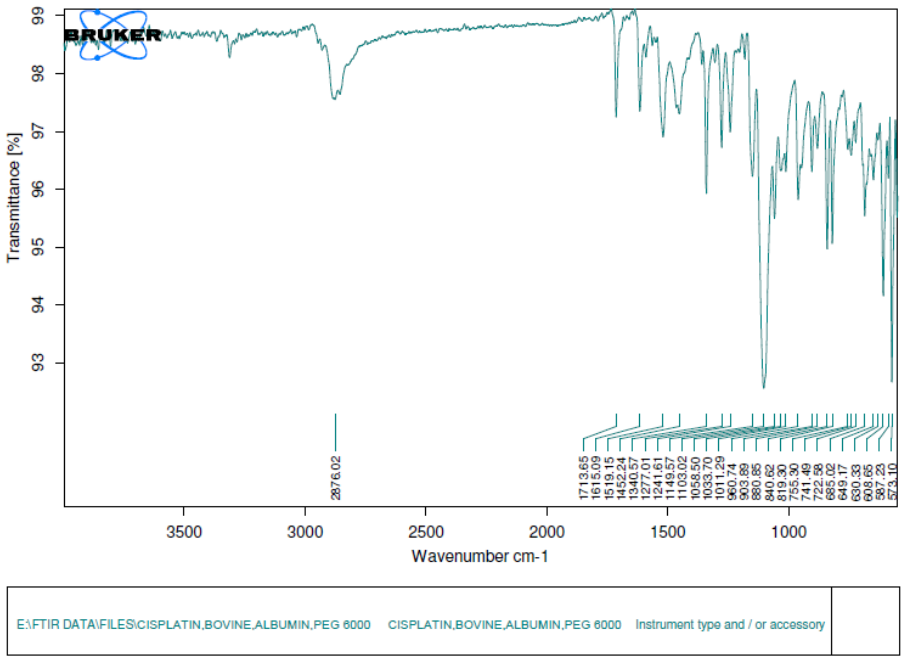

Figure: 10FTIR Spectra of Cisplatin-Bovine Albumin-PEG-6000

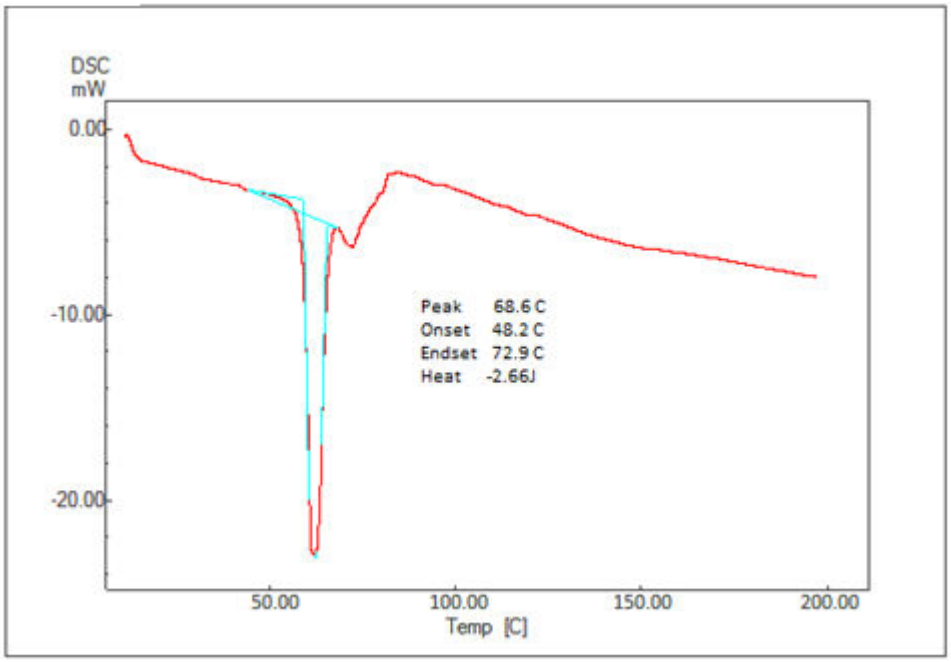

Figure: 11 DSC thermogram of Cisplatin

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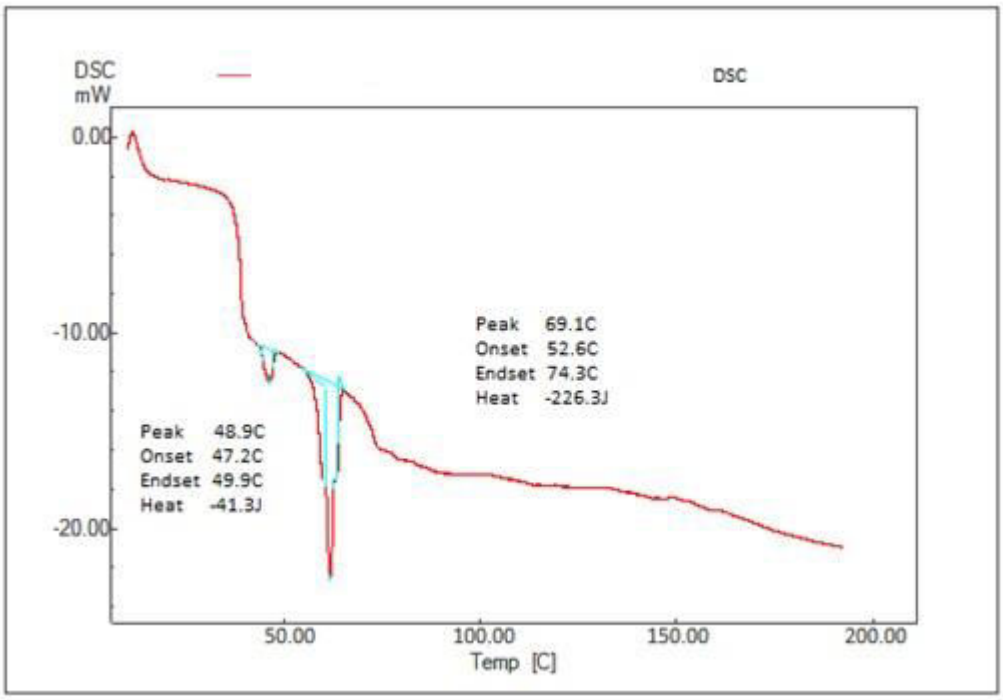

Figure: 12 DSC thermogram of Physical Mixture

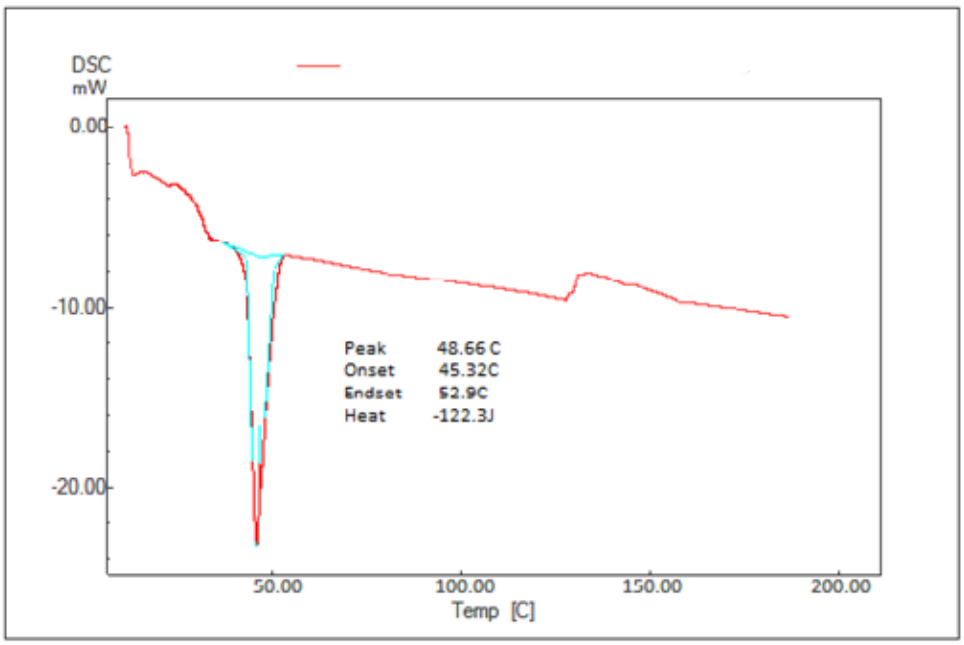

Figure:13 DSC thermogram of CisplatinNanopaticles optimized formulation (F4)