Asian Journal of Chemical and Pharmaceutical Research
CODEN (USA): AJCPHP | ISSN: 2347-8322
Journal Home Page: www.pharmaresearchlibrary.com/ajcpr

Preparation and Characterization of Paclitaxel Nanoparticle by Precipitation Technique

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A b s t r a c t

Paclitaxel is a microtubule-stabilizing agent which promotes polymerization of tubululin causing cell death by disrupting the dynamics necessary for cell division. It has neoplastic activity especially against primary epithelial ovarian carcinoma, breast, colon, and non-small cell lung cancers. Paclitaxel is poorly soluble in aqueous solutions but soluble in many organic solvents such as alcohols. It therefore lends itself well to more advanced formulation strategies. In the present investigation, an attempt was made to prepare Paclitaxel Silver Nanoparticle by precipitation technique. In order to stabilize the size of particle, various concentrations of Silver nitrate and Trisodium citrate to be used. Prepared Nanoparticle to be optimized by their encapsulation efficiency, particle size and Release rate. Zeta potential and SEM study also included in the investigation.

Keywords: Paclitaxel, nanoparticles, Zetapotential

A r t i c l e I n f o

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A r t i c l e H i s t o r y: Received 26 January 2023, Accepted 09 April 2023, Available Online 30 June 2023

Citation: K. Sunil Kumar, *et al. RP-HPLC method development and validation for estimation of voriconazole, azithromycin and metoprolol tartrate in pharmaceutical dosage form. Int. J. Pharm. Natural Med.,* 2023, 11(1): 14-19.

C O N T E N T S

1. Introduction. 14
2. Methodology. 15
3. Results and Discussion. 17
4. Conclusion. 18
5. References. 19

1. Introduction

Nanoparticles play a very important role in cancer research. Due to extremely small size of Nanoparticles they are easily and more readily taken up by the human body. Biological membranes and access cells, tissues and organs are eligible for entrance of Nanoparticles. Nanoparticles are stable, solid colloidal particles consist of biodegradable polymer or lipids and size range 10-1000 nm. Nanoparticles have attracted the attention of scientists because of their multifunctional character. Nanoparticles have greater surface area to volume ratio that helps in the diffusion process. Nanoparticles also leading to special properties

such as increased heat and chemical resistance. A single cancerous cell, giving a strain on the nutrient supply and removal of metabolic waste products. If small tumor has formed, the normal tissue will not be able to oppose the cancer cells for the normal supply of nutrients from the blood.[1]

The advantages of Nanoparticles as drug delivery systems include time controlled drug delivery, reduced drug toxicity, improved bioavailability and enhanced therapeutic efficacy and biodistribution.[2] Nanoparticles can also protect the sensitive drugs from degradation by environmental factors such as stomach acid and enzymes.[3] Polymeric Nanoparticles range in size from about 10-1000 nm, and can be modified with different ligands such as antibodies to create a smart targeting delivery system.[4] Polymeric Nanoparticles of a size around or less than 300 nm coated with surfactants have been proved to be able to transport drugs across the Blood Brain Barrier BBB[5]

Cancer therapy strategies are currently focused on surgery, chemotherapy, radiotherapy, immunotherapy and hormonal therapy. These conventional strategies are limited by the accessibility to the tumor and the lack of selectivity towards tumor cells, the spread of cancer cells throughout the body, and the risk of operating on a vital organ. Regarding cancer chemotherapy, treatment failure is frequently encountered even in the most sensitive cancers to chemotherapy agents.[6] Several reasons have been pointed out for chemotherapy failure: i) the Physicochemical properties of many drugs, e.g., hydrophobicity, promotes the unsuccessful localization at the cancer site ii) Unfavorable pharmacokinetics (rapid clearance and rapid *in vivo* degradation) determine the need of higher doses and rigorous treatment schedules to obtain a therapeutic effect iii) The relative poor selectivity of chemotherapy agents for targeted tumor cells iv) the large bio-distribution and non-intended extravasation with severe side effects in non-targeted sites; and v) The susceptibility to induce drug resistance[7, 8, 9].

Cancer physiology is also responsible for the chemotherapy failure, mainly because of the absence of a non-functional lymphatic system that allows drug escaping out of the tumor, and due to a very high hydrostatic pressure gradient inside the tumor that difficult a uniform drug diffusion inside the tumor.[10,11]

The association of anti-tumor drugs to colloidal delivery systems in cancer treatment has been proposed to improve their efficacy and to reduce their associated toxicity. This strategy could allow obtaining a specific accumulation at the tumor site, an improvement of the pharmacokinetic profile, a prolongation of the exposure of the tumor cells to these active agents and a minimization of the severe side effects.[7,12]

With this, it have been established that a suitable anti-tumor drug delivery system should have the following properties:

i) small size (≤500 nm) to allow a large biodistribution and an adequate perfusion at the target site

ii) ii) the ability to deliver therapeutic drug quantities, without overloading the organism with foreign material;

iii) iii) Physical stability and low drug leakage problems under storage and *in vivo* iv) controlled drug release rates exclusively at the targeted tumor; and v) maximum biocompatibility and biodegradability (with very low toxicity of breakdown products), and minimal antigenicity.[7, 13] These drug carriers are frequently based on vesicular (liposomes and niosomes) and polymeric systems. Special approaches such as surface-functionalization (e.g., with specific ligands to tumor cells) and engineering of stimuli-sensitive materials, could enhance the bio distribution profile of loaded drugs and, thus, resulted in a more efficient tumor therapy[7, 11,14]

One of the most promising materials for the design of Nano carriers loaded with chemotherapy agents is the biodegradable polymer poly (ε-Caprolactone) (PCL). This aliphatic polyester is very suitable for controlled drug delivery due to its high permeability to many drugs and non-toxicity, its exceptional ability to form blends with other polymers, and it's very low degradation rate (compared to other well known drug carriers, such as Poly (D,Lactide-co-glycolide) (PLGA).[15]

2. Materials and Methods

Materials:

Paclitaxel has been procured as a gift sample from Salius Pharma Pvt.Ltd (Mahapi Navi Mumbai) and the other chemicals used were of high laboratory grade.

Methodology:

Method of preparation for Paclitaxel silver nitrate Nanoparticles by precipitation Technique:

Silver nitrate and trisodium citrate were used as starting materials for the preparation of Paclitaxel silver nitrate Nanoparticles (NP). The silver colloid was prepared by using chemical Precipitation method. All solutions of reacting materials were prepared in distilled water. In typical experiment 50 ml of 0.001 M AgNO3 was heated to boil. To this solution 5 mL of Paclitaxel (150 mg/5 ml of methanol) solution was added followed by addition of 5 ml of 1 % of trisodium citrate added drop by drop. During the process, solutions were mixed vigorously and heated until change of color was evident (pale yellow). Then it was removed from the heating device and stirred until cooled to room temperature. The colloidal solution of silver Nanoparticles were characterized by using UV-Visible

spectroscopy and SEM. The entire addition process took about 3 minutes, after which the stirring was stopped and the stir bar was removed. Reaction conditions including stirring time and relative quantities of reagents (both the absolute number of moles of each reactant as well as their relative molarities) must be carefully controlled to obtain stable yellow colloidal silver. If stirring was continued once all of the silver nitrate was added, aggregation began as the yellow solution first turned to darker yellow then violet and eventually grayish after which the colloid broke down and particle settled out.

Fig: 1 Photograph showing the preparation of Nanoparticles

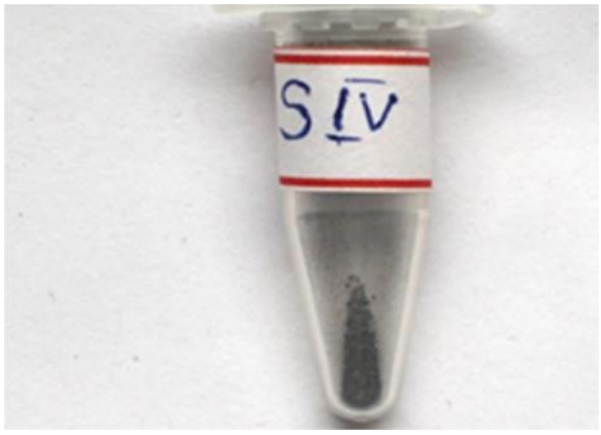

Fig: 2 Silver Nitarte Nanoparticles Product

Drug content and encapsulation efficiency[16]

Precisely weighed 100mg of Paclitaxel Silver Nitrate Nanoparticles were crushed in a mortar and suspended in 100ml of phosphate buffer (pH 7.4) and kept in sonication for 2hrs. Then the samples were centrifuged at 1000rpm for 20mins to remove the supernatant layer, if any. The samples were filtered. From this filtered solution 1 ml of sample was withdrawn and diluted to 100 ml with phosphate buffer (pH 7.4). Then it was analyzed spectrophotometrically at 271nm.

Drug content:

Theoretical drug content =Weight of drug loaded / Total weight of Nanoparticles Practical drug content = Concentration X dilution factor X Conversion factor Encapsulation efficiency = (Actual drug content / Theoretical drug content) X 100

***In-vitro* Drug Release Studies**[17]

In vitro release of Paclitaxel Silver Nitrate Nanoparticles was conducted by a dialysis membrane having a pore size of 2.4 mm (LA-395-5Mt Himedia Pvt. Ltd, Mumbai, India) with 75 ml of pH 7.4 phosphate buffer at 37°C. Briefly in a 100 ml beaker 75ml of pH 7.4 phosphate buffer was taken. A 2 ml of formulation was taken into a dialysis bag and dipped into the buffer solution. The dialysis membrane was activated earlier using by soaking in 1% w/v NaOH overnight. The flask was kept on a magnetic stirrer. Stirring was maintained at 250 rpm and the temperature of the buffer was maintained at 37°C. Sampling was done by withdrawing 1 ml of aliquots from a beaker. Immediately 1 ml of new buffer was added to keep the sink condition. Samples were analyzed after sufficiently diluting with methanol by using a UV/Spectrophotometer (UV/VIS-Double beam Spectrophotometer, V-530, Jasco, Tokyo, Japan) at a wavelength of 271 nm. Each test was conducted thrice and average value taken for the calculation.

Fig:2 Dialysis Membrane

The release data obtained were fixed into various mathematical models like zero order, First Order, Higuchi and Korsmeyer-Peppas to know which mathematical model was best fitting the obtained release profile.

SEM Analysis[18]

Scanning electron microscopy (JEOL 5400, Tokyo, Japan) was used to decide the shape, surface topography and texture as well as to inspect the morphology of cracked or sectioned surface. SEM is a frequently used method for characterizing drug delivery systems, owing in large part to simplicity of sample preparation and ease of operation. Sample spreads on the small square plate and coated with a gold ion for 5-6 mins. The prepared sample was kept inside the chamber and images captured with different magnifications. (10,000, 15,000 and 20,000)

Particle Size Analysis (PSA)[19]

The size division of the Nanoparticles was determined using the particle size analyzer (Beckman Coulter, Delsa

nano C, Brea, USA) prepared with a dry accessory system. About 2ml of the prior prepared suspension has to be transferred into a 4.5 ml disposable plastic cuvette, placed in the analysis device and subsequent analyzed for size analysis and temperature maintained at 25°C.

Zeta Potential Analysis[20]

The zeta potential was measured using the appropriate instrument (Beckman Coulter Delsa Nano C, Brea, USA).using automatic titration regime that adjusts the pH of the sample to pre-defined values by adding 0.1M HCL or 0.1M NaOH titrator a volume of 20 ml suspension is necessary.

3. Results and Discussion

Stability studies:

The objective of the stability testing is to show evidence as how the quality of the drug substance or product varies with time under the influence of variety of environmental factors such as temperature, humidity and light to establish a reset for the drug substance or a shelf life for the drug product and recommended storage condition.

Stability of a drug is defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutically and toxilogical specifications. The optimized batch paclitaxel silver nitare Nanoparticles were subjected for accelerated stability studies. The Paclitaxel Nanocapsule from the batch of PXN6filled in the capsule (size1) equivalent to 150 mg. Lactose was used as diluent to make up total weight of capsule as 290 mg (161+129).The Paclitaxel Nanocapsules were kept in in sigma stability chamber. The samples were analysed at 0,1 and 2 months intervals. The data was analysed for any significant changes from the initial data. The following test were performed:

- Test for physical parameters
- Assay
- *In-vitro* dissolution study.

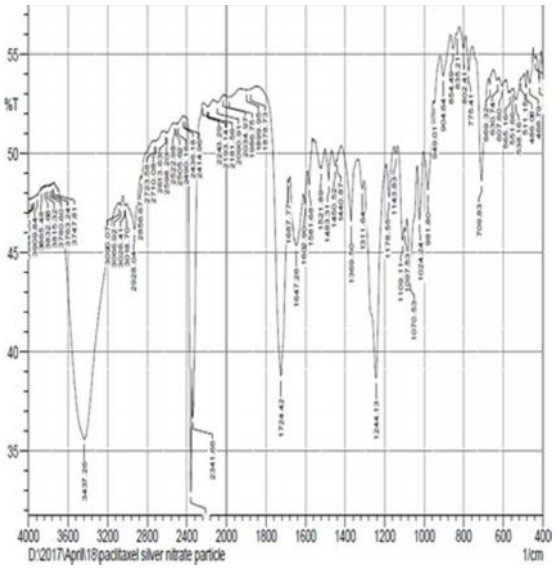

Fig3. FTIR Spectrum of Pure Drug paclitaxel

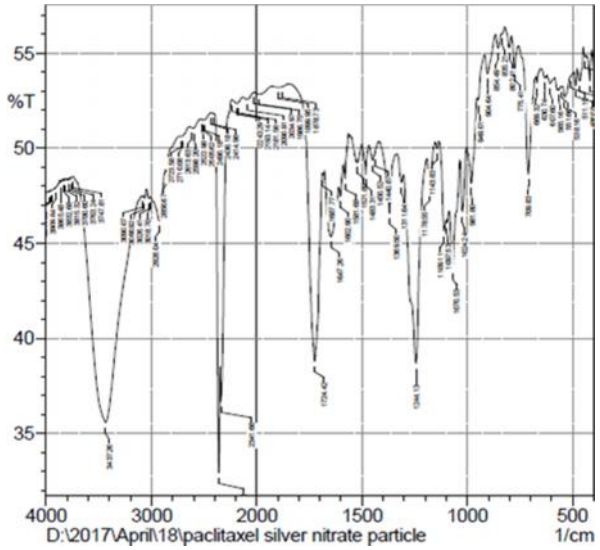

Fig.4: FTIR Spectrum of Formulation paclitaxel

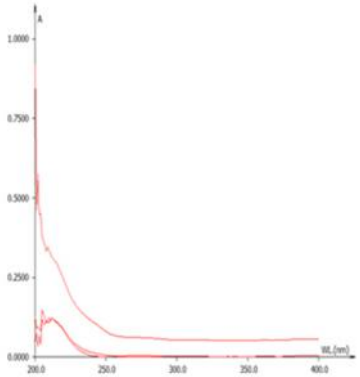

Fig.: 5 λmax of in pH 7.4 at 220nm

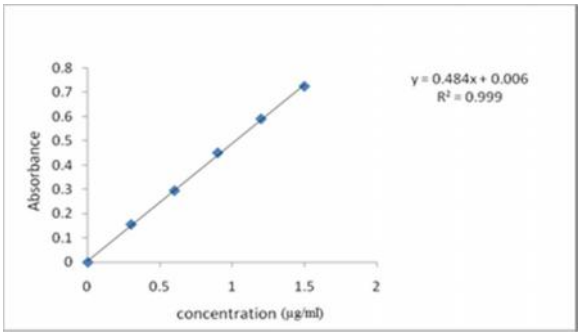

Fig.: 6 Calibration curve of paclitaxel in pH 7.4 at 220nm

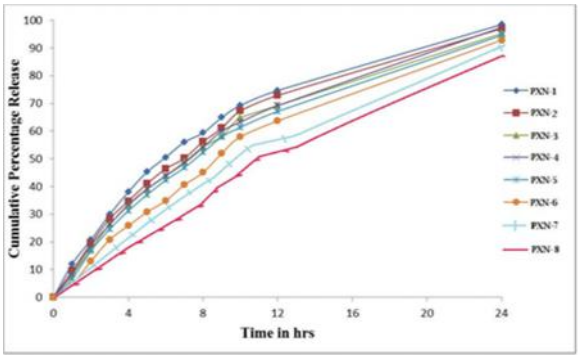

Fig.: 7 Comparative Zero order Release Plot for Formulations PXN-1to PXN-8

Discussion:

Fourier Transformer Infrared spectroscopy (FT-IR):

The development of a successful formulation depends only on a suitable selection of excipients. Hence the physical state of the drug paclitaxel, silver nitrate and the combination of drug and polymers used for Nanoparticles preparation were studied by FTIR (Fourier transform infrared spectroscopy) to know the drug – polymer compatibility. The physicochemical compatibility of the drugs and the polymer was obtained by FTIR studies.Therefore, there was no alteration and no interaction was observed between polymer and drug in combination. All the characteristic peaks of, were present in combination, thus indicating no compatibility between drug and polymers and finally confirm that there was no chemical modification of drug have been taken place. Thus, from IR spectra studies we can draw a conclusion that the drug remains in its normal form without undergoing any interaction with the polymers.

Evaluation of Paclitaxel Nanoparticles:

Nanoparticles size determination:

The particle size of the prepared Nanoparticles was determined by particle size analyzer (Beckman Coulter). The Average particle size of the loaded Nanoparticles were found to be 717.4 ± nm. All the, prepared formulations were found to be in the nano range.

Zeta Potential:

Measurements of zeta potential were also carried out in order to study the stability of nanoparticles as this extremely important for many applications, Surface zeta potentials were measured using the zeta analyzer (Beckman Coulter Delsa Nano C, Brea, USA) Liquid samples of the nanoparticles (5ml) were diluted with double distilled water (30 mL) and the pH was then adjusted to the required value. The samples were shaken for 30 minutes. After shaking the zeta potential of the metallic particles was measured. A zeta potential was used to determine the surface potential of the silver nanoparticles. In each method, an average of two separate measurements was reported. Summarizing the zeta potential measurements of samples in a solution form. For synthesis nanoparticles using different methods, zeta values were measured and found to be – 57.3 mV at pH=7. The value of the zeta potential of Silver NPs provides satisfactory evidence about their little tendency towards aggregation when its negative charges with a diameter of 717.6 nm. This behavior unambiguously suggests the presence of strong electric charges on the particle surfaces to hinder agglomeration. These values were found to fall in the negative side which showed the efficiency of the capping materials in stabilizing the nanoparticles by providing intensive negative charges that keep all the particles away from each other. This result suggests that the Silver NPs particles and thus their solution is stable behavior.

Scanning Electron Microscopy

SEM analysis was performed on the prepared loaded Nanoparticles to access their surface morphological characteristics of prepared Silver NPs using Tri sodium citrate a)Low, Medium and b) high magnifications as shown in **Fig. (a.b.c).**

SEM was performed for best formulations to assess their surface. The polymer surface of the Nanoparticles appeared spherical with smooth texture surface. Discrete nature, and distinct particle size and shape with a smooth surface.

The morphology particles were measured. The silver Nanoparticles in the citrate cross linked were in a spherical form with a well-defined particle size. The particle size strongly depend preparation conditions. The average particle size of the measured particles was as small as 645 nm to 1.12 µm.

Stability study:

The colour and shape of Paclitaxel were found to be unchanged even at the end of 2nd month stability study in all conditions. In order to perform the stability study the Nanoparticles were placed with blister packing material and folded in to the strips, then placed into stability chamber .At the end of the month one set of the colloidal Nanoparticle were analyzed for shape average weight and drug content. There was no change in the colour and shape of Nanoparticles .Also no changed absorved in release behavior up to two months when compared to optimized formulation. Sufficient precautionary measures were taken to prevent the photolytic degradation of Paclitaxel.

4. Conclusion

The present *in vitro* study revealed that the Paclitaxel NP can be a useful means for the colon targeted delivery of drugs. Around 60% of the drug to be released at colon after regular transit (GIT) of 4-6 hour. This work suggests the Paclitaxel NP of the size ranging from 635 nm -717 nm and their solutions are stable at neutral pH. The prepared Nanoparticle found to be stable without any tendency of aggregation and shown higher entrapment efficiency. The hydrophobic surface of the drug with silver release the drug slowly but up to 24 hours only. Because of higher in size of prepared Nanoparticle, release couldnot be prolonged for more than 24 hours: this finding is considered as drawback. The dissolution data indicates that the release of Paclitaxel NP with controlled manner is directly proportional with the size and concentration of Silver nitrate and crosslinking agent. Therefore, nanoparticles release increased with smaller size of particles. In this study, the prepared paclitaxel NP exhibited prolonged intestinal absorption, and prevent gastric release, avoid gastric erosion side effects and thus improve patient compliance. Short term stability study reveal

formulation in Capsule dosage form found to be stable without any major problem. However, further studies are needed to investigate these formulations to prepare Nanoparticles with desired size range of around 200 nm.

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