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Assessment of temperature stability of nanoparticle formulated vinblastine

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ABSTRACT

Catharanthus roseus L. (Madagascar periwinkle) is regarded as a rich source of pharmaceutically important terpenoid indole alkaloids(TIAs) which are valued highly due to their wide spectrum of pharmaceutical application. Among these, vinblastine and vincristine are of particular importance because of their wide use in cancer chemotherapy. Vinblastine can have extensive use for the treatment of lung cancer, breast cancer, head and neck cancer and testicular cancer. It is also used to treat Langerhens cell histocytosis. This study was done to assess the stability of vinblastine. The extracted crude vinblastine was formulated with nanoparticles at different temperature. Stability was determined at varying temperature. Reduction method was used to synthesize silver nanoparticles, LDH was synthesized using co-precipitation method and MMT was purchased from Sigma-Aldrich.LDH and MMT formulated vinblastine showed maximum temperature stability at 60°C and 80°C. The increase in heat stability will help in increasing the shelf-life as well as storage at room temperature, eliminating the need for refrigeration. Study shows that formulation of NP-TIA's has significant potential in drug delivery and enhanced shelf -life and storage condition.

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INTRODUCTION

Catharanthus roseus L. is a renowned medicinal plant, belonging to the family Apocynaceae and is a rich source of alkaloids, which are distributed in all parts of the plant. The leaves and roots are specialized for Monoterpenoids Indole Alkaloids (MIA) biosynthesis. It is known for the production of two potent anti-cancer agents, the bisindole alkaloids Vincristine and Vinblastine. These compounds are classified as terpene indole alkaloids that are used as anti- cancer, anti- malarial and anti-arrhythmic agents. Vinblastine, an anti microtubule drug is used to treat certain cancers like Non- small cell lung cancer, Head and Neck cancer, Hodgkin's lymphoma, Breast cancer and Testicular cancer (Aslam *et al*., 2010). Having such an economic and medicinal

importance, obtaining these drugs on large scale with better accuracy is both tedious and expensive process. The stability of the drug is referred as the capacity of a drug to remain within established specification of identity, quality and purity in a specific period of time. There are various advantages of nanoparticles such as longer shelf-stability, high carrier capacity and ability to incorporate hydrophilic and hydrophobic drug molecules. The metabolism of drugs occurs through basic chemical reactions as soon as the administered compound comes into contact with enzymes that are capable of altering its chemical structure. Many drugs are susceptible to some form of chemical decomposition either interaction with enzymes or through improper storage and use and such degradation often leads to a loss of potency. The main chemical reactions that affect the stability of a drug are oxidation and hydrolysis. The physico-chemical properties such as pH stability, temperature stability, and shelf-life can be determined so as to ensure that it does not degrades or changes its conformation and properties at adverse to adverse conditions and remains in its stable state.

MATERIAL AND METHODS

Synthesis of nanoparticles :

Silver nanoparticle:

The silver nanoparticles were prepared by chemical reduction method. 1ml of 0.001M $AgNO_3$ aqueous solution was added in 100ml of distilled water along with 1ml of 0.1M PEG and 1ml of $NaBH_4$. Then the mixture was vigorously stirred and heated at 70-80^0 C till the colour changed to pale yellow. Then, it was removed from heating and the solution was kept in amber cooled to keep away from sunlight. Confirmation of silver nanoparticle was done using SEM and UV-Vis spectrophotometer to obtain a peak at 430nm. Calibration curve at 430 nm was drawn for further calculations.

Layered double hydroxide (*LDH*) *nanoparticles:*

Synthesis of Mg-Al LDH nanoparticles was done through conventional co-precpitation route in which 20ml of 0.1 M solution of Mg $(NO_3)_2$ and 20 ml of 0.3 M solution of Ag $(NO)_3$ was mixed and titrated against 40 ml of 0.1 M solution of NaOH with vigorous stirring at fixed temperature *i.e.* 50^0 C. The pH value of the mixture was maintained at 9-10 during the entire reaction process and was performed in inert atmosphere. The precipitate was stirred heated and stirred for 30-40 minutes and then washed with distilled water and centrifuged at 5000 rpm for 10 min. and then again washed with distilled water thrice and then resuspended in 20 ml of distilled water. The concentration was calculated by drying 1 ml of prepared LDH on an aluminium foil and taking the weight of foil with and without LDH and characterization was done using SEM.

Montmorillonite nanoparticles:

2g of MMT was dispersed in 10 ml of 0.1N NaCl solution and stirred for 12 hours and centrifuged. This process was repeated thrice. The slurry obtained was centrifuged at 5000 rpm for 10 min. and washed with distilled water. It was then suspended in 20 ml of distilled water and then Na-MMT was purified by sedimentation which was used for further studies. The concentration was calculated by drying 1 ml of prepared MMT on a silver foil and taking the weight of foil with and without MMT and characterization was done using SEM.

Preparation of crude vinblastine :

Extraction and partial purification of Vinblastine from the roots of *Catharanthus roseus* was done by simplified methanolic extraction procedure (Tikhomiroff and Jolicoeur, 2002)

Various dilution of crude vinblastine stock was used for the concentration estimationat varying temperature ranges of 30, 40, 50, 60, 70, 80, 90, 100^o C.

Nanoformulation of vinblastine :

Nanoparticle formulated vinblastine using silver, LDH and MMT was done by adding 0.5 ml of crude vinblastine and 0.5 ml of NPs to 9 ml of distilled water, respectively.The solutions are kept at different temperatures to perform stability test.

Temperature stability :

Stability test was done at different temperature ranges of 30, 40, 60, 80, and 100^0 C, respectively. The solution was prepared by adding 0.5 ml of crude vinblastine to 9.5 ml of DW in case of pure solution of crude vinblastine and to 9 ml of DW and 0.5 ml of NPs to prepare different solutions of NPs, respectively and kept at different temperature ranges. The solutions were

vortexed for 5 min. and then filtered using Whatmann filter paper. The filtered solution was calibrated at 420 nm using UV-Vis spectrophotometer. The stability was checked by calculating the concentration from the linear equation of the graph and compared.

RESULTS AND DISCUSSION

The findings of the present study as well as relevant discussion have been presented under the following heads:

Nanoparticle synthesis and characterization :

Silver nanoparticle :

Silver nanoparticles were prepared by reduction of silver nitrate solution by Sodium borohydride in the presence of PEG. Silver nitrate and PEG is a colourless solution but after adding $NaBH_4$ to the solution the colour turned to pale yellow which indicated formation of silver nanoparticles.

UV- Vis spectroscopy characterization of silver nanoparticle showed peaks at 390nm and 430nm which indicated total conversion of silver ions to silver nanoparticles as shown in Fig. 1.

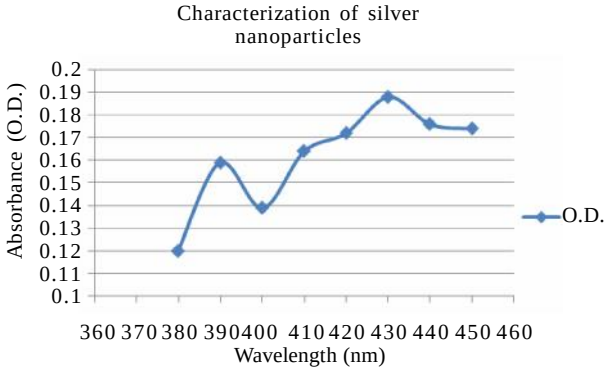

Fig. 1 : Characterization of silver nanoparticles using UV-Vis spectrophotometer

Scanning electron microscope (SEM) image of Silver nanoparticle showing the morphology and the size approximately 50-150nm as shown in Fig. 2.

Synthesis of LDH nanoparticles was done using co-precpitation method. Scanning electron microscopy (SEM) image of LDH nanoparticles shows that the morphology and size distribution of LDH nanoparticle showing the particle size of approximately~300nm as shown in the Fig. 3.

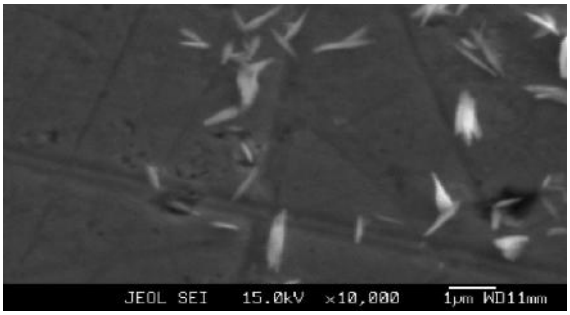

Fig. 2 : Scanning electron microscopy (SEM) image of silver nanoparticle Mg-Al (LDH) nanoparticles

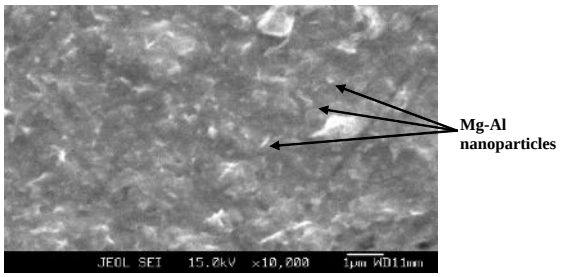

Fig. 3 : Scanning electron microscopy (SEM) image of Mg-Al nanoparticles

MMT nanoparticles :

Scanning electron microscopy (SEM) image of MMT nanoparticles shows the morphology and size distribution shown in Fig. 4.

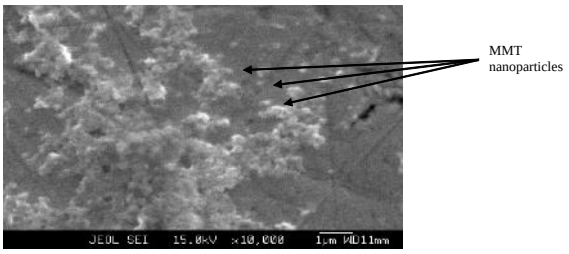

Fig. 4 : Scanning electron microscopy (SEM) image of MMT nanoparticles

Characterization and calibration of crude vinblastine:

Characterization of crude vinblastine was done using UV-Vis spectroscopy which showed a peak at 420 nm as shown in Fig. 5.

Crude vinblastine was calibrated by UV-Vis spectra at 420 nm at different concentrations which is calculated using a relation between OD (y) and concentration (x) equation

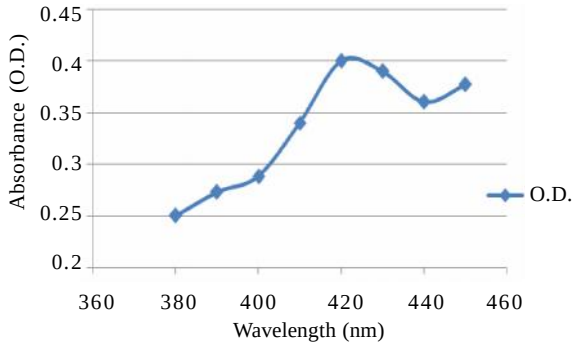

Fig. 5 : Characterization of crude vinblastine using UV-Vis spectra

$$y = 0.003x + 0.014 \quad \text{..... (Eq. 1)}$$

Temperature stability analysis:

Stability curve of pure vinblastine shows that there was minimal decrease in stability with increase in temperature as shown in Fig. 6.

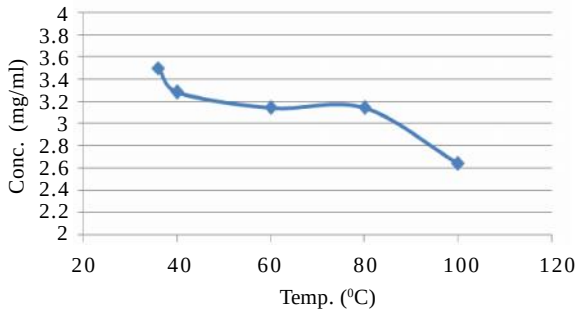

Fig. 6 : Temperature stability of pure vinblastine

Comparative analysis at variable temperature :

The comparative study of temperature stability of pure vinblastine and NP formulated vinblastine showed that LDH and MMT have highest stability at 60°C. The formulation with silver NP showed decrease stability with temperature as shown in Fig. 7.

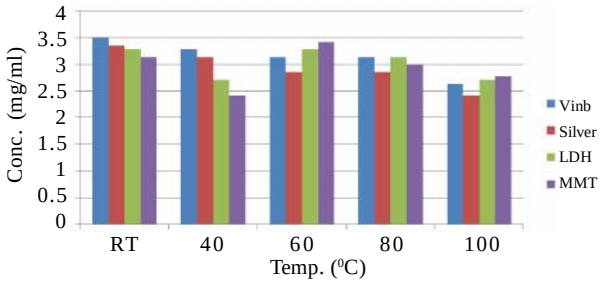

Fig. 7 : Comparative analysis of temperature stability of pure vinblastine and NP formulated vinblastine

Though a lot of researches have been carried out for the increase in production of vinblastine, researches on stability of the drug in pure form and with NP formulation has not been performed extensively. Apart from the production of the drug which matters, its stability under different conditions is also important.

Crude vinblastine was extracted from *Catharanthus roseus* and incorporated into the nanoparticles silver, LDH and MMT. Silver NPs were coated with PEG which acts as a capping agent and a linker between vinblastine and nanoparticles. The current study demonstrates that the attachment of crude vinblastine into the nanoparticles for the enhancement of its stability compared to the pure drug. Vinblastine formulation with silver NP showed the best results in comparison to LDH and MMT. The comparative analysis of stability of pure and nanoparticle formulated vinblastine showed that LDH and MMT formulations had higher stability at 60°C. Silver showed decreasing stability with decrease in temperature and there was no significant effect on the stability of vinblastine.

NPs coated by PEG with a molecular weight of 2~5 kDa gives desirable circulation profile for medical applications. It has also been reported that the long circulating NPs were typically coated with layer of PEG, which is a synthetic hydrophilic polymer. It forms a hydration layer that retards resistance recognitions by satirically inhibiting hydrophobic and electrostatic interactions with plasma proteins (Uchida *et al.*,2005)

Liposome and immune liposome formulations of two *Vinca* alkaloids, vincristine and vinblastine, were prepared using intra liposomaltriethyl ammonium sucrose octasulfate and examined for their ability to stabilize the drug for targeted drug delivery *in vivo* (Noble *et al*., 2009).

Conclusion :

There are several commercially valuable secondary metabolites which are been used as an anti-cancer agent, one of them is vinblastine produced from *Catharanthus roseus*. As it has the ability to inhibit the assembly of microtubules vinblastine has been implicated as possible target sites for certain kinds of cancer including breast cancer and various other cancers. Four types of nanoparticles were formulated with different methods and encapsulated with vinblastine and aspirin to check their physico-chemical properties

The comparative study of stability of pure vinblastine and vinblastine formulated NPs showed that LDH and MMT formulated vinblastine showed high stability at 60^0 C. The formulation with silver NP showed decrease stability with temperature. In case of silver nanoparticle there was no significant effect on the stability of Vinblastine. LDH and MMT formulated drug can be used for the development of new medicines which are safer, to prevent the multi-drug resistance mediated efflux of chemotherapeutic agents and product life extension. Temperature stability and shelf-life can be enhanced so as to ensure that it does not degrades or changes its conformation and properties at adverse conditions and remains in its stable state.

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