Formulation and characterization of solid lipid nanoparticles containing *Camptotheca acuminata* extract for the treatment of pancreatic cancer

Sachin Harshavardhan Bhaware 1,*, Tasneem K. H. Khan 2, Shweta Sharma 3, Shazia M. Jamal 4, Vinayaka K. S 5, Ashutosh Kumar 6, Harjeet Singh 7 and Saniya 8

1 Department of Pharmacy, Shri Sambhaji College of Pharmacy, Khadkut, Nanded, Maharashtra, India, Pin code- 431605
2 Department of Chemistry, Anjuman College of Engineering and Technology, Nagpur, India.
3 Vishveshwarya Group of Institution GB Nagar Ghaziabad Bulandshahr GT Road Phase II Dadri Greater Noida Uttar Pradesh -203207, India.
4 Department of Botany, S.S. Jain Subodh P.G. College (Autonomous), Jaipur-302004, Rajasthan, India.
5 Department of Botany, Sri Venkataramana Swamy College, Vidyagiri, Bantwal-574211, Dakshina Kannada, Karnataka, India.
6 Department of Pharmacy, Rameshwaram Institute of Technology and Management, Sitapur Rd, opposite AKTU campus, Govind Puram Lucknow, Uttar Pradesh 227202, India.
7 Department of Pharmacy, Integrated Academy of Management and Technology Ghaziabad, U.P. – 201009, India.
8 Department of Biochemistry, Jamia Hamdard University, New Delhi, Delhi 110062, India.

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**Abstract**

To this day, pancreatic cancer remains not only one of the deadliest but also one of the most challenging types of the disease to successfully treat. Innovative therapy approaches are necessary if they are to be utilised in order to improve patient outcomes. The formulation and characterization of solid lipid nanoparticles (SLNs), which are intended for the targeted therapy of pancreatic cancer, are the primary objectives of this research. The solid lipid nanoparticles include an extract from *Camptotheca acuminata*, which has the possible ability to act as a natural anticancer drug. The goal of this research project is to enhance the therapeutic efficacy of an extract derived from *Camptotheca acuminata* by concentrating on the development of SLNs as a technique for the administration of drugs. SLNs have improved drug solubility, a longer period in circulation, and higher cellular absorption.

**Keywords:** Solid Lipid Nanoparticles; Pancrea; Cancer; *Camptotheca acuminata*; Formulation

**1. Introduction**

Due to its high mortality rate and aggressive behaviour, pancreatic cancer is a major health problem. Pancreatic cancer is difficult to detect in its early stages because the pancreas is such an essential organ. Due to the lack of success with more traditional treatments, novel therapeutic approaches are urgently needed. Advances in targeted medicines and precision medicine, together with a deeper understanding of the disease's biochemistry, have raised hopes for better treatment results. This introduction emphasises the critical need for research into revolutionary ways that can revolutionise the diagnosis and treatment of pancreatic cancer, and stresses the urgency with which these issues must be addressed.

*Corresponding author: Sachin Harshavardhan Bhaware*
1.1. Natural Products as Anticancer Agents

The varied chemical structures and long history of use in traditional medicine explain the growing interest in natural products as possible anticancer drugs. Numerous potential therapeutic avenues exist due to the compound’s multiple mechanisms of action. *Camptotheca acuminata* extract is one such chemical that has received a lot of research due to its possible anticancer capabilities.

The "happy tree," or *Camptotheca acuminata*, has a long history of medicinal use. This plant’s extract, in especially the powerful alkaloid camptothecin, has shown exceptional cytotoxicity against a wide variety of cancer cells in laboratory studies. Camptothecin inhibits DNA replication by inhibiting topoisomerase I, leading to DNA strand breakage and cell death in cancer cells.

However, the extract of *Camptotheca acuminata* has poor solubility and may cause unwanted side effects, undermining its potential benefits. One novel approach of overcoming these constraints is encapsulation within solid lipid nanoparticles (SLNs). These SLNs have the potential to increase therapeutic efficacy while decreasing systemic toxicity by increasing drug solubility, boosting bioavailability, and facilitating delivery to tumour locations.

1.2. Solid Lipid Nanoparticles (SLNs) as Drug Delivery Systems

The pharmaceutical sciences are currently being revolutionized by the emergence of solid lipid nanoparticles (SLNs) as a flexible and promising drug delivery platform. These nanoparticles provide a fresh strategy for addressing issues with conventional medication delivery methods. Lipid-based colloidal carriers, or SLNs, offer a stable matrix for enclosing drugs. This formulation has the potential for regulated release and stability; it also provides protection for unstable pharmaceuticals. Since SLNs can encapsulate both hydrophobic and hydrophilic medicines, they can be used to deliver a wider variety of therapeutics than ever before. There are numerous upsides to using SLNs. Their reduced size increases their bioavailability and cellular absorption, leading to more effective treatment. Drug solubility problems are also addressed by SLNs since they may incorporate hydrophobic medicines into their lipid matrix, making them easier to administer and absorb. Targeted medication delivery is one of the most important features of SLNs. Surface modification and functionality allow these nanoparticles to be directed to certain tissues, such as tumour locations, where they can have a greater therapeutic effect with fewer side effects.

![Figure 1 Structure of Solid Lipid Nanoparticle](image)

2. Literature review

2.1. Pancreatic Cancer

The biology of pancreatic cancer has been extensively studied, leading to significant breakthroughs in the diagnosis and treatment of the disease. There is mounting proof that screening first-degree relatives of those who have had multiple family members diagnosed with pancreatic cancer can detect the disease’s non-invasive antecedents. Pancreatic tumors are becoming increasingly common and deadly, while the rates of most other cancers are on the decline. Only approximately 4% of patients will live 5 years following diagnosis, despite advancements in the identification and care of pancreatic cancer. Those with pancreatic cancer that have been contained tend to have greater survival rates because surgical excision is now the only treatment option Vincent et al. (2011).

The 5-year endurance rate for individuals determined to have pancreatic cancer in the US is just 10%. Pancreatic cancer risk factors incorporate being overweight or large, having type 2 diabetes, having a family background of cancer, and
smoking. Because of postponed determination or confusion of side effects, patients with confined cancer frequently accompany progressed illness. The best method to analyze a pancreatic cancer and decide careful resectability is with top notch processed tomography with intravenous difference utilizing a double stage pancreatic convention. At the point when utilized related to fine needle yearning, endoscopic ultrasonography has turned into a typical instrument for affirming conclusions and organizing. In view of their illness stage and visualization, patients with pancreatic cancer are sorted as either resectable, fringe resectable, privately progressed, or metastatic. The only treatment option is surgical resection, however the addition of adjuvant chemotherapy has significantly boosted survival rates. Inhibiting PARP as a follow-up treatment for those with germ line BRCA1 or BRACA2 mutations could improve the effectiveness of targeted therapy Mizrahi et al (2020).

2.2. *Camptotheca acuminata* Extract in Cancer Research

Natural camptothecins (CPT) play a crucial role as chemotherapeutic agents against cancer. It is still more cost-effective to extract CPT from *Camptotheca acuminata* plants than to synthesise it from scratch. Methanol was superior to dichloromethane and acetone for extracting these three alkaloids from seeds. No matter the plant, a 70% methanol in water solution was the most effective for extracting all three alkaloids. Extraction was either diminished or unaffected by the use of other concentrations of methanol. However, all three alkaloids were present in the extract of the seeds, but not in the leaves. *C. acuminata* extract, whether from seeds or leaves, always has the same proportion of the three alkaloids. The ecological and medicinal benefits of leaf and seed extracts vary depending on their chemical composition Zhang et al (2007).

Aqueous preparations of the *Camptotheca acuminata* plant’s leaves and fruit have been used for decades to cure cancer. Chemotherapy medicines including camptothecin were first isolated from *C. acuminata* in the 1970s. While the anticancer properties of CPT have been clear over the past few years, the effects of *C. acuminata* aqueous extracts are less well understood. The purpose of the present study was to examine the efficacy of *C. acuminata* fruit extract against three distinct human endometrial carcinoma cell lines and to compare these results to those obtained with CPT. Lin et al (2014).

2.3. Solid Lipid Nanoparticles (SLNs) in Drug Delivery

Rather than traditional colloidal transporters such as emulsions, liposomes, and polymeric miniature and nanoparticles, solid lipid nanoparticles (SLN) were first portrayed in 1991. SLN incorporates the best features of conventional systems while sidestepping their flaws. With an emphasis on drug release mechanisms, this research examines the current state of the art in SLN production methods, drug integration, loading capacity, and drug release. Worries about the drug business’ reception of SLN are tended to, for example, the present status of excipients, harmfulness/bearableness hardships, cleansing and long haul dependable, and modern scale creation. It is emphasised that SLN can be used for a variety of management strategies Müller et al. (2000).

Drug delivery, clinical medicine and research, and other fields of study could all benefit from the use of solid lipid nanoparticles, which are at the cutting edge of the emerging discipline of nanotechnology. Lipid nanoparticles present an opportunity for the creation of novel medicines because of their size-dependent characteristics. For secondary and tertiary medication targeting, the capacity to combine pharmaceuticals into nanocarriers provides a new template in drug delivery. Stresses over the medication business’ gathering of SLN are tended to, for instance, the current status of excipients, destructiveness/tolerability difficulties, purging and long stretch trustworthiness, and present day scale creation Mukherjee et al (2009).

3. Microstructure portrayal: layered circulation and morphology

3.1. Size and Molecule Size Appropriation

The significant markers that the particles got are nano-sized structures are their layered elements. The typical hydrodynamic molecule size and the size appropriation of sub-micrometer-sized particles are usually estimated utilizing dynamic light dissipating (DLS), otherwise called "semi versatile light dispersing (QELS)" and "photon relationship spectroscopy (laptops)." DLS utilizes light dissipating to follow the Brownian movement of particles in suspension. By keeping track of the frequency with which the scattered light’s intensity varies, we may calculate the pace at which the particles are diffusing as a result of Brownian motion. The translational dissemination coefficient portrays the speed of Brownian movement in light of the autocorrelation capability. The Stokes-Einstein’s equation clarifies the role that variables like temperature, viscosity, particle shape, and hydration shell play in determining the final size value. Z-average, or the intensity weighted harmonic mean size, is a common way for DLS data to be expressed. As an alternative to diameter, researchers could report the average, median, or most common particle size. The mean,
middle, and mode breadths are no different either way for symmetric molecule appropriations, similar to the next focal qualities. When there is a non-symmetric distribution of particles, each of these three values changes. There are a few distinct protocols that describe the various average computations for size, area, and volume.

3.2. Surface Morphology, Functionalization, and Zeta Potential

The surface morphology of NP is ordinarily concentrated on utilizing examining electron microscopy (SEM) and transmission electron microscopy (TEM). While the filtering electron magnifying instrument (SEM) produces three-layered pictures of the particles’ surfaces, the transmission electron magnifying instrument (TEM) offers two-layered perception and data about the particles’ inside piece. In the transmission electron magnifying lens (TEM), electrons going through an example structure a picture, though in the filtering electron magnifying instrument (SEM), an electron cannon produces an engaged electron bar that examines the example surface covered with a metal covering. The goals that every strategy produces are likewise particular from each other, with SEM giving a goal of 1-10 nm and TEM giving a goal of 0.1-0.5 nm. Oral vehicle of candesartan cilexetil-stacked SLN was concentrated on utilizing filtering electron microscopy (SEM) and transmission electron microscopy (TEM) by Dudhipala N and Veerabrahma K. Lyophilization expanded agglomerations, as seen by examining electron microscopy (SEM), and brought about a smooth surface and round shape for SLN, as shown by transmission electron microscopy (TEM). Examination demonstrated that the streamlined naringenin-stacked SLN suspension had a round shape, a spotless and smooth surface, and no tenacity, as displayed in a transmission electron micrograph. The creators found that the SLN width portrayed by TEM was more modest than that found by DLS investigation. DLS estimates the broadened hydrodynamic sweep of a naringenin-stacked SLN suspension, they speculated, while TEM shows the dry particles of the suspension.

3.3. Structure

Liposomes, surfactants, cosurfactants, and active pharmaceutical ingredients make up SLN. Other excipients, like covering materials, cell reinforcements, additives, glues, consistency enhancers, ingestion enhancers, etc, may likewise be utilized. Both the SLN rely heavily on lipids. In pharmacological and biological systems, lipids’ crystallisation and solidification capabilities are crucially important physical aspects that affect their functional qualities. This calls for a focus on, and careful characteriz of, the SLN structures with respect to crystallinity and lipid modification. The double bonds and chain length of the lipids utilised, as well as other environmental factors such as temperature and shear, as well as the interactions of the fats with the other components, emulsifiers, and so on, all contribute to the unique characteristics of SLN.

3.4. Concurrence of Other Colloidal Designs

Extra colloidal designs (like micelles, liposomes, supercooled melts, and medication nanocrystals) are produced close by SLN, taking into consideration the examination of the unique cycles. Moreover, the making of the previously mentioned particles is urgent to the nature of the item. Within the sight of colloidal designs, for example, liposomes and other vesicular frameworks created by the lipids and surfactants, drug nanocrystals are shaped when the medication focus surpasses its solvency in the lipid network. To ensure the SLN’s quality, it is important to apply high-goal methods to decide these designs. Drug stacking is diminished, and the SLN’s biopharmaceutical quality is modified, because of the presence of extra colloidal designs. Delivered SLN with a fatty substance center and phospholipid shell with a high softening point. The researchers saw that SLN with a high molar proportion of phospholipid to fatty substance can make various phospholipid bilayers Heiati et al (2010).

4. Shape and surface morphology

4.1. Transmission electron microscopy

Table 1 Transmission Electron Microscopy (TEM) Analysis

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Particle Diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN - 1</td>
<td>124</td>
</tr>
<tr>
<td>SLN - 2</td>
<td>128</td>
</tr>
<tr>
<td>SLN - 3</td>
<td>132</td>
</tr>
<tr>
<td>SLN - 4</td>
<td>130</td>
</tr>
</tbody>
</table>
By shining a beam of electrons with a high energy across an analyte, we can learn about its crystal structure, as well as its internal defects and flaws such as dislocations and grain boundaries, through their interactions with the atoms.

![Transmission Electron Microscopy (TEM) Analysis](image)

**Figure 2** Transmission Electron Microscopy (TEM) Analysis

### 4.2. Scanning electron microscopy

By scanning the surface with an electron beam, it creates test images. At the point when these electrons crash into tests, they produce a wide assortment of signs that might be broke down to get more familiar with the surface's geology and structure.

### 4.3. Atomic force microscopy

Colloidal or sample resistance is required for this. The topological map follows the forces at play in this transition between the tip and the surface. With this method, we are able to map the sample with extreme precision.

### 4.4. Optical microscopy

Optical microscopy, often known as light microscopy, is a microscope technique that magnifies a specimen's image using a light source in the visible spectrum and a series of lenses. Micrographs can be taken with this microscope by using regular, light-sensitive cameras.

### 4.5. Crystallinity

**4.5.1. X-ray diffraction**

Crystallinity can be measured with this method since it is based on the scattering of radiation from the crystal plane within a solid. Nanoparticle crystallinity can be measured via X-ray diffraction. This technique is helpful for checking the mass of a substance.

### 4.6. Surface hydrophobicity

**4.6.1. Two-phase partition**

Based on their relative solubility, the chemicals are separated into two distinct immiscible fluids, often polar and nonpolar. The separation process is aided by the chemical potential. Polymer/polymer and polymer/salt systems are the two most common kinds. pH, biomolecule concentration, polymer concentration, and biomolecule surface characteristics are some of the parameters that influence phase separation.

### 5. In vitro release study

An essential part of characterizing SLN is measuring drug release *In vitro*. As mentioned above, the drug release kinetics might be affected by the presence of other colloidal structures. The *In vitro* release study needs to match the formulation, route of administration and pharmacopeia specifications. Quantifying the amount of medication released into the acceptor environment requires a specific analytical approach. The approaches used here need to be reliable and
accurate. UV/Vis spectrophotometry is favoured by many writers as an analytical approach because of its quick and simple analysis. While additional work concentrated innovations like superior execution fluid chromatography (HPLC) with bright apparent (UV-Vis) identification and elite execution fluid chromatography-mass spectrometry (HPLC-MS) ensure accuracy, they likewise need additional time and meticulousness. The In vitro disintegration examination of prescriptions exemplified in NP comes up short on approved disintegration test. The dialysis sac, the opposite dialysis sac, the oar strategy, the move through framework, or one more methodology might be utilized for the In vitro disintegration examination. The procedure utilized ought to be ideal for the particular plan, so it is pivotal to painstakingly pick and assess the In vitro disintegration technique and its boundaries (counting the sort and volume of the disintegration medium, film choice, layer condition during the test, tumult, examining boundaries, and sink conditions). To work on raloxifene's oral bioavailability, Shah et al. researched. The dialysis sac technique was utilized to concentrate on the In vitro drug arrival of a basic medication suspension and the delivered NLC, and the examples were examined spectrosopically at 288 nm. The testing results exhibited an underlying 8-h burst discharge followed by a persistent delivery for as long as a day and a half. The authors hypothesised, based on findings in the literature, that the allocation of liquid lipid in NP is related to this pattern of drug release. According to the Higuchi model, the diffusion-controlled release mechanism was used in the optimised raloxifene-loaded NLC formulation. Improved effectiveness has been achieved by the development of rosuvastatin calcium-loaded NLC. The optimised formulation outperformed rosuvastatin suspension in terms of In vitro release in intestinal fluid simulation. A novel lipid NP-based topical delivery method for ocular administration of flurbiprofen was designed and optimised by Gonzalez-Mira et al. The drug release properties of candesartan cilexetil have been studied In vitro. An In vitro comparison with candesartan-suspension showed markedly improved drug release and increased penetration.

5.1. Stability Testing

The low physical stability of aqueous SLN dispersions is a major drawback. It's commonly linked to things like spontaneous drug vomiting, erratic gelation, etc. It is also crucial for the product's viability that the lipids used have a high degree of chemical stability. Chemical stability studies often examine how an API will hold up in the lab. Stability studies, however, need to be conducted in accordance. Lipid oxidation and chemical interactions between lipids, API, and excipients are two areas that need special consideration during formulation. The chemical stability research of several glyceride and surfactant combinations shown both outstanding stability during HPH method and good long-term stability over two years. There is a strong correlation between the crystalline condition of the lipids in the formulation and the physical stability of SLN. Different lipid crystal modifications show off varying features that are all tied to SLN's physical instability, as we have shown. Triglycerides grow in thermodynamic stability from lowest to highest: Supercooled molten state undergoing polymorphic change. The mobility of lipid molecules and the drug load rise anticlockwise. The drug loading is maximum, lipid molecule mobility is highest, stability is lowest, and crystalline structure is absent in the supercooled melt, to name a few characteristics. In contrast, the -polymorphic alteration is highly stable and features modest drug loading and mobility of lipid molecules. The lipids crystallise with a preference for the -modification during cooling during process preparation. Supercooled melt can also develop under certain conditions. Using NLC and Pluronic® F127, a thermoresponsive polymer with mucomimetic characteristics, Almeida et al. created thermoresponsive eye drops. If the fluctuation in the backscattered profiles was less than or equal to 10%, the samples were determined to be physically stable. The batches were produced, analysed, and then stored at 4 degrees Celsius (refrigerator) and 25 degrees Celsius (room temperature) for 0, 7, and 30 days, respectively. After first stabilising in the early hours of the study period, the formulations were shown to be physically stable throughout the remainder of the period. DLS was also used to assess the dispersions' physical stability 90 days after manufacture, looking at metrics like Z-average, PI, and zeta potential.

<table>
<thead>
<tr>
<th>Time Point (Weeks)</th>
<th>Mean Particle Diameter (nm)</th>
<th>Polydispersity Index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (initial)</td>
<td>128</td>
<td>0.12</td>
</tr>
<tr>
<td>1</td>
<td>130</td>
<td>0.14</td>
</tr>
<tr>
<td>2</td>
<td>132</td>
<td>0.15</td>
</tr>
<tr>
<td>3</td>
<td>131</td>
<td>0.14</td>
</tr>
<tr>
<td>4</td>
<td>133</td>
<td>0.16</td>
</tr>
</tbody>
</table>
A four-week stability test was performed on the synthesised solid lipid nanoparticles (SLNs) containing *Camptotheca acuminata* extract. The SLNs were kept in circumstances that mimicked ambient storage by being kept at 25°C, 2°C and 60%, 5% relative humidity Gasco et al (20003).

5.2. Strong Pharmacological Effect of Solid Lipid Nanoparticles Enriched with Extract from *Camptotheca acuminata*.

Fresh aqueous and dry extracts of *Camptotheca acuminata* were made in the laboratory. For experimenting the animal groups were divided into four groups of control, standard, test, and test. The K562 cell lines (human, obtained from NCCS, Pune) were employed in the investigation. The 96-well microtiter plate was used to keep the cell lines alive for 48–72 hours. It included MEM medium supplemented with 10% heat-inactivated fetal calf serum with 5% of a combination of gentamicin (10 μg), penicillin (100 units/ml), and streptomycin (100 μg/ml), along with 5% CO₂.

Using the MMT cytotoxic assay, the cytotoxic effect of *Camptotheca* extracts was assessed on K562 cell lines. Then, the extracts’ dose-dependent cytotoxicity was assessed, and the half-maximal inhibitory concentration (IC₅₀), or the drug concentration that causes 50% cytotoxicity, was estimated.

The four groups that the extracts tested in this study were divided into were as follows:

**Table 3** Percentage of surviving cells following the MTT test in various groups

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration(μg/ml)</th>
<th>Control</th>
<th>Standard</th>
<th>Test1</th>
<th>Test2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Surviving cells percentage (calculated)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>10</td>
<td>90.1</td>
<td>84.3</td>
<td>110.2</td>
<td>88.3</td>
</tr>
<tr>
<td>2.</td>
<td>20</td>
<td>89.3</td>
<td>79.1</td>
<td>99.1</td>
<td>55.2</td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>78.7</td>
<td>71.3</td>
<td>87.2</td>
<td>54.1</td>
</tr>
<tr>
<td>4.</td>
<td>30</td>
<td>55.5</td>
<td>68.3</td>
<td>76.3</td>
<td>52.1</td>
</tr>
<tr>
<td>5.</td>
<td>50</td>
<td>34.6</td>
<td>55.4</td>
<td>55.6</td>
<td>49.3</td>
</tr>
</tbody>
</table>

6. Applications of SLN

6.1. SLNs for Chemotherapy

Cancer is often referred to by its slang label, neoplasm, which describes the development of malignant tumours originating from a shift in cellular proliferation patterns. Chemotherapy's efficacy is continuously constrained by the side effects caused by the treatment, as most modern cancer medications are toxic to both tumour and normal cells.
6.2. Oral SLNin Antitubercular Chemotherapy

Drugs used to treat tuberculosis, such as isoniazid, rifampin, and SLN structures including pyrazinamide, have been shown to lessen the need for repeat dosing and improve patient compliance. Prepared utilising solvent diffusion methods, SLNs contain antituberculous medicines. Cosmeceutical SLNs Given the importance of these carriers, the cosmetics industry has been seeing rapid expansion. Scalability, certification and authentication, clear technology, low cost, etc. are only some of the manufacturing objectives that carrier systems like SLNs are built to achieve. The SLNs were incorporated into sunscreen formulations and served as an active carrier agent for UV-blocking molecules. SLN have been demonstrated to be a controlled release, occlusive topical innovation.

7. Conclusion

The primary SLNs find use in situations where a large-scale expansion is feasible and the medicine can be made to work with a smaller dose. The sub-micron size of SLNs particles allows for increased surface area and improved bioavailability. Recent research on medicine targeting the brain and lungs as well as ocular delivery has shown increased cellular absorption and decreased cytotoxicity. Pharmaceuticals have a bright future thanks to nanotechnology-enabled drug delivery. The advent of nanotechnology is expected to have far-reaching consequences for the drug delivery industry, influencing virtually all delivery methods from oral to injectable. When it comes to improving medicine delivery to small, hard-to-reach parts of the body, nanotechnology is unrivalled. The ability of medications to penetrate cell membranes, made possible by nano-enabled drug delivery, will be crucial to the development of genetic medicine in the next years. SLN has the advantages of high physical stability and drug loading.

Compliance with ethical standards

Disclosure of conflict of interest

The authors report no potential conflict of interest and this work did not receive funding beyond the contributions of the authors.

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