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Irinotecan Engineered Proniosomes: *In vitro* and *In vivo* Characterization

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Author's contribution

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Objective: The focus of this research has been to improve efficacy, decrease tolerance and increase the irinotecan pharmacokinetic profile.
Methods: Proniosomesformulated with various surfactants, cholesterol and dicetyl phosphate using the slurry method. A slurry process was used to prepare proniosomes with maltodextrin as the carrier by using surfactants span 20, span 60, tween 20 and tween 80.
Results: The preparations were characterized in terms of shape and specific surface area, entrapment efficacy, *in vitro* release studies, *in vivo* tissue diffusion and stability testing. The proniosome surface was found to be smoother in nature showing thin and compact layer with skim milk powder. For formulation 2 (73.94±2.8%), the maximum entrapment efficacy was found.
Conclusion: The formulation 3 obtained the desired maximum release profile within 24 hours (98.06%). The *in vivo* tissue distribution studies for the proniosomes reveal that the drug was

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preferentially targeted to liver followed by the alveolus and lymphatic system.Stability studies have indicated that the most acceptable condition for storage of the formulation 2 was 4^{o} C. Proniosomes provide an acceptable method to the carrier for targeted therapy. These can be held at specific sites and can release the drug for a prolonged period of time.

Keywords: Irinotecan; Proniosomes; drug entrapment; tissue diffusion and stability testing.

1. INTRODUCTION

The principle of drug delivery to a given spot for the control of specific disease, thereby reducing the side effects of the drug and increasing their therapeutic index is considered as an obstacle [1,2]. The principle of a drug carrier with accuracy has always impressed researchers for generations and limited success has been achieved in this regard over the last decades. So this strategy includes the use of vesicular dosage forms which can provide selective toxicity in conjunction with an optimal drug content [3]. Vesicular formulations in the form of liposomes and niosomes are the most efficiently explored among many carriers used for target-oriented drug delivery. Liposomal formulations limit poor stability and low efficiency of drug absorption, while niosomes show structural uncertainty and frozen product aggregation, fusion, and leakage, thus reducing dispersion shelf life [4,5]. Liposomes were the first vesicle system discovered; however, they have several disadvantages, including cost and decreased stability at various pH levels [6]. Proniosomes are actually surfactant coated water-soluble carriers in the formofadry, free-flowing powder that on agitation with water,are converted to nonionic surfactant vesicles or niosomes. Non ionic surfactants used are are biodegradable, relatively nontoxic, more stable and inexpensive. The use of proniosomes can solubilize the drug with int hen onion icsurfactantvesicles [7]. Proniosomes overcome all the disadvantages of niosomes and provide the potential for targeted drug delivery with a flexible vesicular delivery model. They include dry surfactant-coated carrier compositions which are hydrated to obtain a suspension of niosomes before their use. The enhanced convenience for transport, delivery, storage and dosing makes proniosomes a promising industrial commodity [8,9] .

Chemically, Irinotecan (Camptothecin-11, CPT-11) is a synthetic analogue of the natural camptothecin alkaloid. It is a chemotherapy agent that is an inhibitor of topoisomerase 1 and is used as the drug of choice to treat colon cancer [10]. Another very substantial harmful effects of irinotecan are excessive dehydration with intense immune suppression Irinotecan-associated diarrhoea is serious and highly crucial, often escalating to extreme dehydration prompting inpatient care. The immune response is also affected, which is reflected in the significantly decreased number of white blood cells in the body [11]. As a result, an attempt is being made in the present work to develop an alternative vesicular drug delivery system for irinotecan in the form of proniosomes that will gain control drug release and site specificity, improved drug stability, high drug payload and lack of carrier biotoxicity [12].

The key objective of this study was to improve drug delivery with optimal therapeutic benefits, including safe and efficient disease management.

2. MATERIALS AND METHODS

Irinotecan drug was received as a gift sample from M/S Cipla Ltd , Bangalore, India, While maltodextrin was obtained as a gift sample from Riddhi Siddhi Glucobials Ltd, Gokak, Karnataka, India. Surfactants, cholesterol and dicetyl phosphate have been purchased from Hi media Chem Pvt Ltd, Mumbai, India. The other reagents used were of scientific accuracy.

2.1 Experimental Animals

For the study, Albino Wistar rats of both sexes weighing 200-250 g were chosen. Under optimal conditions, the animals were housed and fed with standard diet pellets (Lipton India Ltd., Mumbai) and basic sanitation. Study carried out in Sri Adichunchanagiri College of Pharmacy, durationof study is six month.

Animals were treated in compliance with the Prescribed Animal Handling Protocols and Appraisal Manual [13].

2.2 Proniosomes Formulation

The optimized proportions of surfactants, cholesterol, and dicetyl phosphate (molar concentration of 47.5:47.5:5, respectively) were used in this research [9]. A slurry process was used to prepare proniosomes with maltodextrin

as the carrier by using surfactants span 20 (PF1), span 60 (PF2), tween 20 (PF3) and tween 80 (PF4) [14]. Maltodextrin powder (10g) was transferred to the 250 ml round-bottom flask and the total volume of the surfactant blend (14.5 ml) was directly added to the vessel. With the rotation speed set at 60 rpm and the temperature at 37^0C, the flask was connected to the rotary evaporator. A vacuum was introduced until it seemed that the powder was dusty and supposed to be free. The container was separated from the evaporator and before its further use the proniosomes were packed in the screw cap vials.

2.3 Particle Imaging by Scanning Electron Microscope (SEM)

Surface morphology testing was done by SEM using the JSM-T330A scanning microscope.
Washed brass sample holder studs were used to place the samples. Wet adhesive, paint was done to these studs and when the paint was wet, the proniosome powder was applied around each stud and dried at room temperature. After that, SEM images were captured [15].

2.4 Drug Charges and Rehydration of Proniosomes

Accurately weighed10mg of irinotecan hydrochloride trihydrate has been dispersed in 10 ml of PBS (pH 7.4). The drug solution was introduced to the vials containing proniosome powder, the vials were capped and then connected to the vortex mixer, where niosomes were agitated at a maximum speed of 2 min.

2.5 Estimation of the Efficacy of Drug Entrapment

The extent of inclusion (entrapment efficiency) of irinotecan hydrochloride trihydrate into the proniosomes was evaluated. Along with the centrifugation of the aqueous proniosome suspension [16], the quantity of the free drug in the reaction mixture as well as the quantity of the injected drug was estimated spectro-photometrically by model UV-1700, Shimadzu Corporation, Japan, at 369 nm. Entrapment efficiency (EE, percent) was calculated from the Equation 1.

$$EE = 100(Wo - Wf)Wo \ldots\ldots\ldots\ldots (1)$$

where

- The total amount of drug used in the preparation of the proniosomes - *Wo*
- The total amount of free drug in the supernatant - *Wf.*

2.6 In Situ Analysis of Drug Release

10 mg of irinotecan proniosomes were taken into a tube with a dialysis membrane wrapped at one end of the tube. The tube was mounted vertically in a glass bottle with 50 ml of phosphate-buffered saline (PBS).

(pH 7.4) in such a manner that it hit the layer of the phosphate solution.The entire set was mounted on a magnetic stirrer spinning at 50 rpm with a buffer temperature operated at 37 ± 1^0C. 1 ml of the release medium (buffer) was collected at different periods of 1, 2, 4, 8, 16, 24 h. and substituted with same amount of PBS at each time. The mixture was screened through a 0.45 mm membrane filtration system (Elix, Mill-Q), diluted suitably and absorbance measured at at 369 nm using UV Spectrophotometer [17].

2.7 Tissue Distribution Study

The goal of this study was to compare the efficiency of targeting drug-loaded proniosomes with that of pure drugs in terms of the extent of trying to target of the different reticuloendothelial (RES) organs, i.e. liver, lung, spleen, kidney, heart and brain [18]. Before the study, nine (9) normal adolescent rats weighing 200-250 g were collected and dieted for 12 h. The pets were divided into 3 groups, each with 3 rats. The group I obtained niosomes (batch PF2) equal to 810 mcg of irinotecan hydrochloride trihydrate i.v in the jugular vein after the dispersion of the niosomes in phosphate Buffer. Group-II rats provided 810 mcg of pure free) irinotecan hydrochloride trihydrate intravenously whereas Group-III rats have been treated as vehicle control and injected with sterile PBS intravenously.Both Group I and II animals were anesthetized with Ketamine HCL (22 mg/kg.i.m) prior to the use of pure medication and formulation (PF1) to alleviate painful symptoms.

After 3 h, rats were sacrificed by spinal fracture and their liver, lungs, spleen and kidneys were isolated. The organs of each rat were preserved separately in 5 ml of ethanol using a tissue

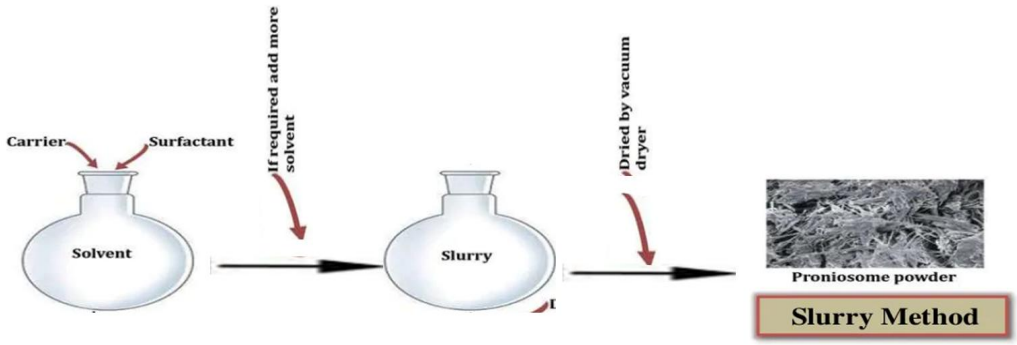


Fig. 1.Proniosomes formulation

centrifuge (Remi Equipments Pvt, Ltd. Mumbai) and the sample solution was centrifuged at 15,000 rpm for 30 min. The precipitate was obtained, filtered through a 0.45 μ filtrate and absorbance was measured at 369 nm after proper dilution with PBS [19].

2.8 Stability Analysis

The objective of the stability test was to determine the stability of the proniosomes throughout periods under a variety of conditions, including temperatures, moisture and illumination. The perfect group of irinotecan-loaded proniosomes (PF2) was used for testing.

All formulations were divided into three sections and stored in conditions of 4 ± 2°C in the refrigerator, 25°C ± 2°C/ 60%±5% RH and 37±2°C/ 65±5% RH. After 90 days, the drug liberation and in situ monitoring of the preparations was calculated using the methods described above [20]

2.9 Analysis of Statistics

The data (mean ± standard deviation) were evaluated by the T - test using Windows Statistica (Version 5.0, Statsoft, Inc, USA).A substantial gap was developed as a probability point of $p < 0.05$.

3. RESULTS

3.1 Dry Proniosome Particle Structure

Surface morphological studies done with the help of scanning electron microscopy (SEM)indicated that vesicle formed in proniosome formulation was spherical, rounded, smooth and there was no sign of aggregation between particles. Further, scanning electron microscopy of dried samples of proniosome-derived niosome dispersions were compared to those prepared by conventional hydration of dried film. SEM micrographs of the dry proniosome sample (Fig 2A) show that the maltodextrin powder has a transparent and continuous coating. Also no major variation in particle size was seen based on the micrograph scale. This indicates that due to surfactant coating, there is no accumulation of the particles. In addition, the scanning electron micrograph of the dried proniosome-derived niosomal dispersion (Fig. 2B) indicates that the proniosome-generated niosomes were concealed and compact.It was observed that niosomes formed by direct hydration are very heterogeneous, but niosomes prepared from proniosomes are more discrete and uniform.

3.2 Efficiency of Drug Entrapment

Table 1 exhibits the results for entrapment. In formulations PF1 to PF4, the entrapment efficacy of the proniosomal formulations ranged from 68.84 ± 2.5% to 73.94 ± 2.8%. On the other side, the entrapment efficiency of generic niosomes was 60.17, which is statistically different due to the greater vesicle dimension of the former. As even the size of the vesicle increased, the surface area decreased, which also contributed to a decrease in drug entrapment

3.3 In Situ Release Profile

The average drug release for PF1 to PF4 after 24 h was 96.24±2%, 95.65±2%, 98. 06±2% and 97.14±2%, respectively, as shown in Fig 2. On

the other side, there was 96.77±2 percent average elease of pure irinotecan in 5 h.

3.4 Tissue Distribution Study

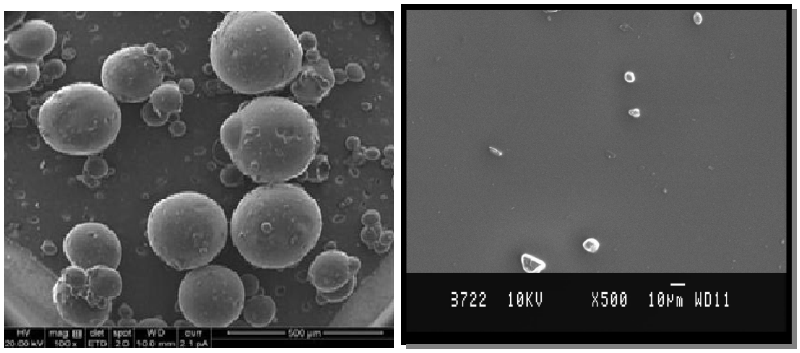
For the in vivo drug target study, Formulation PF2 was preferredappropriate particle size and good trap efficiency and in vivo bioavailability. The release of the drug to different organs after intravenous injection is shown in Fig. 4.

3.5 The Outcomes of Stability

The conclusions of the stability testing as shown Fig. 5, suggest that the angle of repose, drug entrapment and drug release profile of the formulations did not influence (P>0.05) after storage under specific climates for 03 months, thus indicating that the formulations were durable.

4. DISCUSSION

Irinotecan is an active chemotherapeutic agent and is commonly used in colon immunotherapy. Even so, dose-related side effects, such as serious diarrhoea and intense immune system suppression, have hampered its diagnostic application [20]. An alternative vesicular drug delivery system for irinotecan in the form of proniosomes should therefore be given which will have the benefits of controlled drug release, site specificity, improved drug stability, high drug load and avoidance of biotoxicity from the carrier [21].



A. B.

Fig. 2. SEM of (A) proniosomes (PF2), and (B) niosomes derived from proniosomes (n=3)

Table 1. Drug entrapment efficacy of irinotecan-loaded proniosomes (mean SD, n = 3)

Code of formulation	Efficiency of drug entrapment (%)
PF1	71.58±.1.8
PF2	73.94±2.8
PF3	68.84±2.5
PF4	69.46±3.2
Generic niosomes	60.17±2.5

Tissue Distribution Study

Body organs	Mean % drug content
Liver	98±2%
Lungs	9. 84±2%
Spleen	9. 36±2%
Kidney	5.2±2%
Liver	16±2%
Lungs	8.76±2%
Spleen	9. 48±2%
Kidneys	11.10±2%
Heart	18. 16±2%
Brain	12±2%

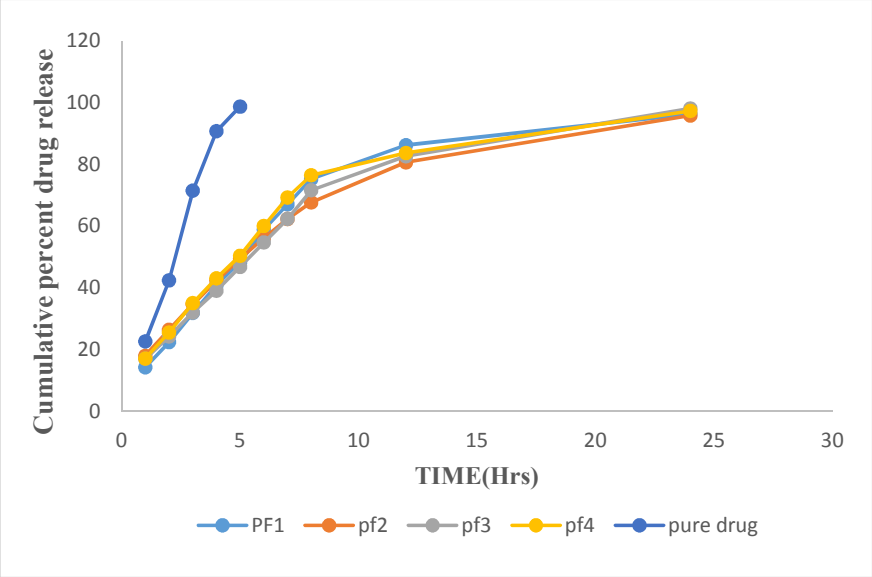


Fig. 3. Combined drug release of proniosomal preparations and pure drugs

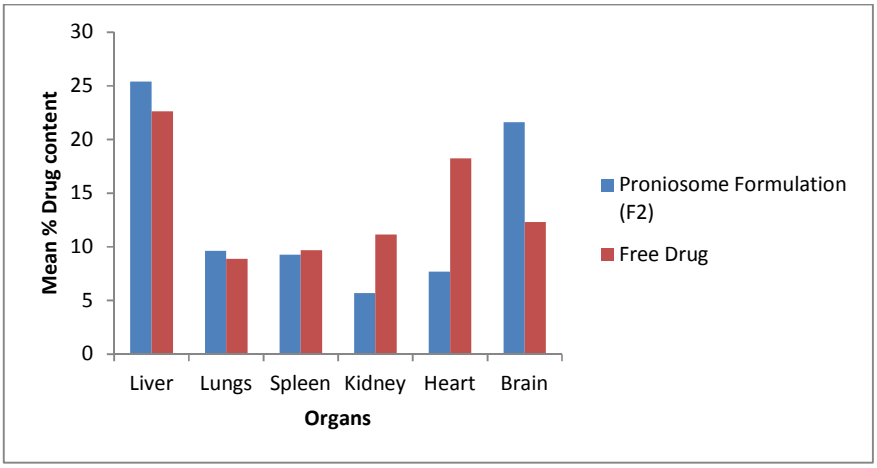


Fig. 4. Controlled release (targeting efficiency) of the form ulation PF2 and free drug in the specified organs (n=3)

It was relatively straightforward to prepare the proniosomes on a maltodextrin carrier, but it was important to integrate the surfactant solution used in very limited quantities to ensure maximum drying before further additions were made. Since proniosomes are a dry powder, additional processing is required [22]. Maltodextrin a polysaccharide as carrier material increases surface of proniosomes which leads to thinner surfactant coating that is suitable for rehydration [23].

In the traditional dry film form, proniosome powder hydration is much simpler than the long shaking process needed to hydrate surfactants and this can be applied in a 'point-of-use' process. Studies of drug entrapment efficacy showed that the improvement in nonionic surfactant had a drastic impact on the entrapment of Irinotecan hydrochloride trihydrate, a hydrophilic drug.

Compared to spans, the entrapment efficacy of proniosomes composed of tweens was relatively poor. Yuksel N et al. [24].

Due to its higher alkyl chain length, higher capture efficiency of vesicles of span 60 was predictable. The Hydrophilic-lipophilic balance (HLB) value of a surfactant is lowered by a larger

alkyl chain and this tends to improve the drug's entrapment performance. All four batches of proniosomes were released in vitro and showcased an interesting bi-phasic release with a rapid release impact during the first hour.

A steady pattern resembling Higuchi matrix release was subsequently accompanied by drug release. In the first hour, the burst release can be related to the drug loaded on the vesicle surface and to the entrapped drug in the niosomal release.

In the lipophilic region (between the bilayers), the drug is adsorbed and can undergo rapid ionization and release the drug until equilibrium is reached Uchegbu IF et al. [25].

When the drug release was regulated by diffusion along the swollen niosomal membrane, the remaining amount of the drug was released up to 24 hours after the initial rapid release.

Compared to other formulations, the total release of F-2 (span 60) was less due to its higher alkyl chain length, higher chain length, slower release. Release rate of proniosome-derived niosomes has been much more controlled than conventional niosomes, so some poorly water soluble drugs with low solubility can give improved bioavailability.

The results of in vivo drug distribution showed that the proniosomes loaded with the drug Exhibited preferential drug targeting to the liver followed by the brain, lungs, spleen, heart and kidneys. Increased levels of the drug were directed at organs such as the liver in the form of proniosomes compared to pure drugs. As many as 70±2% of patients with colorectal cancer will present with (synchronous) liver metastases at the time of their primary diagnosis or develop liver metastases (metachronous) as their disease progresses Kemeny N et al. [26]. Higher concentration of irinotecan hydrochloride trihydrate proniosomes at liver justifies above aspect.The enhanced lipophilicity of irinotecan-loaded proniosomes is due to higher drug targeting of the liver and brain.

Stability tests of the F2 formulation indicate that an average increase in drug release ($P<0.05$) has developed.These results may be due, to some extents in storage, to the phase transition of surfactant and lipid causing vesicle leakage.It can be noticed from the stability data that 4^{o} C is the most acceptable condition of irinotecan-loaded proniosome storage [27].

Thus, problems related to the hydrolysis of the active ingredient or surfactants are avoidedproducing a dry proniosomal formulation. When suspension is developed, precipitation and accumulation are prevented. Maltodextrin-based proniosomes meet all of these criteria, suggesting that proniosomes are a potential drug carrier.

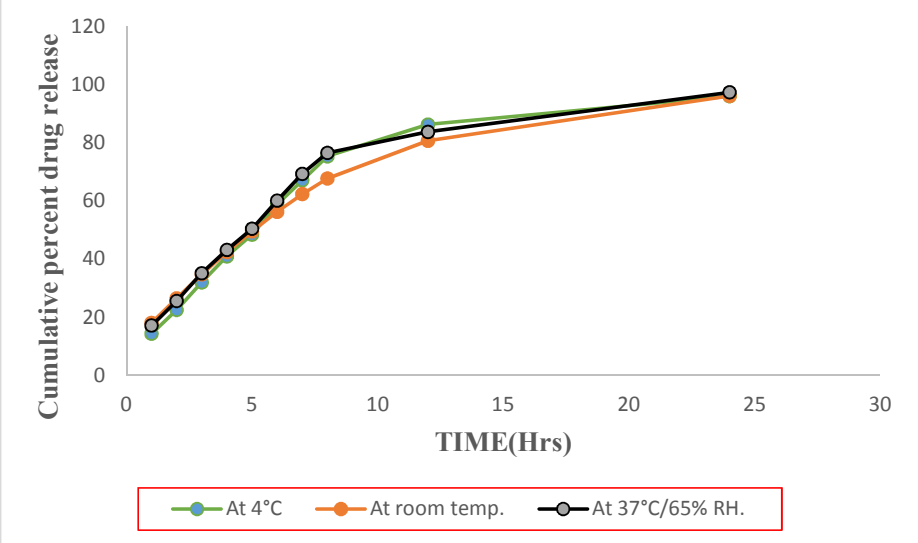


Fig. 5. Cumulative release of PF2 formulation upon 3 months of storage

5. CONCLUSION

The slurry method used is simple and suitable for the preparation of Irinotecan proniosomes on a laboratory scale. In achieving drug targeting, Proniosomes provides an alternative colloidal carrier strategy as irinotecan proniosomes have been maintained at targeted sites and are able to release drugs for an extended period of time.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The testing approach was approved by the Sri Adichunchanagiri College of Pharmacy Institutional Animal Ethics Committee B. G. Niagara, Karnataka, India (Reg No. 377/CPCSEA).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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