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Research Article

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Design and characterization of vinblastine sulphate loaded proniosome for cancer therapy

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ABSTRACT

The aim of this investigation was to prepare, characterize and optimize the vinblastine sulphate loaded proniosomes for overall improvement in the physical stability and to prolong the release time in a controlled manner there by increasing its efficacy and to reduce its toxicity and to study the suitability of proniosomes as the carrier of drug. Proniosomes of vinblastine sulphate were prepared by slurry method. The formulations were characterized with respect to shape and surface morphology, entrapment efficiency, invitro drug release profile, and by cell line studies and stability under specific conditions. The formulated proniosomes were smoother indicating a thin and uniform coating over maltodextrin powder. The vesicular size of the optimized formulation showed the vesicular size of 250-300nm. The evaluation of entrapment efficiency showed that it played a significant role by varying the concentration of cholesterol and Span, the highest entrapment efficiency was found in formulation F6 with 84.41±0.636%. Highest cumulative percent drug release was observed with formulation F5 with 97.13% in 48 h. The cell line study result of formulated proniosomes reveals that the drug was showing its efficacy for a 48 hours and the percentage cell line inhibition of optimized formulation was 2 times double when compared to marketed formulation. The results of investigation demonstrated that vinblastine sulphate loaded proniosomes offers an alternative colloidal carrier approach in increasing its physical stability. The results obtained for the present study clearly revealed that proniosomes containing vinblastine sulphate are capable of releasing their drug for the extended period of time there by increasing the efficacy of drug.

Key words: Proniosomes, drug carrier, cancer therapy, cell line study

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INTRODUCTION

Decreasing the adverse effects and improving its therapeutic index, is considered as a challenge in the cancer therapy. Vinblastine is having stability problems [1] ,constant efforts have been pursued in order to design such an ideal drug delivery system, which improves therapeutic index , decreases the adverse effects and also increases the stability of drug. Vesicular drug delivery system in the form of liposomes or niosomes are investigated. Liposomes have limitation of poor stability where as niosomes exhibit physical instability, aggregation, fusion, leaking of entrapped drug thus limiting shelf life of dispersion [2,3] . Proniosomes are dry formulations of surfactant coated carrier and hydrated before use to obtain a suspension of niosomes. The additional convenience are transportation, distribution, storage and dosing which make it a cost effective industrial product.[4]

EXPERIMENTAL SECTION

Materials

Vinblastine sulphate was purchased from yucca enterprises Mumbai. Maltodextrine and span 60 from Loba chemie Pvt. Ltd, Mumbai and cholestrol from SD Fine chemical Ltd.

Formulation of proniosomes

Proniosomes were prepared by slurry method [5]. Proniosomes with maltodextrine as the carrier were prepared by using surfactants like span 40 , span 80 span 85 and tween 60. Precisely optimized proportions of Surfactants, cholesterol, and lecithin (molar ratio 47.5:47.5:5, respectively), were used in this work. A stock solution of surfactants in chloroform was prepared with 164 mmol/L surfactant, 164 mmol/L cholesterol, and 17.2 mmol/L dicetylphosphate. 10 g of maltodextrine powder was added to a 250-mL round-bottom flask and the entire volume of surfactant solution (14.5 mL) was added directly to the flask. The flask was attached to the rotary evaporator with the rotation speed set at 60 RPM and temperature to 37˚c. Vacuum was applied until the powder appeared to be dry and free flowing. The flask was removed from the evaporator and the proniosomes were sealed in screw caped vials until further use.

Measurement of angle of repose[6]

Funnel method was employed to determine angle of repose of proniosome powder. Briefly, the maltodextrine powder or proniosome powder was poured into a funnel which was fixed at a position so that the orifice of the funnel is 10 cm from base. The powder flowed down from the funnel to form a cone on the surface and angle of repose was then calculated by measuring height of the cone and diameter of the base.

Vesicular Size Evaluation [7,8]

A small quantity of proniosome was hydrated with 10 ml of phosphate buffer (pH 7.4). The dispersion of proniosomes was shaken manually occasionally so that lumps of proniosomes are disintegrated into individual proniosomes. A drop of the dispersion was placed onto the slide and examined under the microscope. Circular vesicle bodies were observed with uniform small size was observed 100 x.

Size distribution

Vesicular size distribution studies were evaluated by Dynamic Light Scattering method. 100mg of proniosomes was hydrated with 10 ml distilled water with manually shaking. The instrumental setting was fixed as temperature-20πC, viscosity-0.01 poise, and refractive index-1.333.

Scanning Electron Microscopy

The proniosome powder was placed on each stud and allowed to dry the sample was observed in scanning electron microscope and photographs were taken.

Entrapment Efficiency [9]

The proniosome loaded with drug was hydrated with phosphate buffer and was sonicated in a bath Sonicator. The vinblastine sulphate loaded niosomes were separated from unentrapped drug by centrifuging at 17,000 rpm at 4πC for 45 minutes. The supernatant was taken and diluted with phosphate buffer. The vinblastine sulphate concentration in the resulting solution was assayed spectrophotometrically at 269nm. The percentage of drug encapsulated was calculated by the following equation:-

$$\text{Percentage Entrapment Efficiency} = [C_t - C_f]/C_t * 100$$

Where, Ct= total concentration of drug
C_f= concentration of free drug
Zeta Potential determination

The zeta potential of the optimized formulation was determined by Zetasizer.

In-vitro Drug Release [8]

Release studies were carried for all the proniosomes prepared.100mg of the proniosomes hydrated in 10 ml phosphate buffer were placed in a cellophane membrane immersed into 30 ml of dissolution medium of phosphate

buffer of pH 7.4 medium. To simulate the human condition, during the experiment, temperature was maintained 37±0.5□C.

The 1ml samples were withdrawn at predetermined rate 1, 2, 3, 4, 5, 6, 7, 8, 24and 48 hour, and replaced with equivalent amount of the samples. The withdrawn samples were analyzed spectrophotometricaly at 269nm. The amount of drug released was calculated and the percentage drug released was plotted against time.

Stability studies [10,11]

The optimized formulation were kept for stability studies for 45 days at room temperature (30 ± 2°C) and at refrigerator temperature (4 ± 2°C) to determine physical and chemical stabilities. The amount of drug degraded at different time intervals was analyzed. The formulation was evaluated visually and for entrapment efficiency after 7, 15, 30 and 45 days.

Cell line studies [12]

The A549 lung cancer cells were taken and grown in 96 well plates and using the drug induced apoptosis.The lyophilized JC-1 reagent was reconstituted with 500 µl DMSO to obtain 100X stock solution.JC-1 reagent was diluted to immediately prior to use (2µl/ml) of the optimum medium.The cell culture media was removed and replaced with enough diluted 1xJC reagent sufficient to concentrate the cells(50µl/well).The cells were incubated at 37˚C in a 5%carbon dioxide incubator in 30 minute. Dye was removed and washed with serum free medium.50µl of serum free medium was added and observed under fluorescent microscope. The aggregate red form has an absorbance at 585-590 nm and monomer green has an absorbance at 510-527nm.

RESULTS AND DISCUSSION

Morphology of dry proniosome powder

Scanning electron microscopy of uncoated maltodextrine and dry proniosome powder reveals that there appears to be a slight difference in the appearance of the surfaces. The powder in appears to be smoother indicating a thin and uniform coating over the maltodextrine powder. Also, based on the scale on micrograph, no significant change in size of particles was seen. This observation clearly shows that, there is no sign of aggregation between particles, due to surfactant coating. Further, scanning electron microscopy of dried samples of proniosome-derived niosome dispersions suggests that niosomes prepared from proniosomes were discrete and uniform. It was observed that preparing proniosomes on maltodextrine was easy but it was necessary that the solution be incorporated in small amounts and complete drying is ensured before further additions are made.

Angle of repose

Measurements of the angle of repose of proniosome formulations and pure maltodextrine indicate that the angle of repose of dry proniosome formulations is smaller than that of pure maltodextrine. This is consistent with the scanning electron microscopic observation of proniosome powder, in which it was observed that the proniosome surface was smoother. Angle of repose measurements indicated that the fluidity of proniosome dry powder is equal to or better than that of maltodextrine powder (Table 1).

Table 1: Angle of repose of formulations

FORMULATION	ANGLE OF REPOSE
F1	32.2±0.75
F2	31.9±1.62
F3	32.7±1.07
F4	31.4±2.04
F5	32.91±0.25
F6	34.23±1.22
F7	36.55±1.36
F8	38.12±0.95
Maltodextrine	32.8±1.87

Results of measurements of the angle of repose of proniosome formulations and pure maltodextrine indicate that the angle of repose of dry proniosome formulations is smaller than that of pure maltodextrine. As the amount of surfactant increases angle of repose increases as flow property decreases. This is consistent with the scanning electron microscopic observation of proniosome powder, in which it was observed that the proniosome surface was

smoother and was shown in figure 1. Angle of repose measurements indicated that the fluidity of proniosome dry powder is in the range of maltodextrine powder.

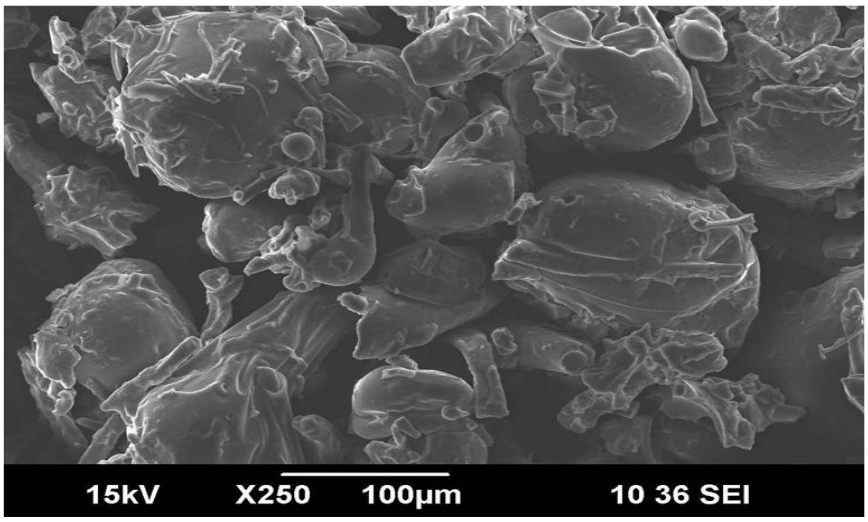


Figure 1: Scanning Electron Microscopy of dry proniosomes

Vesicular Size Evaluation

Hydrated niosomes from the proniosomes were observed under optical microscope at 100 x and observed that the formed vesicles were of uniform size.

Size distribution

The vesicular size and size distribution was evaluated by using dynamic light scattering,(figure 2 and 3) the results showed that increase in ionic-surfactant concentration increases the mean vesicular size whereas the increase in cholesterol concentration decreases the mean vesicular size. The vesicular size was between 135-306 nm. Vesicular size higher means large amount of drug will get entrapped in it. The polydispersity index was found to be low, shows that the particles were of low value shows that niosomes formed by hydration was of uniform size [13]. (Table 2)

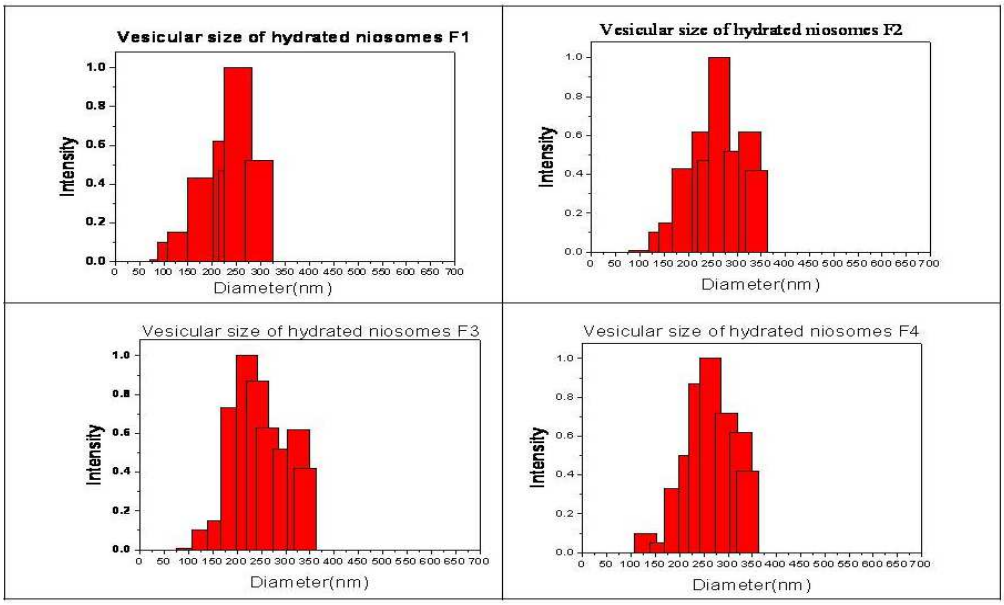


Figure 2: Vesicular size of proniosomes F1 to F4

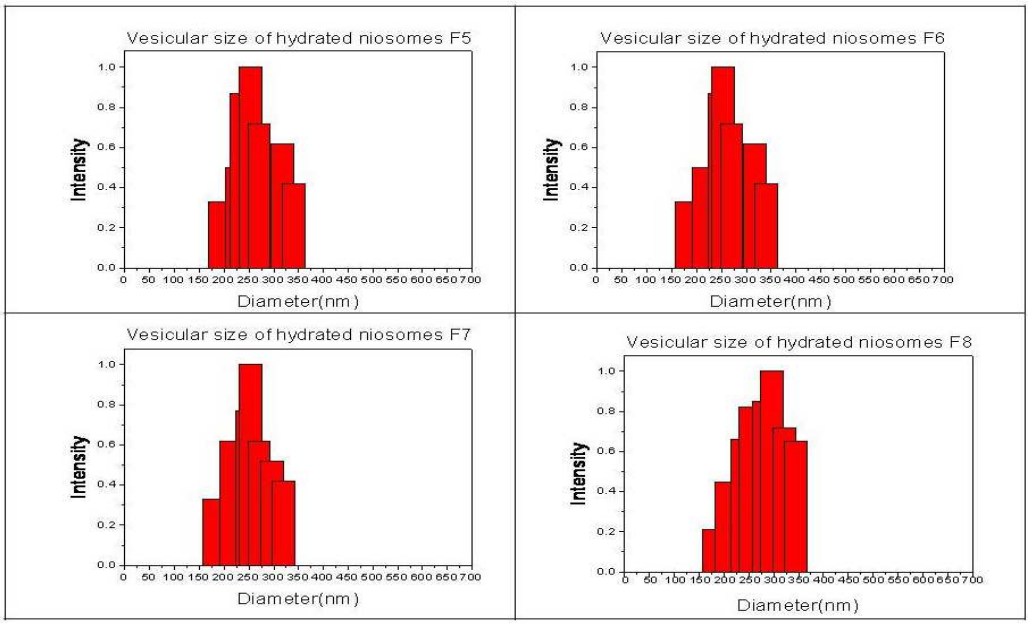


Figure 3: Vesicular size of proniosomes F5 to F8

Table 2: Size Distribution and Polydispersity of Formulations

Sl. No	Formulation Code	Vesicular size(nm)*	Polydispersity**
1	F1	220.17±32.51nm	0.147
2	F2	214.83±36.72nm	0.170
3	F3	246.51±30.32nm	0.122
4	F4	260.32±26.85nm	0.103
5	F5	274.61±28.58nm	0.104
6	F6	282.38±25.61nm	0.090
7	F7	223.15±33.28nm	0.149
8	F8	216.62±35.81nm	0.165

** Data obtained from Nicomp 380 DLS, **PI=Standard deviation/mean vesicular size*

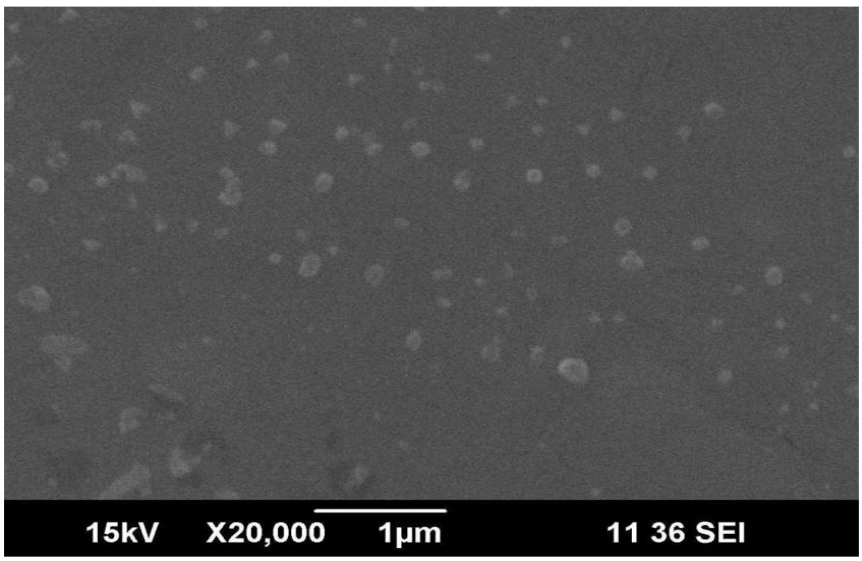


Figure 4: Scanning Electron Microscopy of hydrated niosomes

Scanning electron microscopy

From the figure 4, it is clear that the particle size of the optimized formulation was confirmed to be 250-300nm. This was in accordance with the particle size of niosomes in the literature.

Entrapment Efficiency

Entrapment efficiency is the measure of solute retention. High entrapment efficiency means that less time and effort is needed to remove the unentrapped drug. Vesicular entrapment efficiency is an important parameter that convey on the stability of vesicles and this depends upon the amount of surfactant and amount of cholesterol used. The entrapment efficiency of these formulations varies from 57.68 to 84.41% and was found statistically significant at $p<0.05$. The entrapment efficiency of various formulations is tabulated in table 3 and it is represented in the figure 5. From the data it is clear that entrapment efficiency depends upon both surfactant and cholesterol.

Table 3: Entrapment Efficiency of Formulations

Sl. No	Formulation Code	Entrapment Efficiency(%)*
1	F1	57.68±0.75055
2	F2	60.52±1.032731
3	F3	67.1±0.995339
4	F4	73.38±1.206248
5	F5	83.09±0.545008
6	F6	84.41±0.636632
7	F7	75.74±0.986425
8	F8	68.38±1.021437

**The values are expressed as Mean ± SD; n = 3;*

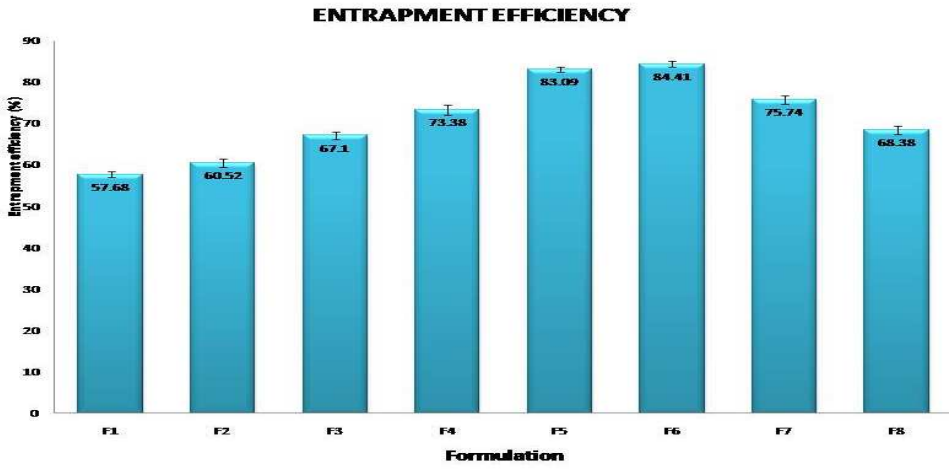


Figure 5: Entrapment efficiency

Effect of Surfactant amount

Surfactant is an important component in the vesicle formation and the variation in the concentration will affect the entrapment efficiency. The concentration of Span 60 was varied from 85mg to 475 mg. The variation in the surfactant amount showed a significant increase in the entrapment efficiency ($p<0.05$) when the concentration is increased from 90 to 180 mg.

Zeta Potential Determination

The magnitude of zeta potential gives a potential stability of the colloidal dispersion. If the particles have, large positive or negative charge reveals that they repeal each other and there is dispersion stability. The zeta potential of the optimized formulation showed that the sample is sample is highly stable. It was found as -50.43, and hence this indicates that the prepared formulation is stable.

In-Vitro Release Studies

The release profile of a drug predicts how a delivery system might function and gives valuable insight into its in vivo behavior. In-vitro release studies for 48 hours were performed for various formulations. The kinetic study

reveals that the proniosomes loaded with vinblastine sulphate follows higuchi matrix release kinetics as the regression coefficients approaches unity, indicating the drug release is independent of drug concentration(figure 6 and 7 and table 4).

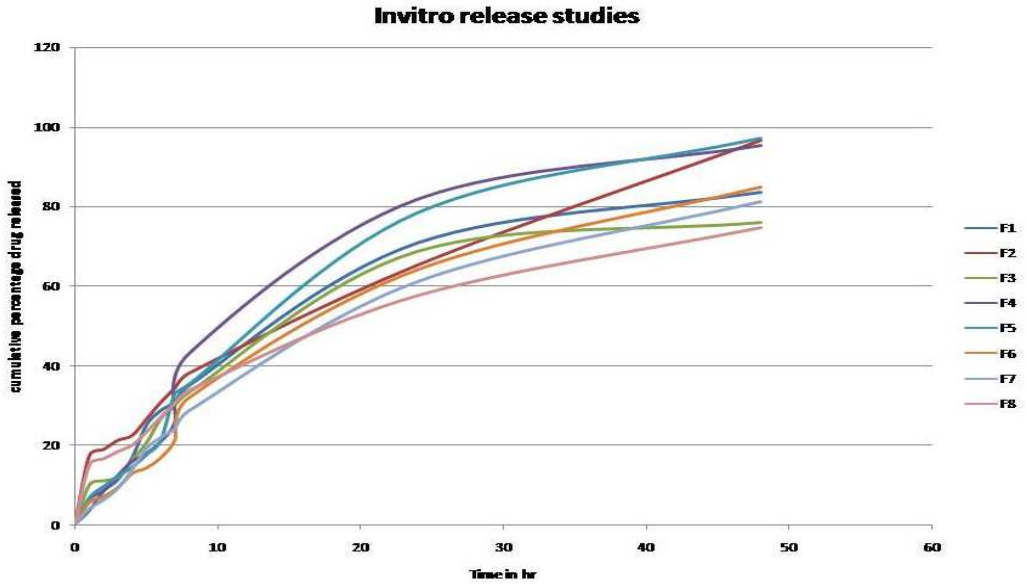


Figure 6: Percentage cumulative drug release at 48 hr of proniosomes

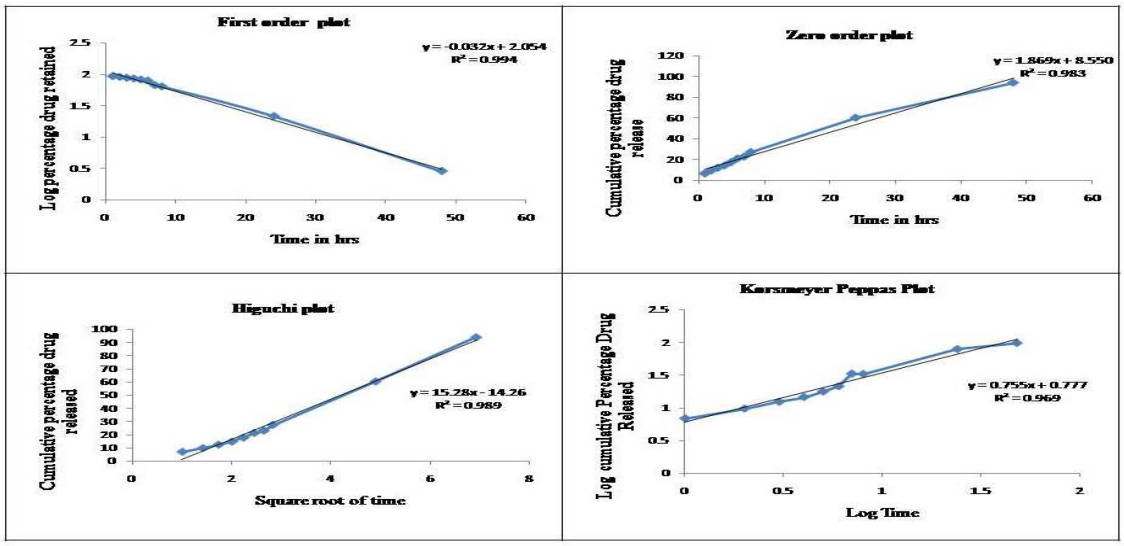


Figure 7: Kinetic plot of optimized formulation

Table 4: Percentage cumulative drug release at 48 hr of formulations

Formulation	Percentage cumulative drug release at 48 hr (%)
F1	83.60133±0.342348
F2	96.60607±0.335145
F3	76.02853 ±0.28625
F4	95.2727±0.442966
F5	97.1376±0.186625
F6	84.8509±0.382153
F7	81.23843±0.408325
F8	74.75013±0.400965

Stability studies

The stability studies of the optimized formulation at room temperature (30 ± 2°C) and at refrigerator temperature (4 ± 2°C) were carried out for 45 days, the physical appearance showed that it does not showed any changes when to the freshly prepared formulation. The entrapment efficiency evaluated on 7th, 15th, 30th, 45th day is represented in the table and shows that there are no significant changes the entrapment during the storage for 45 days in both conditions. However, the formulation is more stable at low temperature compared to the room temperature(table 5).

Table 5: Stability study of optimized formulation

TEMPERATURE	ENTRAPMENT EFFICIENCY (%)					ANGLE OF REPOSE			
	Freshly Prepared	7th Day	15th Day	30th Day	45th Day	7th Day	15th Day	30th Day	45th Day
Refrigerator (4 ± 2°C)	83.09.	80.98	79.64	79.42	79.15	32.91	32.90	32.4	31.93
Room Temperature (30 ± 2°C)	83.09	80.75	79.54	79.39	77.91	32.91	32.89	32.89	32.85

Cell line studies

Based on the data of percentage cumulative amount of drug released ,entrapment efficiency and physical stability formulation F5 was optimized and the anti cancer activity of formulation was analyzed by cell line studies. The results of cell line studies revealed that the optimized formulation was showing its activity against cell line A549 (wild type) lung cancer cells and and apaptosis of the cells was seen even at the 48th hour for the optimized formulation and was found to be 2 fold active when compared to the marketed formulation. Since controlled release of the formulation was confirmed.(figure 8 and 9, table 6).

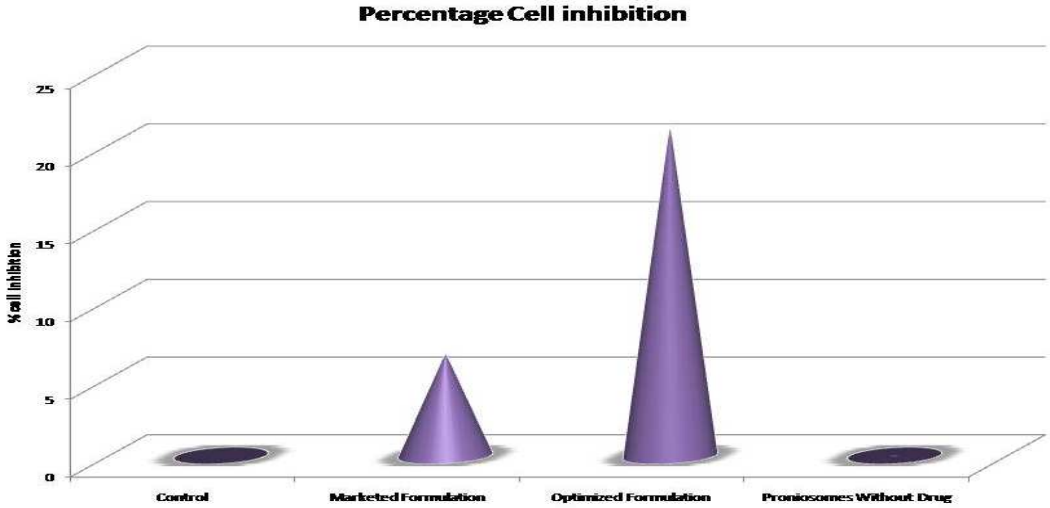
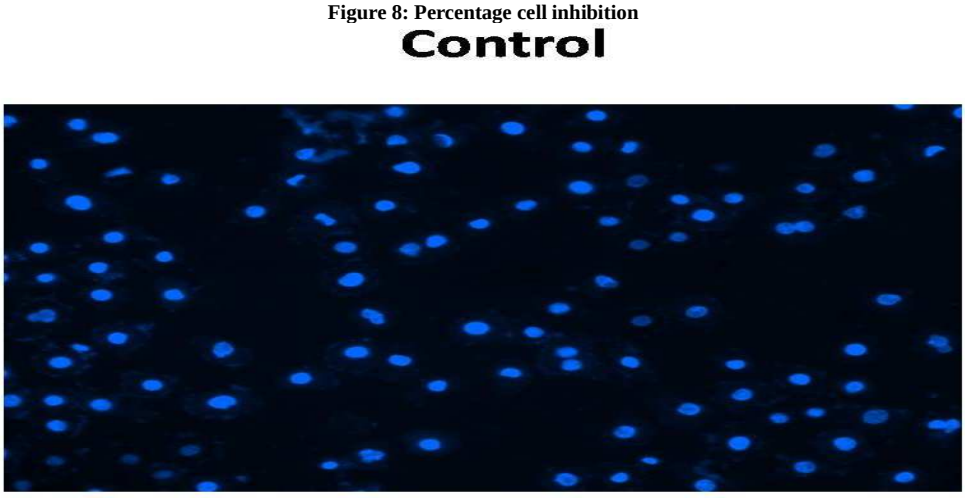


Figure 8: Percentage cell inhibition



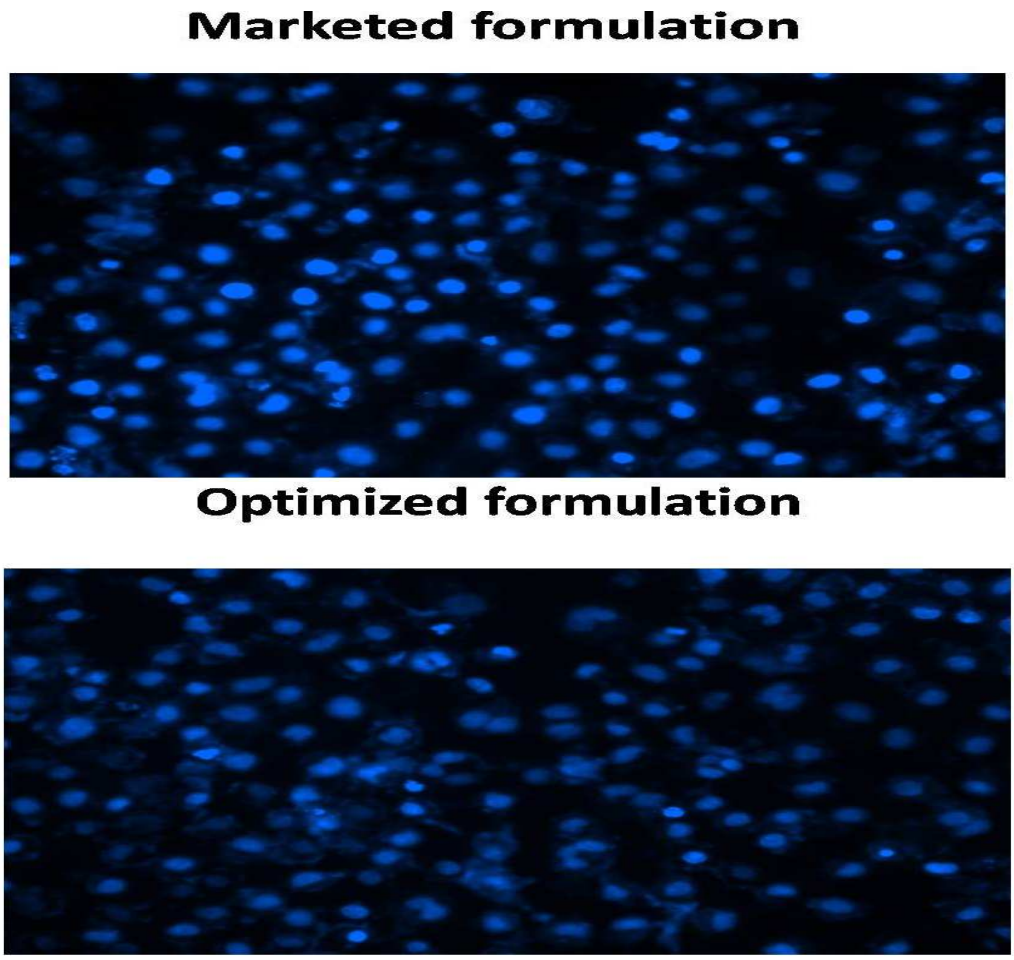


Figure 9(a),9(b),9(c): Photograph of cell line studies

Table 6 : Cell line study of optimized formulation and marketed formulation

Formulation	% cell inhibition
Control	0
Marketed Formulation	6.62
Optimized Formulation	21.32
Proniosomes with out drug	0.013

CONCLUSION

Vinblastine sulphate is an effective anticancer agent and widely used in lung cancer and breast cancer therapy. However, its clinical use has been limited because of its less physical stability and by dose-related toxicity,. Therefore, it is necessary to provide an alternative vesicular drug delivery system for vinblastine sulphate in the form of proniosomes which will have advantages of controlled drug release and site specificity, increased drug stability, high drug pay load and no bio-toxicity of carrier. Proniosomes of vinblastine sulphate were prepared by slurry method using different ratios of surfactants and cholesterol. The slurry method was found to be simple and suitable for laboratory scale preparation of proniosomes. All formulations were evaluated for vesicular size and found that particle size ranged from 200-400nm, whereas the optimized formulation showed a particle of 250-300nm. In-vitro drug release study showed that release from proniosomes was in a controlled manner and follows higuchi kinetics. The results of investigation demonstrated that proniosomes offers an alternative colloidal carrier approach in achieving the physical stability as well as prolonged duration of action .The percentage cell line inhibition of optimized formulation was 2 times double when compared to marketed formulation. The results

obtained for the present study clearly revealed that proniosomes containing Vinblastine sulphate are retained at cancer cells and are capable of releasing their drug for the extended period of time.

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