



## Non-Ionic Surfactant Vesicles of Silymarin: Formulation Development and Evaluation

Bhupendra Kumar, Mahasweta Roy, Nishant Kumar, Babita Kumar, Pradeep Kumar Tyagi, Kohinoor Khan\*

College of Pharmacy, Shree Ganapati Institute of Technology, Ghaziabad, Uttar Pradesh, India.

\*Corresponding author's E-mail: [bhupharma84@gmail.com](mailto:bhupharma84@gmail.com)

Accepted on: 05-04-2014; Finalized on: 31-10-2014.

### ABSTRACT

The present study was to prepare and evaluate niosomes of Silymarin using non ionic surfactants for targeted drug delivery system. Silymarin niosomes were prepared from Proniosomes, by using surfactants like span 40, span 60, span 80, tween 20, and tween 80 and were evaluated for physicochemical properties including particle size, entrapment efficiency, and in vitro drug release using "Franz diffusion cell" through goat intestine. Preformulation studies were performed to check the compatibility of drug and excipient and no interaction was found. A total of five formulation were developed (F1 to F5) and it was found that span80 formulation (F3) has best result for all the parameters as it had maximum entrapment of 80.45% among other five formulations followed by span60 formulation (F2) 74.21% entrapment efficiency. The in vitro drug release profile shows that span80 formulation (F3) have more sustained release profile as compare to other formulation, because %CDR (cumulative drug release) of F3 is 44.90% in 12 hours. The shape and surface morphology of niosomes were characterized by optical and Transmission electron microscopy. This study was also conducted to evaluate the hepatoprotective activity of Silymarin niosomes. Hepatotoxicity was induced by CCl<sub>4</sub> in mice, the Silymarin niosomes have shown very significant (P<0.001) hepatoprotection against CCl<sub>4</sub> induced hepatotoxicity in mice by reducing serum enzyme and total bilirubin level. Span 80 formulation (F3) showed greater hepatoprotective activity as compared to Span 60 formulation (F2). The developed Silymarin niosomes exhibited sustained drug release for at least 12hr, and therefore, could potentially improve patient compliance.

**Keywords:** Cholesterol, CCl<sub>4</sub>, Hepatotoxicity, Niosomes, Proniosomes, Silymarin.

### INTRODUCTION

Niosomes are a novel drug delivery system, in which the medication is encapsulated in a vesicle. They are microscopic lamellar structures which are formed on the admixture of non-ionic surfactant of the alkyl or dialkyl polyglycerol ether class and cholesterol with subsequent hydration in aqueous media. The vesicle is composed of a bilayer of non-ionic surface active agents and hence the name niosomes. The niosomes are very small, and microscopic in size. Their size lies in the nanometric scale. They are used in cosmetic formulations and experimentally as drug carriers.<sup>1,2</sup>

Proniosomes; semisolid liquid crystal gel products of non-ionic surfactants easily prepared by dissolving the surfactant in a minimal amount of an acceptable solvent ethanol and the least amount of water could solve the problem.<sup>3</sup> Then these Proniosomes was converted into niosomes immediately upon hydration.<sup>4,5</sup>

Non ionic surfactant vesicles are ideal means of drug delivery that can enhance bioavailability of encapsulated drug by various mechanisms and provide therapeutic activity for prolonged period of time. However they are suffered with aggregation, fusion, leaking, sedimentation of vesicles, difficulty in sterilization, so to overcome these problems a newer approach was employed known as pro vesicular carriers here in this review we elaborate one of the pro vesicular carrier, widely known as proniosome which is semisolid liquid crystal (gel) products of non ionic

surfactants converted into niosomes (non ionic surfactant vesicular system) upon hydration.<sup>6</sup>

Silymarin, a flavonolignan from 'milk thistle' (*Silybum marianum*) plant, in the family Asteraceae, is used from ancient times because of its hepatoprotective action. It is a mixture of three flavonolignan, Silybin, Silidianin, and Silychristine, with Silybin being the most active. Its mechanism of action includes inhibition of hepatotoxin binding to receptor sites on the hepatocyte membrane, reduction of glutathione oxidation to enhance its level in the liver and intestine, antioxidant activity, and stimulation of ribosomal RNA polymerase, protein synthesis in the damaged cells.<sup>7</sup> Impressive anticancer effect against several human carcinoma cell lines.<sup>8,9</sup> In addition antidiabetic activity, anti-inflammatory, antifibrotic, neuroprotective.<sup>10,11</sup>

Silymarin is insoluble in water, and therefore, an acidic medium is required for its dissolution. The peak plasma levels after an oral dose are achieved in 4-6 hrs.<sup>12</sup> Its dose is 70-140mg thrice a day and has low bioavailability. The low bioavailability of drug is due to rapid biotransformation in the liver and has biological half life of 6hr.<sup>13</sup> Its short half life, poor bioavailability, and lipophilic nature make it a suitable candidate for sustained drug delivery system.

### MATERIALS AND METHODS

Silymarin was received as a gift from Micro Lab India. Sorbitan monolaurate (span 20), sorbiton monostearate



(span 60), sorbiton mono-oleate (span 80), polyoxyethylene 20 sorbitan monooleate (tween 80), polyoxyethylene 20 sorbitan monolaurate (tween 20) and cholesterol were purchased from sigma chemical Co., St. Louis, MO, USA, ethanol were purchased from E. Merck India Ltd. Mumbai. All other chemicals and solvents used were of analytical grade.

Male and Female healthy Albino mice weighing between (30g-35g) were obtained from the animal house of Pinnacle Biomedical Research Institute. The experiment was conducted as per the permission of institutional animal ethical committee (IAEC) of PBRI (Regd. No.1283/c/09/CPCSEA).

## Methods

Identification of Drug: Determination of Melting point

### Determination of absorption maxima of ( $\lambda_{max}$ ) by U.V spectrophotometer

The absorption maxima of Silymarin (10  $\mu$ g/ml) were determined by running the spectrum (200-400 nm) in Systronic 2022 double beams UV/VIS spectrophotometer using distilled water as blank.

### Preparation of Standard Calibration Curve of Silymarin in Distilled Water

100 mg of Silymarin was dissolved in 100 ml volumetric flask with distilled water. Stock solution was prepared by taking 1ml of this solution and diluting it to 10 ml with distilled water. From this stock solution further dilution were made to prepare solutions of 2  $\mu$ g/ml-12  $\mu$ g/ml concentration.

## Drug and Excipient Compatibility Studies

### By FT-IR Spectroscopy

Compatibility studies were conducted to detect any changes in chemical constitution with excipients using FT-IR spectral analysis.

### Determination of Partition coefficient

Partition coefficient determination was done using n-butanol and Distilled Water.

### Preparation of Proniosomes

Accurately weighed surfactants (40mg) were mixed with cholesterol (60mg) in separate vials. Silymarin (15 mg) was dissolve in ethanol (1ml) separately. Both the mixtures were mixed together and the prepared vials were tightly sealed and warmed in water bath (55°C-60°C, 5 min). To it hot distilled water (0.16ml, 55°C-60°C) was added and the mixtures were warmed in the water bath for 3-5 min till a clear or translucent solution, or white creamy proniosomal gel was obtained. It was allowed to cool down at room temperature and was kept in dark to obtain Proniosomes. (Refer Table 1).

To the above mixture, 7ml phosphate buffer (7.4 pH) was added and heated again in a water bath (60°C-65°C, 10 min) and vortexing was done simultaneously two to three times while heating. The final volume was adjusted to 20 ml by the same buffer to obtain Niosomes. (This method is known as Hydration step of niosomes).<sup>3,14</sup>

**Table 1:** Formulation design of Silymarin niosomes

Formulation No.	Non-ionic surfactant	Non-ionic surfactant (mg)	Cholesterol (mg)	Drug (mg)	Ethanol (ml)	Water (ml)	Phosphate buffer (ml)
F1	Span 20	40	60	15	1	0.16	7
F2	Span 60	40	60	15	1	0.16	7
F3	Span 80	40	60	15	1	0.16	7
F4	Tween 20	40	60	15	1	0.16	7
F5	Tween 80	40	60	15	1	0.16	7

### Particle size distribution study

Particle size distribution study was carried out by using ocular micrometer.

### Shape of Niosomes

Shape of Niosomes study was carried out using TEM (Transmission Electron Microscope) and ocular micrometer using Olympus microscope.

### Procedure (TEM)

A drop of niosome dispersion was applied to a carbon-coated copper grid and left for 1 min to allow some of the particles to adhere to the carbon substrate and excess dispersion was adsorbed off with a piece of filter paper. A

drop of 1% phosphotungstic acid solution was applied to the adhered particles and the excess solution was removed. The sample was then air-dried. The final sample was observed under a ZEISS EM 900 Transmission electron microscope at an accelerating voltage of 70 kV.

### Evaluation

#### *In Vitro* Drug Release Studies by "Franz diffusion cell" through Goat Intestine

*In vitro* drug permeation of optimized formulation was carried out using modified Franz diffusion cell using Goat intestine.<sup>15</sup>



**In Vivo Drug Release**<sup>16-18</sup>**CCl<sub>4</sub> Induced Hepatotoxicity**

Mice were divided into five groups each containing 6 animals.

- **Group I:** Controls received the vehicle of normal saline (2 ml/kg, p.o.).
- **Group II:** Received CCl<sub>4</sub> (2 ml/kg, s.c.) at every 72 h for 10 days.
- **Group III:** Received silymarin 100 mg/kg p.o. for 10 days and simultaneously administered CCl<sub>4</sub> (2 ml/kg, s.c.) at every 72 h.
- **Group IV:** Received niosome formulation, containing surfactant a span 60 (100mg/kg, p.o) for 10 days and simultaneously administered CCl<sub>4</sub> (2 ml/kg, s.c.) at every 72 h.
- **Group V:** Received niosome formulation, containing surfactant a span 80 (100mg/kg, p. o) for 10 days and simultaneously administered CCl<sub>4</sub> (2 ml/kg, s.c.) at every 72 h.

At the end of experimental period, all the animals were sacrificed by cervical decapitation. Blood samples were collected, allowed to clot. Serum was separated by centrifuging at 3500 rpm for 15 min and analyzed for various biochemical parameters.

**Biochemical Studies**

The biochemical parameters were determined after 24 hr fasting after administration of the last dose of treatment. Blood was obtained from all animal by puncturing retro-orbital plexus. The blood samples were allowed to clot for 45min at room temperature. Serum was separated by centrifugation at 3500 rpm at 4°C for 15min and utilized for the estimation of various bio-chemical parameters namely Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP), Aspartate Amino Transferase (AST), and serum bilirubin.

**Statistical analysis**

The values were expressed as mean ± SEM. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

**RESULTS AND DISCUSSION****Identification of drug****Melting point**

Melting point was found to be in the range of 164°C - 180°C which was in compliance with the official value.

**Determination of absorption maxima ( $\lambda_{max}$ ) by UV Spectrophotometer**

The Silymarin shows the absorbance maxima at 281.6 nm in ethanol.

**Partition coefficient**

$$P_{O/W} = (2.611_{oil}/1.806_{water}) = 1.44$$

The value obtained suggested that silymerin is a good candidate for niosome entrapment

**Characterization of Formulation****Particle size distribution study****Table 2:** Particle size analyses of different Formulations

Formulation code	Mean Particle size ( $\mu\text{m}$ )
F1	3.0 ± 0.97
F2	3.56 ± 1.31
F3	8.62 ± 3.14
F4	2.25 ± 1.38
F5	1.5 ± 0.70

Table 2 shows that keeping the cholesterol concentration constant for all formulations, the use of Span 80 in F3 results in maximum mean particle size i.e 8.62 ± 3.14  $\mu\text{m}$  as compared to the other formulations with different surfactants. F2 formulation containing Span 60 was close to the F3 having Mean Particle size to be 3.56 ± 1.31  $\mu\text{m}$  followed by F1 formulation containing Span 20 having Mean Particle size to be 3.0 ± 0.97  $\mu\text{m}$ .

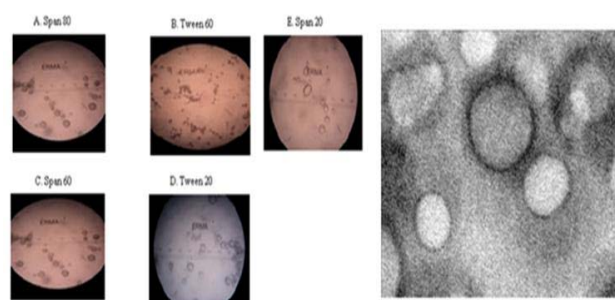
**Shape of niosome**

Fig 1: Olympus microscope view

Shape of the Niosomes by TEM

**Drug entrapment efficiency**

The entrapment efficiency is one of the important parameters in the design of vesicular formulations. High encapsulation efficiency ensures more bioavailability and also high concentration of drug targeted may help in the reduction of dose required for therapy and thereby decreasing the dose related systemic side effects. The entrapment efficiency is one of the important parameters in the design of vesicular formulations. High encapsulation efficiency ensures more bioavailability and also high concentration of drug targeted may help in the reduction of dose required for therapy and thereby decreasing the dose related systemic side effects Vesicle entrapment efficiency relies on the stability of the vesicle which is highly dependent on the type and amount of surfactant forming the bilayers, the amount of both cholesterol and the drug load.<sup>19</sup>

**Table 3:** Percent Drug entrapment of different formulations

Formulation Code	Absorbance*	concentration [µg/ml]	% Un entrapped drug	% Entrapped Drug	Mean Particle size (µm)
F1	1.325±0.1145	236.60	31.54	68.45±0.3891	3.0 ± 0.97
F2	1.083±0.1142	193.39	25.78	74.21 ± 0.3476	3.56 ± 1.31
F3	0.821±0.1149	146.60	19.54	80.45 ± 0.4113	8.62 ± 3.14
F4	1.504±0.1148	268.57	35.80	64.19 ± 0.3254	2.25 ± 1.38
F5	1.933±0.1151	345.17	46.02	53.97 ± 0.2561	1.5 ± 0.70

\* (10 times dilution at 286.0nm)

**Evaluation parameters****In vitro drug release studies by "Franz diffusion cell" through Goat Intestine****Table 4:** Diffusion parameter

Dissolution medium	PBS buffer pH 7.4
RPM	500
Apparatus	Magnetic stirrer
Temperature	60 ± 65 °C
Sampling time	Up to 12 hrs with 1 hr of interval
Volume withdrawn	1 ml

**Table 5:** Drug release profile (%CDR) of all formulations<sup>20</sup>

Time (hr)	%CDR				
	F1	F2	F3	F4	F5
0	0	0	0	0	0
1	29.14	21.71	19.57	34.81	41.0
2	33.09	24.33	23.38	36.82	42.81
3	36.24	27.43	25.67	41.0	45.90
4	38.33	30.09	27.86	43.90	47.57
5	42.05	32.80	28.62	47.38	51.62
6	44.33	34.71	31.09	55.67	53.24
7	46.52	37.29	34.24	58.43	61.57
8	53.48	38.52	36.90	61.19	64.62
9	55.67	41.24	38.52	62.52	68.24
10	58.19	42.76	40.76	65.29	71.48
11	60.14	45.29	43.0	69.29	78.62
12	63.57	47.57	44.90	75.67	88.24

**Table 6:** Effect of niosome formulation on CCl<sub>4</sub> induced activity on mice<sup>21,22</sup>

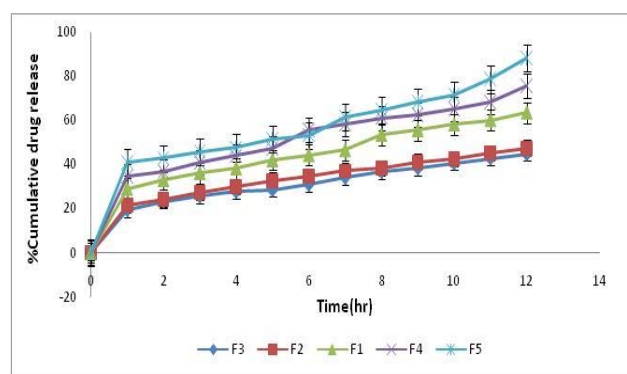
Groups	Treatment	AST/SGOT (IU/L)	ALT/SGPT (IU/L)	ALP (IU/L)	Total bilirubin (mg/dL)
Group 1	Control	74.56±3.05	45.35±2.88	67.78±4.66	0.59±0.04
Group 2	CCl <sub>4</sub>	285.16±8.53	241.22±5.56	263.56±4.45	5.12±0.19
Group 3	Silymarin+ CCl <sub>4</sub>	94.81±5.02	108.86±4.02	101.11±8.12	3.02±0.16
Group 4	F2+ CCl <sub>4</sub>	276.61±7.02	237.31±5.57	260.16±5.40	4.89±0.05
Group 5	F3+ CCl <sub>4</sub>	243.32±6.61	225.13±5.43	231.50±4.11	4.72±0.13

In the group I (control) normal saline treated animals, showed the level of marker enzymes were not significantly elevated. The level of marker enzymes of group II CCl<sub>4</sub> induced animals were significantly increased (P<0.05) when compared to the control animals. But

F3 formulation (Span 80) showed promising results of % CDR as it releases almost 50% of the drug in first 12 hrs. It concluded that it is an effective sustained release formulation as compared to others which is also revealed from the Figure 2.

**In vivo Drug Release Studies: Hepatoprotective Activity CCl<sub>4</sub> Induced Hepatotoxicity**

The damage of the liver caused by CCl<sub>4</sub> was evident by the alteration in serum marker enzymes concentration beside the clinical signs and histopathology. The levels of serum Aspartate amino transaminase (AST), Alanine amino transaminase (ALT) and Alkaline Phosphate (ALP) were taken as an index for hepatotoxicity induced by CCl<sub>4</sub>. The levels of AST, ALT and ALP were analyzed in serum samples of different groups of albino rats shown in table 1. Serum marker enzymes such as ALT, AST and ALP were analyzed for the control and experimental animals.

**Figure 2:** Graph of % CDR v/s Time of Formulation F1-F5

there was a significant decrease of the enzyme level (P<0.001) in the Silymarin and CCl<sub>4</sub> treated animals (group III). The (F3) formulation treated animals of Group-IV: showed significant (P<0.001) reversal of toxic effects in the liver cells followed by F2 formulation (table 6).



## CONCLUSION

Drug incorporation in the niosomes to target specific sites is an effective drug delivery model. It is concluded from this study that study showed that the formulation of niosome could be used as a favourable delivery systems where for the silymarin, a hepatoprotective agent can be loaded for effective drug release. Silymarin is a favoured drug for different liver diseases because of its oral effectiveness, good safety profile, availability in India. It has established efficacy in the restoration of liver function and regeneration of liver cells. It may make a breakthrough as a new approach to protect other organs in addition to liver.

It provided successful preparation with efficient encapsulation of silymarin using non ionic surfactants especially span 80. The shape and surface morphology of niosomes were characterized by optical and Transmission electron microscopy. A total of five formulation were developed (F1 to F5) and it was found that span80 formulation (F3) has best result for all the parameters as it had maximum entrapment of 80.45% among other five formulations. The in vitro drug release profile shows that span80 formulation (F3) have more sustained release profile as compare to other formulation, because %CDR (cumulative drug release) of F3 is 44.90% in 12 hours. The Silymarin niosomes have shown very significant ( $P < 0.001$ ) hepatoprotection against  $CCl_4$  induced hepatotoxicity in mice by reducing serum enzyme and total bilirubin level.

They present a structure similar to liposomes and hence they can represent alternative vesicular systems with respect to liposomes, due to the niosome ability to encapsulate different type of drugs within their multi environmental structure. Niosomes are promising vehicles at least for lipophilic drugs.

## REFERENCES

- Jaleh VB, Abbas P, Abdol hossein R, *In vitro* study of Polyoxyethylene alkyl ether niosomes for delivery of insulin, *International Journal of Pharmaceutics*, 328, 2007, 130–141.
- Yoshida H, Lehr CM, Kok W, Junginger HE, Verhoef JC, Bouwistra JA, Niosomes for oral delivery of peptide drugs, *J Control Rel.*, 21, 1992, 145-153.
- Vora B, Khopade AJ, Jain NK, Proniosome based transdermal delivery of levonorgestrel for effective contraception, *J. Control. Rel.*, 54, 1998, 149–165.
- Vasistha, Peeyush, Ram, Alpana, non-ionic pro vesicular drug carrier: an overview, *Asian Journal of Pharmaceutical & Clinical Research*, 2013, 38-42.
- Fang JY, Yu SY, Wu PC, Huang YB, Tsai YH, *In vitro* skin permeation of estradiol from various proniosome formulations, *Int. J. Pharm.*, 215, 2001, 91- 99.
- Varshosaz J, Pardakhty A, Mohsen S, Baharanchi H, Sorbitan onopalmitate based proniosomes for transdermal delivery of chlorpheniramine maleate, *Drug Deliv*, 12(2), 2005, 75-82, ISSN: 1071-7544.
- Singh RP, Mallikarjuna GU, Sharma G, Dhanalakshmi S, Tyagi AK, Chan DC, Agarwal C, Agarwal R, Oral silibinin inhibits lung tumor growth in athymic nude mice and forms a novel chemo combination with doxorubicin targeting nuclear factor kappaB-mediated inducible chemoresistance, *Clin Cancer Res.*, 10, 2004, 8641–8647.
- Yang SH, Lin JK, Chen WS, Chiu JH, Anti-angiogenic effect of silymarin on colon cancer LoVo cell line, *J Surg Res.*, 113, 2003, 133-138.
- Rao PR, Viswanath RK, Cardio protective activity of silymarin in ischemia-reperfusion induced myocardial infarction in albino rats *Exp Clin Cardiol*, Winter, 12(4), 2007, 179–187.
- Radko L, Cybulski W, Application of silymarin in human and animal medicine, *J Preclin Clin Res*, 1, 2007, 22-26.
- Saller R, Meier R, Brignoli R, % use of silymarin in the treatment of liver diseases, *Drugs*, 61, 2001, 2035-2063.
- Garg R, Gupta GD, Preparation and Evaluation of Gastro retentive Floating Tablets of Silymarin, *Chem. Pharm. Bull*, 57(6), 2009, 545-549.
- Liu T, Guo R, Investigation of PEG 6000/Tween 80/Span 80/H2O niosome microstructure, *Colloid Polym. Sci.*, 285, 2007, 711-713.
- Bhaskaran S, Lakshmi PK, Comparative evaluation of niosome formulations prepared by different techniques, *Acta Pharmaceutica Scientia*, 51, 2009, 27-32.
- Dubey V, Mishra D, Jain NK, Melatonin loaded ethanolic liposomes: physicochemical characterization and enhanced transdermal delivery, *European Journal of Pharmaceutics and Biopharmaceutics*, 67(2), 2007, 398–405.
- Raj Kapoor B, Palanivel MG, Senthil KR, Einstein JW, Kumar EP, Kumar MR, Kavitha K, Hepatoprotective and Antioxidant Effect of *Pisonia aculeata* L. against  $CCl_4$ - Induced Hepatic Damage in Rats, *Sci Pharm.*, 76, 2008, 203–215.
- Vogel GH, *Drug discovery and evaluation: Pharmacological assays*, 2nd ed Heidelberg, Springer-Verlag, 2002, 942-943.
- Uchegbu IF, Vyas SP, Non-ionic surfactant based vesicles (niosomes) in drug delivery, *Int. J. Pharm.*, 172, 1998, 33– 70.
- Shyamal S, Latha PG, Shine VJ, Suja SR, Rajashekhran S, Devi T, Hepatoprotective effects of *Pittosporum neelgherrense* wight & arn, a popular Indian ethno medicine, *Journal of Pharmaceutical Science*, 107, 2006, 151-155.
- Tangri P, Khurana S, Niosomes: formulation and evaluation, *Int j biopharm*, 2(1), 2011, 47-49.
- El ridy Mohamed S, Badawi alia A, Safar Marwa M, Mohsen amira M, Niosomes as a novel pharmaceutical formulation encapsulating the hepatoprotective drug silymerin, *Int J Pharm Sci*, 4(1), 2012, 549-559.

Source of Support: Nil, Conflict of Interest: None.

