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Formulation and Evaluation of Decitabine Loaded Niosomes

J.Gomathi [1]*, P.Palanisamy[1], J.Jayalakshmi[1], J.Loganathan[1]
1.Department of Pharmaceutics, Vinayaka Mission's College of Pharmacy, Vinayaka Missions University, Salem-636008 Tamilnadu

ABSTRACT

Nonionic surfactant vesicles (niosomes) were formulated with an aim of enhancing the oral bioavailability of Decitabine, an anti-cancer drug. Niosomes were formulated by conventional thin film hydration technique with different molar ratios of surfactant, cholesterol and dicetyl phosphate. The formulated niosomes were found spherical in shape, ranging from 2.95 μm to 10.91 μm in size. Vesicles with 1 : 1 : 0.1 ratios of surfactant : cholesterol : dicetyl phosphate with each grade of span were found to have higher entrapment efficiencies, which were further selected for *in vitro* studies. Vesicles formulated with sorbitan monostearate were found to have maximum drug release (99.091%) at the end of 24 hours and followed zero order release kinetics. In conclusion, noisome could be a promising delivery for Decitabine with improved oral bioavailability and prolonged release profiles.

Keywords: Niosomes, Decitabine, Non-Ionic Surfactants.

*Corresponding Author Email: palanisamy2907@gmail.com
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INTRODUCTION

Nonionic surfactant vesicles (niosomes) formed from self-assembly of hydrated synthetic nonionic surfactant monomers are capable of entrapping a variety of drugs and have been evaluated as an alternative to liposomes. Nonionic surfactants form unilamellar and multilamellar vesicles that have similar physical properties to liposomes and are relatively inexpensive drug delivery system. In niosomes, soluble drug molecules are present in the aqueous compartments between the bilayer whereas insoluble ones are entrapped within the bilayer matrix. The use of niosomes for drug delivery can alter the biodistribution to provide a greater degree of targeting of the drug to selected tissues, sustained release and altered pharmacokinetics.

Decitabine is indicated for treatment of patients with myelodysplastic syndrom(MDS). Decitabine is slightly soluble in ethanol/water/water. Decitabine requires control release due to its short biological half life (30 mins), low bioavailability, narrow therapeutic index and moreover it is primarilyabsorbed from stomach. All the drawbacks necessitated the development of niosomal formulation for improving residence of dosage form in GIT which could utilize all the efficacy of Decitabine, thereby reduce dosing frequency and enhance bioavailability. Therefore, drug loaded niosomes are promising candidate for delivery of Decitabine for treatment of MDS patients.

MATERIALS AND METHOD

Materials:

Decitabine was supplied as a gift sample from blueberry pharmaceuticals (Chennai), India. Cholesterol (CHOL), span 20, span 40, span 60, span 80, chloroform, were purchased from Qualichem Specialties Pvt. Ltd., Mumbai. All other chemicals were of analytical grade and procured from the authentic sources. Dialysis membrane (30/15mm flat width/diameter) was purchased from Himedia, Mumbai.

Formulation of Drug Loaded Niosomes:

Drug loaded niosomes were formulated by conventional thin film hydration technique. Different grades of span such as span 20, span 40, span 60, and span 80 in different molar ratios of Surfactant :CHOL as 2.5 : 1 , 2 : 1 , 1.5 : 1 , 1 : 1 and 1 : 1.5 were used. 10 mg Dicetyl phosphate which is charge-inducing substance is added in all batches. Accurately weighed quantities of surfactant, CHOL were dissolved in 10mL diethyl ether using a 100mL round bottom flask. The lipid solution was evaporated by rotary flash evaporator under reduced pressure at a temperature of 60 ± 2 ^{0}C. The flask was rotated at 120 rpm until a smooth and dry lipid film was obtained. The film was hydrated with 10mL phosphate buffer saline (PBS) of pH 7.4 containing drug for 3 hours

at 60 ± 2 ^{0}C with gentle shaking. The niosomal suspension was further stabilized by keeping at 2–8^{0}C for 24 hours.

Visual Observation:

All the prepared batches were visually observed for turbidity and flocculation in transparent containers.

Vesicle Size Measurement:

The average vesicle size of the prepared niosomes was measured by using optical microscope and the vesicle size distribution studies were performed on the optimized batches by measuring the size of randomly selected 100 niosomes vesicles from each formulation. The vesicles were also studied by scanning electron microscopy (SEM) technique to check their shape at higher magnification values.

Zeta Potential Measurement*:*

The surface charge of the vesicles plays an important role in the *in vivo* performance of niosomes. The significance of zeta potential is that its value can be related to the stability of vesicular formulations. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion system. Zeta potential of suitably diluted niosomal dispersion was determined using Zetasizer Nano ZS-90 (Malvern Instruments Ltd., UK) at 25^{0}C. The working principle of the instrument is electrophoretic light scattering (ELS), which determines electrophoretic movement of charged particles under an applied electric field from doppler shift of scattered light, for zeta potential determination.

Entrapment Efficiency:

Entrapment efficiency of drug loaded niosomes was determined after separation of unentrapped drug, which was performed by cooling centrifugation (Remi, C-24DL) at 12,000 rpm for 30 min at 4^{0}C. The supernatant liquid was collected separately. The separated vesicles were washed with PBS and the washings were mixed with supernatant liquid. The vesicles were suspended in 3mL PBS and placed in a dialysis bag. The dialysis bag after tying at both ends was immersed in 200mL PBS, maintained at 37^{0}C and stirred overnight by using magnetic stirrer. Decitabine was estimated spectrophotometrically at λmax of 252 nm, against PBS as blank. The percentage of entrapped drug was calculated by applying the following equation:

$$\% \text{ Entrapment} = (DE \times 100)\ (DI)\ , \qquad (1)$$

where DE is the amount of entrapped drug and DI is the initial amount of drug.

The entrapped drug was also verified by estimating the unentrapped drug in the supernatant liquid separated in the initial step.

***In Vitro* Drug Release Study:**

In vitro release pattern of niosomal suspension was carried out. The formulations with each grade of surfactant, which showed the better entrapment efficiencies (F4, F9, F14, and F19), were further selected for carrying out *in vitro* release studies. Buffers of different pH were used for *in vitro* drug release studies to simulate stomach and blood pH and also to evaluate the effect of pH on drug release. Dialysis tube containing the measured amount of drug loaded niosomal dispersion was initially placed in magnetically stirred 200mL of 0.1N HCl at 37 ± 5 ^{0}C, and then after the completion of 2 hours of the study, the test media were replaced with PBS pH 7.4 and the test was continued for a total period of 24 hours. Aliquots of 5mL samples of dialysate were withdrawn periodically at 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 18 and 24 hours and immediately replenished with the same volume of buffer medium. The drug content was determined spectrophotometrically at λmax of 252 nm. Results are the mean of three runs.

Release Kinetics Modeling:

For the characterization of the release kinetics studies and to determine the release mechanism of drug, the results of *in vitro* studies were fitted with several kinetics models as follows.

Zero order rate equation: $Q_t = Q_0 + K_0 t,$ (2)

where Q_t is the amount of drug dissolved in time t, Q_0 is initial amount of drug in solution, and K_0 is zero order release constant.

First order rate equation: $\log C = \log C0 - K_t / 2.303$, (3)

where C_0 is the initial concentration of drug, K is first order release constant, and t is time.

Higuchi's model: $Q = K_H t$ ½ , (4)

where Q is the amount of drug released in time t per unit area, K_H is Higuchi dissolution constant.

Hixson-Crowell model: W_0 1/3 $-W_t$ 1/3 = κt, (5)

Where W_0 is the initial amount of drug in the niosomes, W_t is the remaining amount of drug in the niosomes at time t, and κ (kappa) is a constant incorporating the surface-volume relation. To find out the mechanism of drug release, the *in vitro* release data of all niosomal formulations were fitted into **Korsmeyer and Peppas equation :** $M_t / M_\infty = K t^n$, (6)

where M_t/M_∞ is a fraction of drug release at time t, K is the release rate constant, and n is the release exponent. The value of exponent (n) indicates the mechanism of drug release.

RESULTS AND DISCUSSION

Visual Observation:

All the prepared batches were visually observed for turbidity and flocculation in transparent containers and were found to be turbid and whitish in colour. However, the selected batches (with good entrapment efficiencies) were also evaluated for sedimentation while keeping at 4^0C for 3 months in the transparent containers. The results revealed that in all the batches, except F14, sedimentation started after 30 days of the storage but the niosomal formulation, formulated with span60, was found in good dispersible form indicating the good physical stability (Table 2).

Table 1: Bottom view of container showed different degrees of sedimentation.

Bottom view of container (differentiate from initial look)	Degree of sedimentation	Indications
No colour change 	No sedimentation	–
Partially dark 	Partial sedimentation (1–25%)	+
Intently dark 	Near to complete sedimentation (26–75%)	++
Entirely dark 	Complete sedimentation	+++

Table 2: Degree of sedimentation of Decitabine loaded niosomes after being stored at 4^0C for 3 months.

Formulation code	0 day	15 days	30 days	45 days	60 days	90 days
F4	-	-	+	+	++	+++
F9	-	-	+	+	++	++
F14	-	-	-	-	+	+
F19	-	-	+	+	++	++

Table 3: Composition and characterization of niosomal formulations

Formulation Code	Surfactant grade	Surfactant : CHOL : DCP ratio	Mean vesicle diameter (μm)*	Zeta potential (mV)*	Entrapment efficiency (%)*	% Cumulative drug released (at the end of24 hours)*
F1	Span 20	2.5 : 1 : 0.1	7.12 ± 0.75	−91 ± 0.19	37 ± 0.56	-
F2	Span 20	2 : 1 : 0.1	8.01 ± 0.61	−87 ± 0.13	48 ± 0.69	-
F3	Span 20	1.5 : 1 : 0.1	8.70 ± 1.24	−56 ± 0.23	58 ± 0.42	-
F4	Span 20	1 : 1 : 0.1	9.03 ± 0.76	−29 ± 0.12	78 ± 1.08	83.86 ± 3.72
F5	Span 20	1 : 1.5 : 0.1	10.91±0.86	−25 ± 0.15	75 ± 0.73	-
F6	Span 40	2.5 : 1 : 0.1	5.70 ± 0.75	−61 ± 0.16	38 ± 0.71	-
F7	Span 40	2 : 1 : 0.1	7.84 ± 0.96	−52 ± 0.21	52 ± 0.82	-
F8	Span 40	1.5 : 1 : 0.1	8.65 ± 0.81	−48 ± 0.24	63 ± 0.36	-
F9	Span 40	1 : 1 : 0.1	8.90 ± 1.30	−26 ± 0.18	83 ± 0.71	94.12 ± 4.82
F10	Span 40	1 : 1.5 : 0.1	9.85 ± 0.78	−22 ± 0.17	81 ± 0.37	-
F11	Span 60	2.5 : 1 : 0.1	3.98 ± 0.34	−46 ± 0.15	48 ± 0.52	-
F12	Span 60	2 : 1 : 0.1	4.31 ± 0.67	−39 ± 0.17	59 ± 1.01	-

F13	Span 60	1.5 : 1 : 0.1	4.93 ± 1.16	−28 ± 0.23	71 ± 0.65	-
F14	Span 60	1 : 1 : 0.1	6.82 ± 0.76	−23 ± 0.21	95 ± 0.51	99.09 ± 4.78
F15	Span 60	1 : 1.5 : 0.1	8.55 ± 0.86	−22 ± 0.18	89 ± 0.79	-
F16	Span 80	2.5 : 1 : 0.1	2.95 ± 0.87	−45 ± 0.19	52 ± 0.63	-
F17	Span 80	2 : 1 : 0.1	4.17 ± 0.86	−37 ± 0.20	62 ± 0.54	-
F18	Span 80	1.5 : 1 : 0.1	4.59 ± 0.56	−25 ± 0.21	74 ± 0.74	-
F19	Span 80	1 : 1 : 0.1	6.31 ± 0.75	−21 ± 0.11	96 ± 0.66	89.90 ± 3.99
F20	Span 80	1 : 1.5 : 0.1	8.42 ± 0.65	−16 ± 0.14	89 ± 0.82	-

*The data were reported as an average of 3 measurements (mean ± S.D.).

Vesicle Size Measurement.

The formulated niosomal vesicles were found to be spherical in shape (Figure 1), ranging from 2.95 μm to 10.91μm in size. The effect of surfactant HLB value on vesicles size has come forward; as the HLB value of the surfactants moves towards the hydrophilicity, the vesicles size was found to be increasing

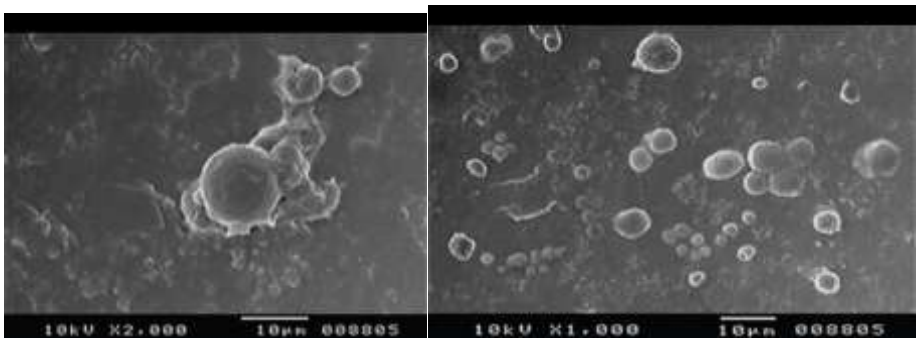

Figure 1: (a) SEM image of Decitabine loaded Niosomes (b) spherical structure of niosome at higher magnification.

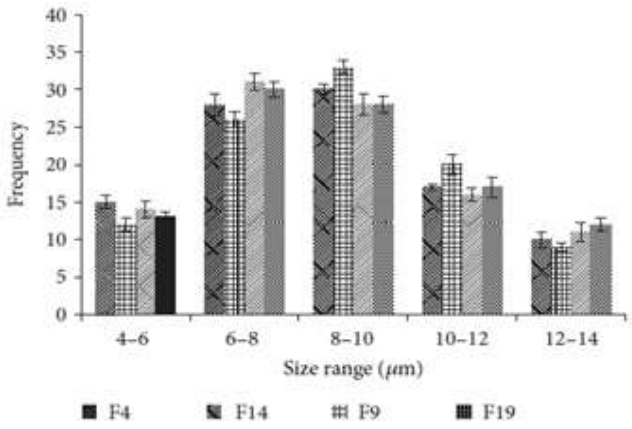

Figure 2: Size distribution plots of all optimized batches.

Zeta Potential Measurement.

The values of zeta potential for all the batches are illustrated in Table 3, which were found in range of −16 to −91.The results, revealed that the zeta values of the vesicles increase towards negative

with increasing the HLB values of the surfactants. The niosomal vesicles formulated with span80 were found to have the least zeta values, span20 showing higher zeta values ranging from −25 to −91 Increase in surface energy of the vesicles leads to increase the values of zeta potential towards negative.

Entrapment Efficiency

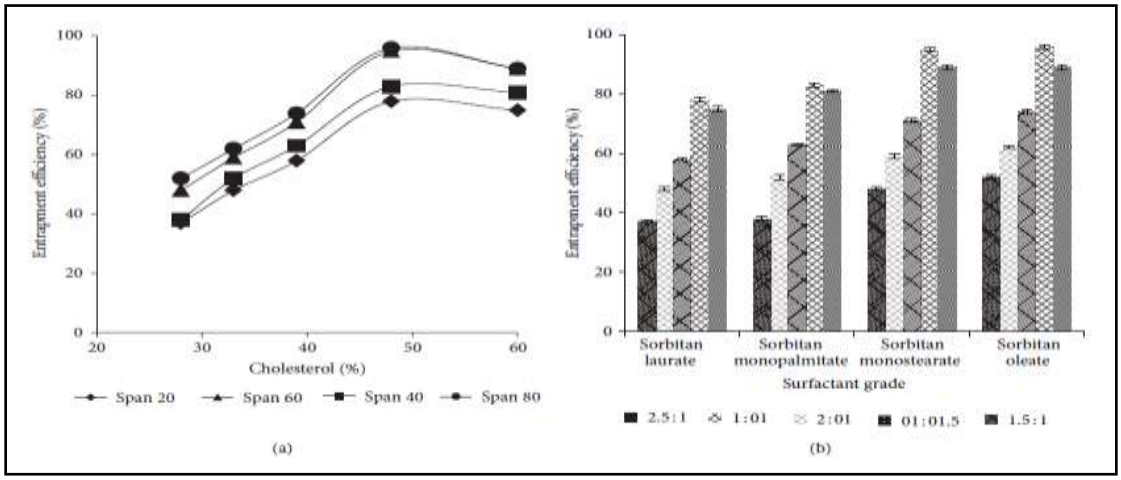

Figure 3: (a) % entrapment efficiency versus % CHOL indicating the effect of CHOL concentration on % entrapment efficiency, (b) %entrapment efficiency versus surfactant grades with several surfactant :CHOL ratios.

The entrapment efficiency is an important parameter for the characterization of niosomal vesicles which was found ranging from 37% to 96% and tabulated in Table 3. The percentage of drug entrapment was found to be good with surfactant : CHOL ratio 1 : 1 with all grades of surfactants but span60 showed the highest drug entrapment compared to other grades (Figure 3).

In Vitro Drug Release Study:

In vitro release studies were performed by dialysis method. The release profile of Decitabine is given in Figure 4(a), shown to be retarded for 24 hours. The percentage of Decitabine released at the end of 24 hours is given in Table 3. The results revealed that the maximum percentage of Decitabine (99.091%) was released from F14 (with span60), followed by F9, that is, 94.12% (with span40), F19, that is, 89.901% (with span80), and F4, that is, 83.86% (with span20) at the end of 24 hours.

Release Kinetics Modelin:.

The *in vitro* release data was fitted to various release kinetics models to predict the release mechanism of drug from the niosomes. The results revealed that all the formulations were best explained by zero order release (plots show highest linearity) followed by Higuchi release kinetics indicating that the concentration was independent of drug release. But the formulation F14 showed

the highest linearity among all the formulations indicating the best zero order release kinetics (Figure 4).

Table: 4 Release kinetic of niosomal formulations

Formulation code	Zero order		First order		Higuchi		Hixson-Crowell model		Korsmeyer-Peppas model	
	r^2	Ko(h^{-1})	r^2	K_1(h^{-1})	r^2	K_H	r^2	KHC($h^{-1/3}$)	r^2	n
F4	0.996	2.994	0.891	0.082	0.990	19.183	0.855	0.086	0.963	0.54
F9	0.997	3.343	0.942	0.126	0.986	21.333	0.899	0.118	0.967	0.53
F14	0.999	3.752	0.979	0.184	0.982	23.841	0.963	0.156	0.968	0.61
F19	0.997	3.282	0.953	0.119	0.989	20.962	0.892	0.102	0.964	0.58

CONCLUSION

All the formulated niosomal vesicles were found to be spherical in shape ranging from 2.9 µm to 10.91µm in size and zeta values within range. The percentage of drug entrapment was found to be higher with surfactant : CHOL ratio 1 : 1 with all grades of surfactants but span60 showed the highest drug entrapment compared to other grades. *In vitro* study revealed that formulation F14 (with span60) showed maximum 99.091% drug release at the end of 24 hours. Further, the *in vitro* release profile was fitted to various release kinetics models to predict the release mechanism of drug from the niosomes and the results revealed that all the formulations were best explained by zero order release. So the prepared Decitabine loaded niosomal vesicles could be the promising drug delivery system for controlled release of Decitabine.

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