

## Reviewer's Comments

### Formulation and evaluation of elastic liposomes of Decitabine prepared by rotary evaporation method

#### ABSTRACT

Decitabine is a cytidine deoxynucleoside analog, which acts by inhibiting DNA methyltransferase, and is used for the treatment of acute myeloid leukemia. Decitabine has a short half-life (25 minutes), and is sensitive to harsh conditions. Elastic liposomes are an effective tool that can be used to overcome this disadvantage. Elastic liposomes also known as transfersomes are modified lipid carriers that enable drug to reach deeper skin layers and/or the systemic circulation. These vesicular formulations are several orders of magnitudes, more deformable than the standard liposomes and thus well suited for skin penetration. The objective of present study is to develop and evaluate the elastic liposomes of Decitabine so as to provide the sustained release and improve its bioavailability. Elastic liposomes were prepared by rotary evaporation method using Span 80 as a surfactant. The prepared Elastic liposomes were evaluated for entrapment efficiency, vesicle size, *in vitro* drug release. The drug release profiles from different elastic liposomes-in-vehicle formulations were in agreement with the physicochemical properties of the formulations. Based on different parameters formulations of batch ELS1 was found to be the best formulations. Stability study was performed on the selected formulation ELS1. Study concludes that Decitabine can also be formulated in the liposomal carrier which finds its best way for the topical administration.

**Keywords:** Decitabine, Elastic liposomes, *in vitro* drug release, Span 80.

#### Introduction

At present scenario, the most recent development in vesicle design for transcutaneous bioactive delivery is the use of elastic liposomes, which differ from conventional liposomes due to their characteristic fluid membrane with high elasticity<sup>1</sup>. Elastic liposomes have been defined as specially designed vesicular particles, consisting of at least one inner aqueous compartment surrounded by a lipid bilayer with appropriately tailored properties. Elastic liposomes consist of phospholipids, surfactants such as edge activators, and an inner aqueous compartment enclosed within a lipid bilayer capable of encapsulating hydrophilic (in an aqueous chamber) and lipophilic (in a lipid bilayer) molecules<sup>2</sup>.

Drug delivery systems using vesicular carriers have soft, flexible, self-regulating, and self-optimizing vesicular characteristics. Greater flexibility of elastic liposomal membranes is achieved by mixing suitable surface-active components in the proper ratios<sup>3</sup>. These properties allow them to penetrate more easily into deeper layers of the skin and circulation. In Elastic liposomes, elasticity is stress controlled, owing to the composition dependence of the membrane bending energy<sup>4</sup>. They are elastic, very deformable vesicles which consist of phosphatidylcholine in combination with an edge-active surfactant like sodium cholate and span 80. Elastic liposomes are applied non-occluded to the skin and are reported to permeate through the stratum corneum lipid lamellar regions as a result of the hydration or osmotic force in the skin<sup>5</sup>. Elastic liposomes passage through the normally confining pores is then governed by the basic principles of elastic mechanics. However, elastic liposomes resemble lipid vesicles, liposomes, in morphology but functionally, elastic liposomes are sufficiently deformable to penetrate pores much smaller than their own size. They are metastable, which makes the vesicle membrane ultra flexible and thus the vesicles are highly deformable<sup>6</sup>.

Decitabine is a cytidine deoxynucleoside analog, which acts by inhibiting DNA methyltransferase, inducing DNA hypomethylation<sup>7,8</sup>. It is used for the treatment of acute myeloid leukemia (AML) in patients aged  $\geq 65$  years. However, it can only be administered intravenously due to very low oral bioavailability and a large distribution volume. Decitabine is a hydrophilic drug (log  $P = -2.2$ ), with a short half-life (25 minutes), and is sensitive to harsh conditions<sup>9</sup>.

Thus the present study was to refine the formulation of elastic liposomes of Decitabine in order to enhance its bioavailability.

#### Materials and methods

Decitabine was obtained from AC Drugs Limited, Enugu State, Span 80 and Span 60 were obtained from Afrab-Chem Limited, Lagos State. Cholesterol was obtained from Asad Pharmaceuticals Ltd, Kano, Nigeria. All other chemicals were of analytical grade.

#### Preparation of Elastic liposomal formulations

The Decitabine elastic liposomes were prepared by rotary evaporation method<sup>10</sup>. Different batches of Decitabine elastic liposomes were prepared using different proportions of surfactant, phospholipids and drug. The accurately weighed amounts of phospholipids and surfactant were taken in a clean, dry, round-bottom flask and this lipid mixture was dissolved in small quantity of chloroform-methanol mixture (2:1). The organic solvent was removed by rotary evaporation under reduced pressure at 40°C. Final traces of solvents were removed under vacuum overnight. The deposited lipid film was hydrated with 7 % v/v ethanol i.e. solution of drug by rotation at 60 rev/min for 1 hr. The resulting vesicles were swollen for 2 h at room temperature; these were bath sonicated for 10 min. Compositions of different batches of elastic liposomes as shown in Table 1.

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**Table 1: Compositions of different Decitabine elastic liposomal formulations.**

Formulation code	Decitabine (mg)	Phosphatidylcholine (mg)	Span 80 (mg)	Span 60 (mg)	Cholesterol
ELS1	20	80	20	-	30
ELS2	20	80	20	-	25
ELS3	20	75	10	10	20
ELS4	20	70	-	20	18
ELS5	20	70	-	20	15

#### Drug loading in elastic liposomal formulation

To determine the maximum amount of drug that can be added in vesicles, increasing amounts of Decitabine (4, 6, 8, 10 and 12mg) was added during the preparation of elastic liposomal formulations. All the drug loaded vesicular formulations were examined for maximum entrapment efficiency and for the appearance of drug crystals over a period of 14 days using optical microscope<sup>11</sup>.

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#### Characterisation of elastic liposomal formulation

##### Particle size analysis

All the prepared batches of Decitabine elastic liposomes were viewed under microscope to study their size. Size of liposomal vesicles from each batch was measured at different location on slide by taking a small drop of liposomal dispersion on it and average size of liposomal vesicles were determined<sup>12</sup>.

##### Percentage yield of liposomes

The prepared Decitabine elastic liposomes were collected and weighed. The measured weight was divided by the total amount of drug and ingredients which were used for the preparation of the liposomes<sup>13</sup>.

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##### Entrapment Efficiency

For determination of entrapment efficiency the untrapped drug was separated by the use of the mini column centrifugation method. The amount of drug entrapped (Total amt. of drug- untrapped drug) in the vesicles was then determined by disrupting the vesicles using methanol followed by filtration and amount of drug was quantified spectrophotometrically<sup>14</sup>.

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##### Redispersibility

The Decitabine elastic liposomal formulation was centrifuged at 4000 rpm for 18 min at 4 °C temperature by using remi cooling centrifuge to separate the free drug. A supernatant contains the liposomes in suspending stage and free drug on the wall of centrifuge tube. The supernatant was again centrifuged at 12000 rpm for 38 min at 4 °C temperature. As a result, a transparent solution of supernatant and liposome pellet was attained. The pellet was then diluted to 10 ml with deionized water. The solution was then taken in 14 ml Eppendorf tubes and vortexed on a vortex mixture at speed 3 for 5 min. After vortexing the solution was then taken in transparent cuvettes and particle size was determined after 10 min<sup>15</sup>.

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##### Zeta potential analysis

Zeta potential is a physical property which is exhibited by any particle in suspension. It can be used to optimize the formulations of suspensions and emulsions. It is also an aid in predicting long-term stability. The magnitude of the zeta potential gives an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential then they will tend to repel each other and there will be no tendency for the particles to come together.

A dynamic light scattering instrument was used to measure zeta potential of the liposomal formulation. The sample was analyzed at 25 °C, using a dispersant refractive index of 1.33. In the current study, the zeta potential of the prepared liposomal formulation was measured in disposable folded capillary cells. Samples should be bubble free for accurate measurement of zeta potential. Measurement was done with Malvern nano series zeta sizer instrument at 25 °C<sup>16</sup>.

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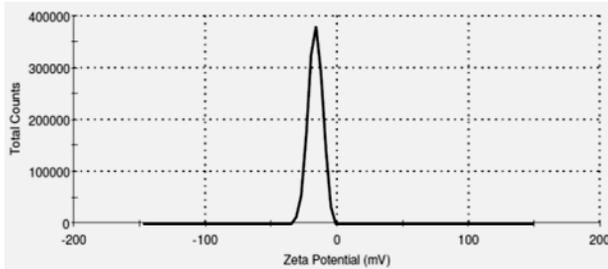


Figure 1: Zeta potential distribution of Decitabine elastic liposome of batch ELS1

#### Elasticity Measurement

For the measurement of elasticity of vesicular membrane, Decitabine elastic liposomal formulations were extruded at 2.5 bars through the polycarbonate filter membrane having a pore diameter of 60 to 200 nm using a stainless steel pressure holder for 25-mm diameter filters with 200-ml capacity barrel<sup>17</sup>. The elasticity of vesicle membrane was calculated by using the following formula -

$$E = J * (r_v/r_p)^2$$

Where,  $E$  = elasticity of vesicle membrane;  $J$  = amount of suspension, which was extruded during 10 min;  $r_v$  = vesicles size (after extrusion); and  $r_p$  = pore size of the barrier

Table 2: Physicochemical characterization of the Decitabine elastic liposomes

Code	Particle size (nm)	% Entrapment efficiency	% Yield	Zeta Potential (Mv)	Elasticity	Redispersion (nm)
ELS1	145 ± 11	70.2 ± 1.3	90.28	-18.4 ± 0.09	28.3 ± 2.6	166
ELS2	153 ± 12	66.5 ± 2.4	89.43	-16.7 ± 0.16	36.7 ± 3.4	170
ELS3	165 ± 11	63.3 ± 2.2	88.52	-15.4 ± 0.31	42.8 ± 1.5	159
ELS4	174 ± 15	55.2 ± 1.5	85.15	-12.5 ± 0.17	54.0 ± 2.6	155
ELS5	182 ± 13	53.4 ± 2.8	80.34	-13.2 ± 0.26	43.6 ± 1.8	148

Value represented as mean ± SD (n = 3)

#### In vitro drug release studies through cellophane membrane

In-vitro drug release studies were carried out in modified Diffusion cell using Dialysis membrane. The membrane was soaked in Phosphate buffer pH 6.8 for 9-12 hours and it was clamped carefully to one end of the hollow glass tube of dialysis cell (2.3 cm diameter; 4-16 cm<sup>2</sup> area). Then Decitabine elastic liposomal gel was spread uniformly on the dialysis membrane. 50 ml of Phosphate buffer pH 6.8 was taken in a beaker, which was used as receptor compartment. The donor compartment was kept in contact with the receptor compartment. This whole assembly was kept on a magnetic stirrer and the solution on the receptor side was stirred continuously using a magnetic bead. The temperature of the cell was maintained at 37°C. Sample (5 ml) was withdrawn at 1 hour time intervals up to 24 hours and replaced with equal amounts of fresh dissolution media. Samples were analysed spectrophotometrically at 260 nm and % cumulative drug release was calculated<sup>18</sup>.

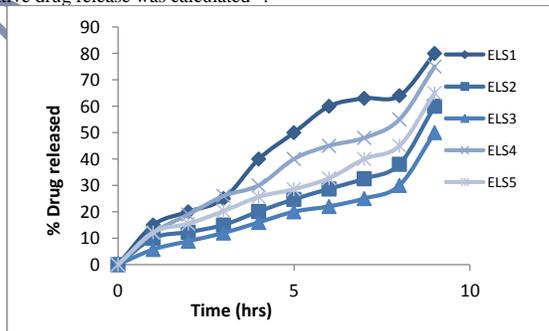


Figure 2: Comparative drug release of all Decitabine elastic liposome formulations

#### Stability Analysis

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The stability of a pharmaceutical delivery system may be defined as the capability of a particular formulation, in a specific container. The behaviour of the Decitabine elastic liposome to retain the drug was studied by storing the liposome at 4 different temperature conditions, i.e., 4-8°C (refrigerator RF), 25±2°C (room temperature RT), 37±2°C and 45±2°C for a period of 1 month. The liposomal preparations were kept in sealed vials. Periodically samples were withdrawn and analyzed for the drug content<sup>19</sup>.

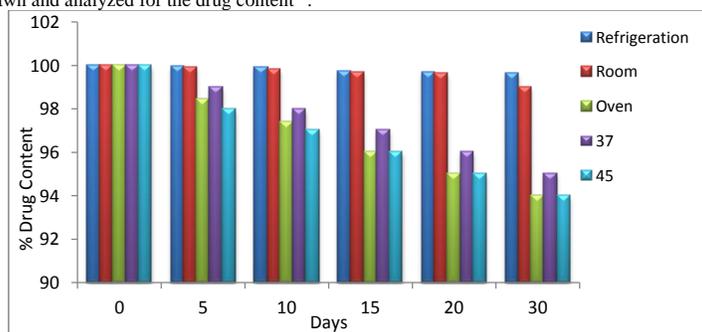


Figure 3: Stability studies for the Decitabine elastic liposome of batch ELS1

### Results and discussion

Different batches of Decitabine elastic liposomes were prepared with Span 80 and Phosphatidylcholine using rotary evaporation method. Span 80 was selected as edge activator surfactant as it is biocompatible and pharmaceutically acceptable.

Phosphatidylcholine was used as bilayer forming agent.

Decitabine elastic liposomes were evaluated by various parameters like vesicle shape, vesicle size, entrapment efficiency, no. of vesicles, *in-vitro* drug release, *ex-vivo* skin permeation study, stability study and microbiological assay. The particle size distribution of the tested liposomal formulation showed uni-modal normal symmetrical frequency distribution patterns. The results of zeta potential for freshly prepared multi lamellar liposomal dispersion were given in Table 2 and Figure 1.

The mean particle diameter of multi lamellar vesicles was found to be in between 145±11 and 182±13 and Zeta potential was -12.5±0.17 and -18.4±0.09 respectively.

The results showed Decitabine elastic liposomes entrapment efficiency was varied with lipid composition and cholesterol content. The percentage entrapment efficiency of Decitabine increased with increasing the cholesterol content. Formulation ELS1 has shown maximum entrapment efficiency (70.2±1.3).

Vesicle size distribution is required to check how much % intensity of particles of Elastic liposomes distributed in the size range. Vesicle size of optimized ELS1 formulation was found to be 145 ± 11 nm i.e. in the normal size range (1-10,000 nm)<sup>20</sup>.

Redispersibility is carried out to check the change in particle size after aggregation. Data suggested from Table 2 particle size ranges from less than 148nm to 170 nm for all hydration volumes.

The maximum % release (80.46±0.47) was shown by formulation of batch ELS1 when compared to all other formulations after 9 hrs. The *in-vitro* drug release studies were given in Figure 2. Cholesterol shows a significant effect on drug release studies as the concentration of cholesterol increases the drug release seems to be controlled and prolonged. After evaluating all the parameters the liposomal Formulations were tested for their stability studies by subjecting them to different temperatures for a period of one month and were found to be stable with very negligible variations.

The stability studies of the selected Decitabine elastic liposomes formulation ELS1 are shown in Figure 2. There were no physical changes observed throughout the study till 30<sup>th</sup> day at refrigerator temperature.

The formulation, stored at room temperature, 37°C and 45°C for 30 days showed varied drug content.

### Conclusion

Elastic liposomes are especially optimized particles or vesicles which can provide a novel solution for transport related problems.

Five different Decitabine elastic liposomes formulations were prepared and evaluated for different parameters like particle size, % entrapment efficiency, zeta potential, % yield, elasticity etc. On the basis of different parameters, formulations of batch ELS1 were found to be best. The selected elastic liposomes formulations of batch ELS1 showed good stability profile without any much declination in their properties at different temperature condition for a study of 30 days.

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This study concludes that Decitabine can also be formulated in the liposomal carrier which finds its best way for the topical administration.

#### Conflict of interest

"No conflict of interest associated with this work".

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