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FORMULATION AND CHARECTERIZATION OF ARTEMISININ LIPOSOMES

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ABSTRACT

The active pharmaceutical ingredient Artemisinin was evaluated for its Organoleptic properties and solubility. The results obtained were satisfactory. Artemisinin liposomes were prepared by solvent evaporation, followed by hydration with water and the Phosphatidyl choline concentrations were optimized by various trials.In the present study liposomes containing Artemisinin was prepared. The effect of increase in Phosphatidyl choline concentration in various parameters like particle size, zeta potential and *in vitro* release profile were studied. The Artemisinin liposomes were formulated and evaluated for its drug content, entrapment efficiency, particle size analysis, zeta potential and *invitro* drug release profile. Based on the results of Artemisinin liposomes formulations (AL-1- AL-5) formulation AL-5 wasselected as the best formulation in which the particle size was 252. 4 nm and the entrapment was 88. 76%. The *in vitro* % drug release of AL-5 formulation was 98. 54 % and it was found to be suitable formulation for the treatment of Malaria. Hence it can be concluded that the newly formulated controlled release liposomal drug delivery systems of Artemisinin may be ideal and effective in the treatment of Malaria by allowing the drug to release continuously for 24 hrs.

KEYWORDS: Formulation, Charecterization, Artemisinin, Liposomes.

INTRODUCTION

Liposomes are self-assembled (phospho)lipid-based drug vesicles that form a bilayer (uni-lamellar) and/or a concentric series of multiple bilayers (multilamellar) enclosing a central aqueous compartment.[1] The size of liposomes ranges from 30 nm to the micrometer scale, with the phospholipidbilayer being 4–5 nm thick.[2] The field of liposomology was launched by the British scientist Alec Bangham and colleagues at Babraham Cambridge in the mid-1960s[3], and they first published the structure of liposomesin 1964.[4] Since then, liposomes have been widely investigated as delivery vehicles for small molecular drugs, protein, nucleic acid, and imaging agents.[5,6,7,8,9] Different administration routes, such as parenteral, pulmonary, oral, transdermal, ophthalmic, and nasal routes, have been developed to improve therapeutic efficacy and patient compliance.[10,11,12,13,14] In addition, liposomes have been widely applied in the fields of food[15] and cosmetics.[16]

As drug vehicles, liposomes exhibit outstanding properties, such as protecting the encapsulated substances from physiological degradation[17], extending the half-life of the drug, controlling the release of drug molecule s[18], and excellent biocompatibility and safety. Furthermore, liposomes can selectively deliver their payload to the diseased site through passive and/or active targeting, thus decreasing the systemic side-effect, elevating the maximum-tolerated dose, and improving therapeutic benefits.[19,20]

Unlike normal tissue with tight intracellular junctions (2–6 nm) between endothelial cells[21], abnormal tissues such as a solid tumor or inflammatory site have highly porous capillaries (100 nm–2 μm depending upon the size and type of tumor tissue[22]). Liposomes can cross over the discontinuous neovasculature and be passively accumulated and detained at the abnormal tissues, which is called the enhanced permeability and retention (EPR) effect. Actively targeting employs specific interactions between the ligands and receptors on the surface of liposomes and tumor cells, respectively. Tumor cells may overexpress specific receptors, such as vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), folic acid (FA), integrin, CD44 (a cell surface glycoprotein), CD13, and prostate-specific membrane antigen.[23] According to these receptors, specific ligands, such as antibody[24], nuclear acid (e.g., aptamers[25]), protein (e.g., transferrin[26]), peptides (e.g., iRGD[27], iNGR[28]), small molecules (folic acid[29]), and carbohydrates (e.g., dextran, mannose, and galactose[30], targeting macrophages) were proposed for the surface modification of liposomes.

As of 2018, the exact mechanism of action of artemisinins was not known because of the complex chemical interactions involved. Artemisinins do not

directly attack malarial parasites or cells. They have to undergo chemical changes in the blood. Their functional group endoperoxide ring has to be activated first. Activation is done by cleavage of the endoperoxide ring. As the drug molecules come in contact with the haem (inside the haemoglobin of the red blood cells), the iron(II) oxide breaks the endoperoxide ring. This process produces free radicals that in turn damage susceptible proteins, resulting in the death of the parasite.[34] In 2016 artemisinin was shown to bind to a large number of targets suggesting that it acts in a promiscuous manner. Unlike other antimalarials which are active only on a particular stage of malarial parasite, artemisinin is able to kill all the life cycle stages.

The main aim of present study is to prepare and evaluate the liposomes for the selected drug Artemisinin.

MATERIALS AND METHODS

Table 1: Materials used.

MATERIALS	SUPPLIER
Artemisinin	Sigma aldrich pvt. ltd
Egg Phosphatidyl choline	Sigma aldrich pvt. ltd
Cholesterol	Sigma aldrich pvt. ltd
Chloroform	M/S SD Fine Chemicals, Mumbai, India
Methanol	M/S SD Fine Chemicals, Mumbai, India
Potassium di hydrogen phosphate	M/S SD Fine Chemicals, Mumbai, India
Ortho phosphoric acid	M/S SD Fine Chemicals, Mumbai, India

METHODS

PREFORMULATION STUDIES

Preparation of calibration graph for Artemisinin

Preparation of calibration curve in pH 1. 2, pH 7. 4 and pH 6. 8 buffer solutions

An accurately weighed amount of Artemisinin 100mg was dissolved in small volume of buffer solutions in each of three 100 ml volumetric flask and the volume was adjusted to 100 ml with 1. 2 pH buffer in first volumetric flask, 7. 4 pH buffer in second volumetric flask and the third one was adjusted to 100 ml with 6. 8 pH buffer. A series of standard solution containing in the concentration range from 10 to 50 µg/ml of Artemisinin were prepared for 1.2 pH buffer solution, 7. 4 pH buffer solution and 6. 8 pH buffer solution separately, absorbance was measured at 195 nm and calibration graph was plotted using concentration versus absorbance.

Drug-excipient compatibility study by DSC

Differential scanning calorimetry (DSC)

Samples of individual components as well as each drug-excipient were weighed (Mettler Electronic balance) directly in pierced aluminum crucible pans (5-10 mg) and scanned in the 50-300°C temperature range under static air, with heating rate of 10 ºC /min, using shimadzu DSC-60 equipment.

METHOD OF PREPARATION

Table 2: Formula used for the preparation of Artemisinin Liposomes.

S. NO	FORMULATION	DRUG (mg)	Phosphatidyl Choline(mg)	Cholesterol(mg)
1.	**AL-1**	100	50	100
2.	**AL-2**	100	75	100
3.	**AL-3**	100	100	100
4.	**AL-4**	100	125	100
5.	**AL-5**	100	150	100

METHOD

PREPARATION OF LIPOSOMES

- Liposomes of Artemisinin were prepared by evaporation of solvent followed by hydration. Briefly, the selected lipids, drug and cholesterol were dissolved in a mixture of chloroform and methanol (ratio 2:1 v/v) in a 250ml round bottom flask.
- The solvent was evaporated in the rotary flash evaporator. The thin dry lipid film thus formed was hydrated using aqueous hydrating medium distilled water at 65°C.
- The procedure was repeated for the preparation of five batches of Artemisinin liposomes using various concentrations of phospholipids **(AL-1 to AL-5).**
- The formed liposomal dispersion was sonicated in probe sonicator using ice bath to prevent temperature induced distortion of liposomes.

CHARACTERIZATION STUDIES

- Particle size and Zeta potential
- Encapsulation efficiency
- Drug content
- *In vitro* drug release

Particle size and Surface charge

Surface charge is important in adhesion and interaction of particle with cells. The zeta- potential is used to measure the cell surface charge density. It can be

measured using Malvern-Zeta sizer. The prepared liposomes were evaluated for their particle size and surface charge by photon correlation spectroscopy (PCS) using zeta sizer. The formulations were diluted to 1:1000 with the aqueous phase of the formulation to get a suitable kilo counts per second (kcps). Analysis was carried out at 25°C with an angle of detection of 90°. In this experiment six replicates were taken for the measurement. The results were given in results and discussion section.

Drug Content

1gm of Artemisinin liposomes were accurately weighed and transferred into a 25ml volumetric standard flask. The sample was dissolved with methanol. 1ml of this solution was diluted to 25ml with the purified water. The standard Artemisinin was dissolved and diluted with same methanol and water respectively.

Then the standard and sample absorbance was measured at 195 nm using UV-Visible spectrophotometer. The percentage of drug content was calculated.

The results were given in results and discussion section.

Entrapment efficiency

The drug loaded liposomes in buffer solutions were subjected to centrifugation at 15000 rpm for 30 min. The supernatant liquid was separated and 1ml of this solution was diluted with buffer solution and the absorbance was measured at 195 nm. The amount of Artemisinin unentrapped in the supernatant was calculated. The amount of Artemisinin entrapped was determined by subtracting amount of free unentrapped Artemisinin from the total amount of Artemisinin taken for the preparation. The results were given in results and discussion section.

***In vitro* release**

In vitro release studies were performed for 24 h using dialysis membrane by using the Franz diffusion cell. The prepared Artemisinin liposomes formulations were placed inside a dialysis membrane and immersed in buffer pH 6. 8. At predetermined time intervals the sample was withdrawn and the amount of Artemisinin released was determined by measuring the absorbance at 195 nm using a UV-Visible spectrophotometer. From the absorbance values the cumulative percentage drug release was calculated. The results were given in results anddiscussion section.

RESULTS AND DISCUSSION

Preformulation studies

Preparation of calibration graph for Artemisinin

Standard calibration data of Artemisinin in pH 1. 2, 7. 4 and 6. 8 buffers at 195 nm.

Table 3: Absorbance of Artemisinin in buffer solutions.

S. no	Concentration (µg/ml)	Absorbance		
		pH 1. 2	pH 7. 4	pH 6. 8
1	10	0. 045	0. 051	0. 065
2	20	0. 091	0. 103	0. 131
3	30	0. 137	0. 154	0. 196
4	40	0. 182	0. 205	0. 263
5	50	0. 274	0. 257	0. 327

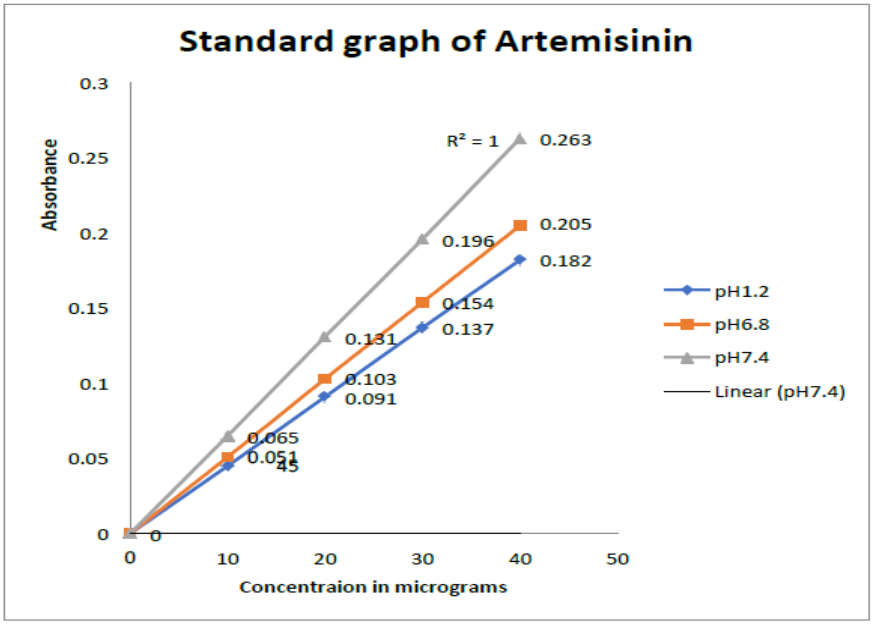

Fig. 1: Calibration curve of Artemisinin in pH 1. 2, 7. 4 and 6. 8 buffers.

Standard calibration curve of Artemisinin was carried out in 1. 2 pH, 7. 4 pH and 6. 8 pH buffer at195 nm. The r^2 value in the entire medium shows nearly 1, which signifies linearity.

DSC analysis

DSC of Artemisinin showed a sharp endothermic peak at about 206. 26^0C (melting point). The physical mixture of Artemisinin with other excipients also showed the same thermal behavior (206. 28^0C) as the individual component. DSC results also revealed that the physical mixture of Artemisinin with excipients showed superimposition of the thermogram. There was no significant change observed in melting endotherm of physical mixture of Artemisinin and excipients.

Hence from the DSC study, it was found that there was no interaction between Artemisinin and other excipients used in the formulation.

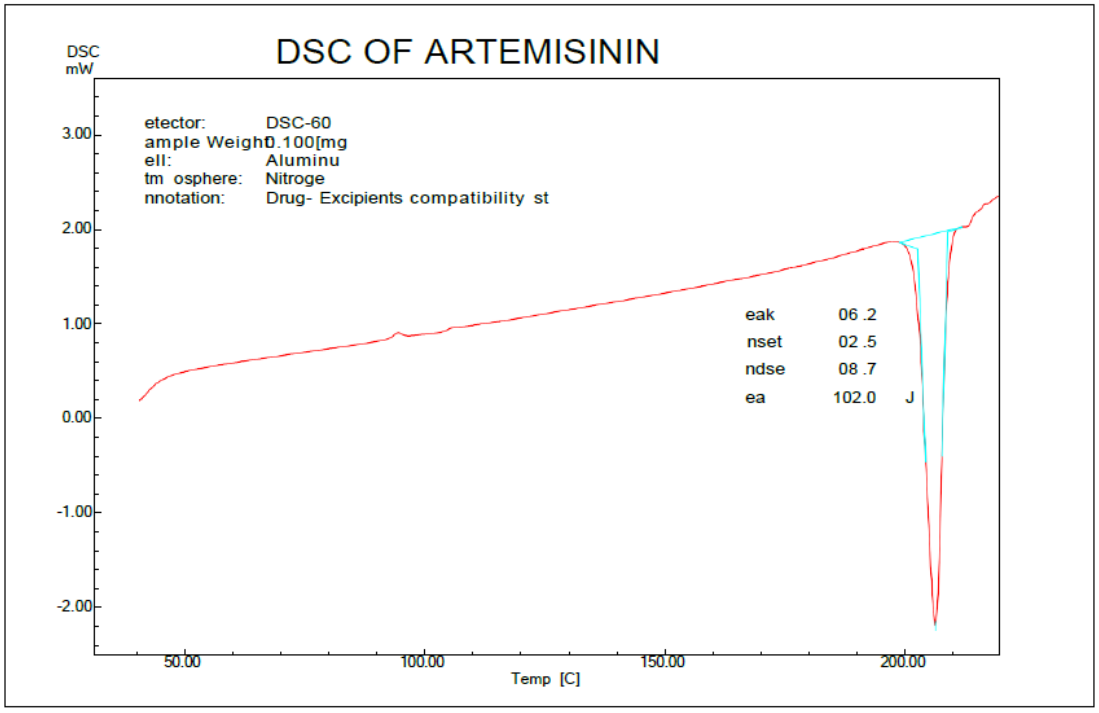

Fig. 2: DSC Thermogram of Artemisinin and Artemisinin LiposomesDrug –Excipients accelerated compatibility study - Physical observation and assay.

Upon analysis of the drug excipient mixture for their physical characteristics no colour change was observed. Based on the chemical evaluation it was found that there was no significant change observed indicating that the drug is compatible with the added ingredients.

Table 4: Physical characteristics of Artemisinin.

S. No	Physical parameters	Results
1	Description	Off white powder
2	Melting point	196°C
3	Loss on drying	0. 35%
4	Assay	98. 58%

Table 5: Physical characteristics of individual drug and excipients.

S. No	Sample ID	Initial description	Final description
1.	Artemesinin	Off white powder	No change
2.	Phosphatidyl choline	Yellowish brown semisolidmass	No change

Table 6: Physical characteristics of Drug-Excipient mixture.

S. No	Sample ID	Initial description	Final description
1	Artemesinin	Off white powder	No change
2	Artemesinin + Phosphatidyl choline	Yellowish powder	No change

Table 7: Chemical characteristics of Drug-Excipient mixture.

S. No	Sample ID	Initial assay (%)	Final assay (%)
1.	Artemesinin	98. 52%±0. 24	98. 51%±0. 23
2.	Artemesinin+Phosphatidylcholine	98. 53%±0. 18	98. 53%±0. 11

n = 3; Mean ± S. E. M.

Table 8: Drug content and Entrapment efficiency Particle size and Zeta potential ofArtemisinin Liposomes.

Trials	Zeta potential (mV)	Particle size (nm)	Entrapment Efficiency (%)	Drug Content (%)
AL-1	-17. 6	236. 8	37. 84	98. 37
AL-2	-18. 3	239. 5	56. 79	98. 45
AL-3	-19. 2	243. 7	61. 53	98. 49
AL-4	-20. 7	248. 8	76. 47	98. 50
AL-5	**-23. 4**	**252. 4**	**88. 76**	**98. 53**

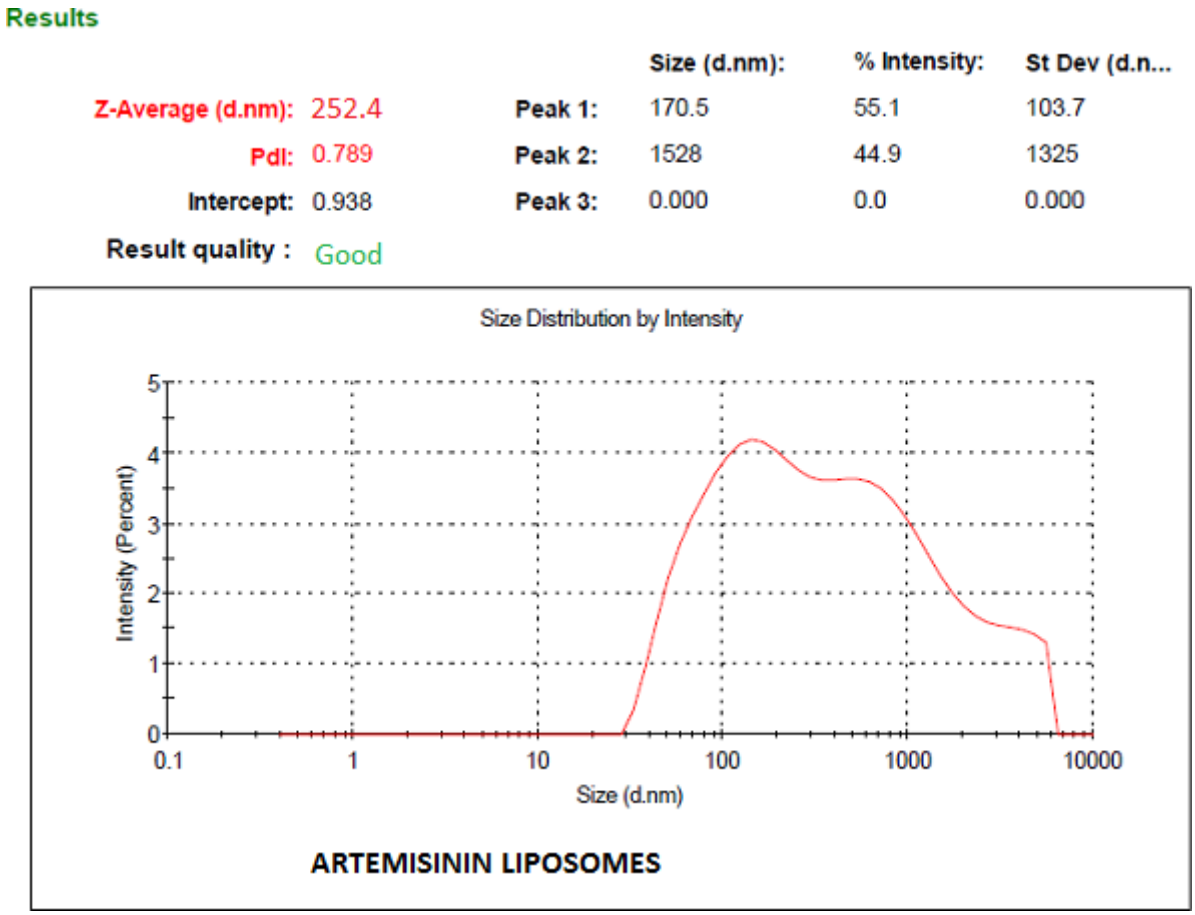

Fig. 3: Particle size of optimized Artemisinin liposomes (AL-5).

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -23.4	Peak 1:	6.33	100.0	6.40
Zeta Deviation (mV): 6.40	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.0714	Peak 3:	0.00	0.0	0.00
Result quality : Good				

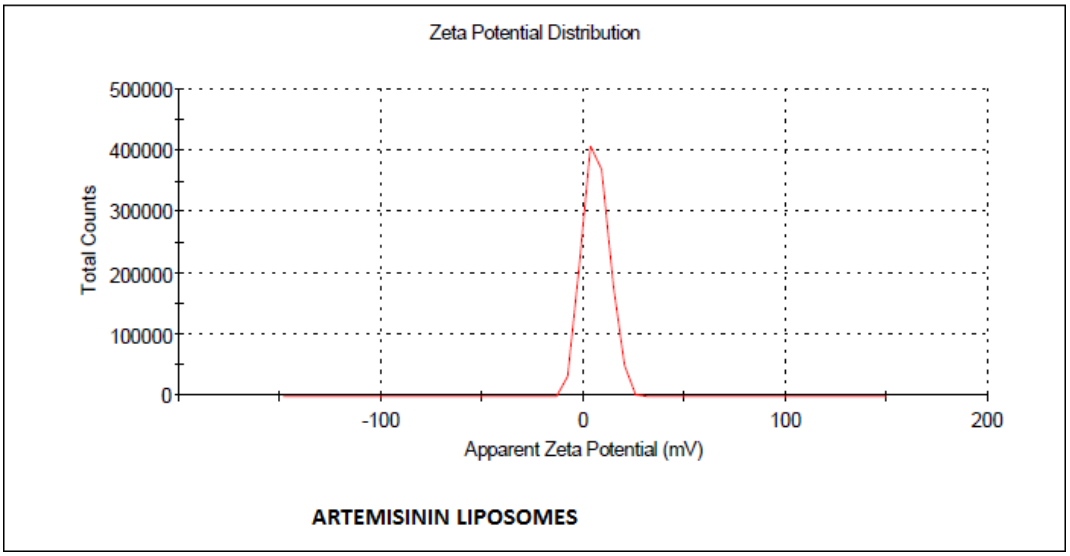

Fig. 4 Zeta potential of optimized Artemisinin liposomes (AL-5).

- Particle size and entrapment efficiency of the **Artemisinin Liposomes (AL-1-AL-5)** were increased with increasing **Phosphatidyl Choline** concentration.
- This may be due to high amount of availability of Phospolipid to encapsulate the drug, upon increasing the **Phosphatidyl Choline** concentration, number of layers coated the drug was increased, this resulted in increased particle size and entrapment efficiency.
- Further increase in the **Phosphatidyl Choline** concentration **(AL-1-AL-5)**, there is no much increase in the entrapment efficiency due to the availability of the drug to be incorporated is low which is not enough for further encapsulation of drug by **PhosphatidylCholine**.
- Based on the results of Particle size and entrapment efficiency of the **Artemisinin Liposomes** (**AL1-AL-5**), the trial **AL-5** which contains **150mg of Phosphatidyl Choline** concentration was selected as the best formulation.

***In- vitro* drug release**

Table 9: *In vitro* release studies of Artemisinin Liposomes.

S. NO	Time(Hrs)	%CUMULATIVE DRUG RELEASE				
		AL-1	AL-2	AL-3	AL-4	AL-5
1	0. 5	48. 67	40. 57	29. 87	20. 77	15. 84
2	1	76. 92	68. 64	57. 94	45. 86	32. 75
3	6	**98. 52**	90. 63	79. 37	67. 93	53. 27
4	12	98. 51	**98. 51**	87. 58	76. 65	65. 39
5	16	98. 50	98. 48	**98. 55**	84. 39	74. 67
6	20	98. 48	98. 47	98. 54	**98. 53**	87. 29
7	24	98. 46	98. 48	98. 52	98. 49	**98. 54**

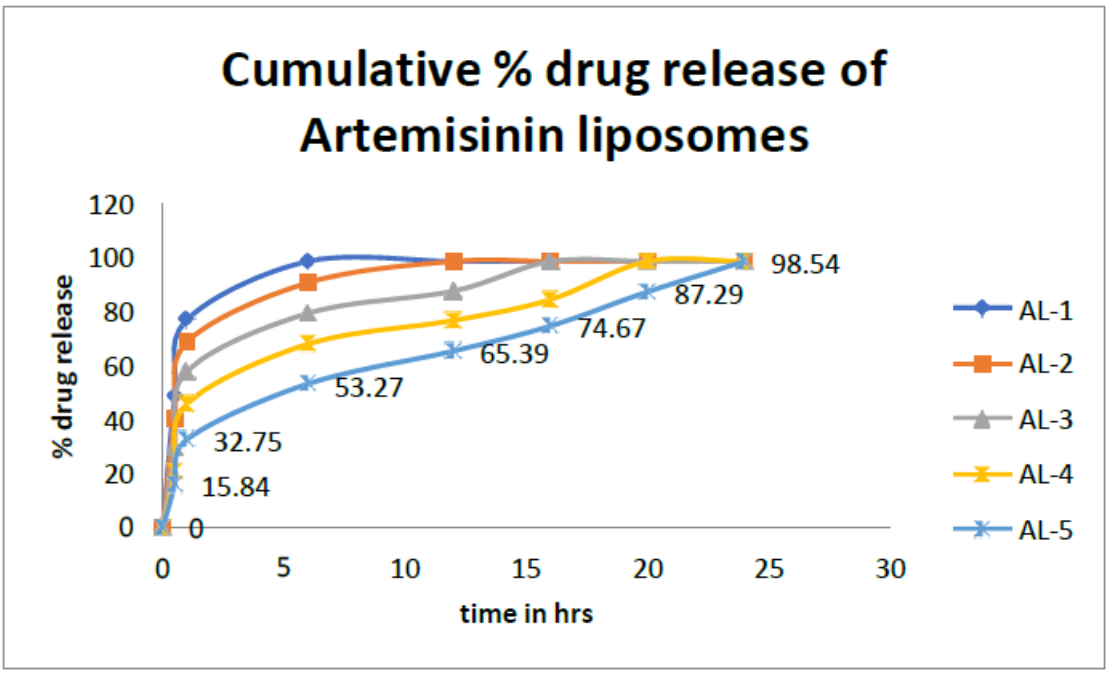

Fig. 5.

- From the *in vitro* drug release study results, the maximum percentage drug release **98. 54** at the end of 24h was observed with trial **AL-5** which contains **150mg of Phosphatidyl choline**
- Below **150mg of Phosphatidyl choline** concentration as in the case of trials **AL-1 to AL-4,** the maximum percentage drug release **98. 52 %, 98. 51%, 98. 55** and **98. 53%** were obtained at the end of 6h. 12h, 16h and 20h respectively which was not desirable.
- Above **150mg of Phosphatidyl choline** concentration, reduction in drug release was observed for all the trials **(AL-1 to AL-5)**. The maximum percentage drug release for **AL- 5** was found to be **98. 54%** at the end of 24h was obtained.
- From the *in vitro* drug release data for **AL-1 to AL-5,** it was observed that increase in **Phosphatidyl choline** concentration delays the drug release due to increased particle size and reduced surface area of the prepared liposomes.
- From all the formulations, **AL-5** was selected as best formulation due to its ideal particle size (**252. 4 nm**), Zeta Potential **(-23. 4)**, high entrapment efficiency (**88. 76%**) and desirable drug release **98. 54 %** at the end of 24 h.

SUMMARY AND CONCLUSIONS

The active pharmaceutical ingredient Artemisinin was evaluated for its Organoleptic properties and solubility. The results obtained were satisfactory.

Artemisinin liposomes were prepared by solvent evaporation, followed by hydration with water and the Phosphatidyl choline concentrations were optimized by various trials In the present study liposomes containing Artemisinin was prepared. The effect of increase in Phosphatidyl choline concentration in various parameters like particle size, zeta potential and *in vitro* release profile were studied.

The Artemisinin liposomes were formulated and evaluated for its drug content, entrapment efficiency, particle size analysis, zeta potential and *invitro* drug release profile.

Based on the results of Artemisinin liposomes formulations (AL-1- AL-5) formulation AL-5 was selected as the best formulation in which the particle size was 252. 4 nm and the entrapment was 88. 76%.

The *in vitro* % drug release of AL-5 formulation was 98. 54 % and it was found to be suitable formulation for the treatment of Malaria. Hence it can be concluded that the newly formulated controlled release liposomal drug delivery systems of Artemisinin may be ideal and effective in the treatment of Malaria by allowing the drug to release continuously for 24 hrs.

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