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FORMULATION AND EVALUATION OF NIOSOMES ENCAPSULATED LEUCOVORIN

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ABSTRACT

The present study was to investigate the feasibility of using niosomes as a drug delivery system for Leucovorin. By entrapping the drug in niosomes, dose could be reduced. Niosomes were prepared by thin film hydration technique with the mixture of cholesterol and surfactant. Particle size, zeta potential, entrapment efficiency and *in vitro* drug release studies of Leucovorin niosomes were evaluated. From the results of the present Leucovorin experimental investigation, it may be concluded that formulation LNF 4 containing drug with 2:1 (surfactant: cholesterol) ratio was showing small vesicles size, high percentage of entrapment with the desired sustained release of Leucovorin.

KEYWORDS

Leucovorin, Thin film hydration technique, Entrapment efficiency and *In vitro* release and study.

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INTRODUCTON

Niosomes are non-ionic surfactant based vesicles composed primarily of synthetic surfactants and cholesterol incorporation as an excipient. Niosomes are lamellar structure and are microscopic in size. Niosomes are found to improve therapeutic efficacy of cancer, parasites and microbial diseases. Niosomes are promising vehice for drug delivery and being non-ionic it is less toxic and improves therapeutic index of drug by restricting its action to target cells. Niosomes are also used as a vehicle for poorly absorbed drugs for designing novel drug delivery system. The use of niosomes for drug delivery can alter the biodistribution to provide greater degree of targeting of drug to selected tissues, sustained release and altered

pharmacokinetics. The presence of cholesterol improves rigidity of the bilayer and its important component of cell membrane and its presence in membrane affects bilayer fluidity and permeability. The present work was to invest investigate the influence of various preparation technique on the formulation of Leucovorin niosomes by using span40 and cholesterol followed by evaluating the parameters such as drug content, entrapment efficacy, particle size, shape, and *in vitro* drug release and drug release kinetics.

MATERIAL AND METHODS

Leucovorin, Cholesterol, Span 40, Diethyl ether, Methanol, from Technico Laboratory, Coimbatore, Sonicator, Digital balance, Magnetic stirrer. All the chemicals used were analytical grade.

Formulation of Leucovorin Niosomes

Niosomes were prepared by thin film hydration technique. Accurately weighed quantity of cholesterol and surfactant were dissolved in diethyl ether methanol mixture ratio (2:1v/v) in a 100ml round bottom flask. The weighed quantity of drug, Leucovorin was added to the solvent mixture and shaken for 30minutes until the formulation of thin film on the wall of the flask. The dried lipid film was hydrated with 6ml phosphate buffer saline of pH7.4 at a temperature of 60°C for a period of 2hrs until the formation of niosomes. All the batches were subjected to sonication process for 2 min using probe sonicator.

Evaluation of transdermal patches

Particle size and zeta potential

Vesicle properties such as particle size and zeta potential were determined by Malvern particle size analyser.

Entrapment efficiency

The percentage of drug encapsulated was increased with increase in span 40. Niosomes prepared by hand shaking method have good entrapment efficiency. After preparing niosomal dispersion, unentrapped drug is separated and the drug remained entrapped in niosomes is determined by complete vesicle disruption using 50% n-propanol or 0.1% Triton X-100 and analyzing the resultant solution by appropriate assay method for the drug. It can be represented as:

Entrapment efficiency (EF) = (Amount entrapped / total amount) × 100

Percentage Drug Entrapment

The entrapped Leucovorin within niosomes was determined after removing the unentrapped drug by dialysis. The dialysis was carried out by taking niosomal dispersion in dialysis bag which was dipped in a beaker containing 200ml of PBS with a pH of 7.4 the beaker was placed in a magnetic stirrer run for 24 hrs. Then, the solution inside the receptor compartment was studied for an un entrapped Leucovorin at 276 nm using a UV spectra photometer. The PDE in the niosomes was calculated from the ratio of the difference of the total amount of drug added and the amount of an unentrapped drug detected to the total amount of drug added.

In vitro release study of leucovorin niosomes

Niosomal preparation was taken in dialysis membrane of 5cm length and suitably suspended in beaker containing 200ml of diffusion medium (phosphate buffer saline pH7.4). The medium was maintained at temperature of 37-37.5^{o}c. It was stirred by means of magnetic stirrer at a constant speed. Sample of 1ml (diffusion medium) was withdrawn at every 1hour for 24hours and replaced the diffusion medium. So, that the volume of diffusion medium was maintained constant at 200ml. The sample were measured spectrophotometrically at 253nm.

Drug release kinetics

Zero order kinetics

The diffusion model of Leucovorin niosomes follows zero order kinetics. The graph is plotted on as Time Vs Cumulative drug release.

Higuchi Plot

The graph is plotted on as Square root of time Vs Cumalative of drug released.

RESULTS AND DISCUSSION

Particle Size and Zeta Potential

Particle size result that reveals that the niosomes prepared using span40 shows size range of 307nm, the smaller vesicle size account for higher entrapment efficiency.

Zeta potential of the formulation was found to be -1.05mV which confirms the particle size of the

formulation remains stable and prevents the particle from aggregation.

Entrapment efficiency

The percentages of drug encapsulated in niosomes are given in the Table No.1, percentage of drug encapsulated was increased with increase in span 40, Niosomes prepared by hand shanking method have good entrapment efficiency.

In vitro drug release was studied for all the batches of niosomes. The studies were performed upto 24 hours for all the batches. The cumulative percentage release of formulation (LNF_1, LNF_2, LNF_3, LNF_4) was found to be 68.28%, 74.35%, 82.39%, 92.43%. The niosomes exhibit an alkyl chain length dependent release due to the surfactant span 40. Increase in surfactant concentration increase the release of drug from surfactant vesicles in formulation LNF_4. Thus optimized formulation LNF_4 has high amount of drug release due to good entrapment efficiency.

Table No.1

S.No	Formulation code	Entrapment efficiency (%)
1	LNF_1	73.5
2	LNF_2	78
3	LNF_3	80
4	LNF_4	87

***In vitro* drug release studies**

S.No	TIME	LNF1	LNF2	LNF3	LNF4
1	0	0	0	0	0
2	2	2	4	2	4
3	4	6	6.2	4.1	10.13
4	6	10.3	10.3	8.2	17.34
5	8	14.5	16.5	12.4	24.9
6	10	18.7	26.08	26.6	31.6
7	12	24.09	38.13	32.13	39.41
8	14	26.12	44.19	44.16	47.28
9	16	34.13	54.22	52.22	55.39
10	18	38.17	62.27	68.26	64.28
11	20	48.19	68.31	74.34	73.9
12	22	56.24	70.34	78.37	82.39
13	24	68.28	74.35	82.39	92.43

Kinetic Studies

Table No.2

S.No	Zero order kinetics	Higuchi plot
1	R^2	R^2
2	0.99	0.96

Size Distribution Report by Intensity

v2.2

Malvern

Sample Details

Sample Name: Leucovorin niosomes 1
SOP Name: mansettings.nano
General Notes:

File Name:	F1 S.dts	**Dispersant Name:**	Water
Record Number:	6275	**Dispersant RI:**	1.330
Material RI:	1.59	**Viscosity (cP):**	0.8872
Material Absorbtion:	0.010	**Measurement Date and Time:**	Monday, March 11, 2019 11:1...

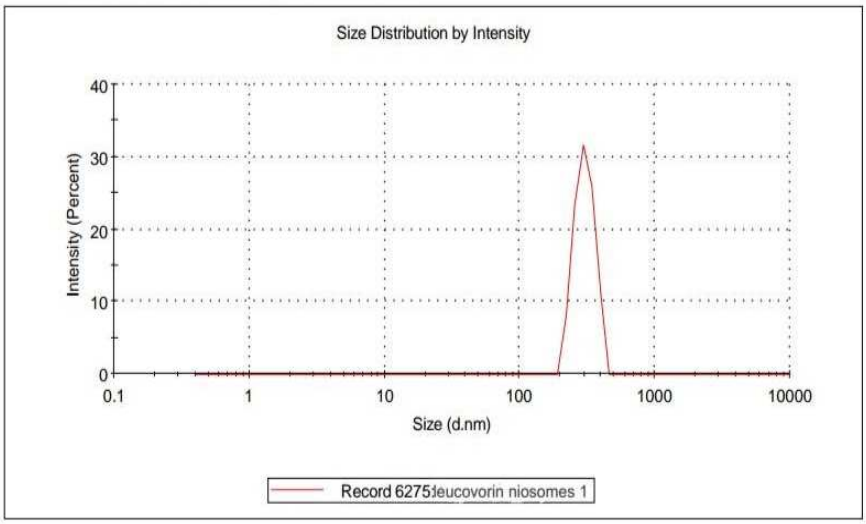
System

Temperature (°C):	25.0	**Duration Used (s):**	20
Count Rate (kcps):	376.1	**Measurement Position (mm):**	4.65
Cell Description:	Disposable sizing cuvette	**Attenuator:**	10

Results

			Size (d.nm):	% Intensity:	St Dev (d.n...
Z-Average (d.nm):	307.7	**Peak 1:**	303.2	100.0	49.69
PdI:	0.406	**Peak 2:**	0.000	0.0	0.000
Intercept:	0.705	**Peak 3:**	0.000	0.0	0.000

Result quality : Refer to quality report



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Zetasizer Ver. 7.11
Serial Number : MAL1063652

File name: F1 S
Record Number: 6275
11 Mar 2019 11:20:55 AM

Zeta Potential Report

v2.3

Malvern

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Sample Details

Sample Name: leucovorin niosomes z 1
SOP Name: mansettings.nano
General Notes:

File Name: F1 S.dts
Record Number: 6276
Date and Time: Monday, March 11, 2019 11:15:2...
Dispersant Name: Water
Dispersant RI: 1.330
Viscosity (cP): 0.8872
Dispersant Dielectric Constant: 78.5

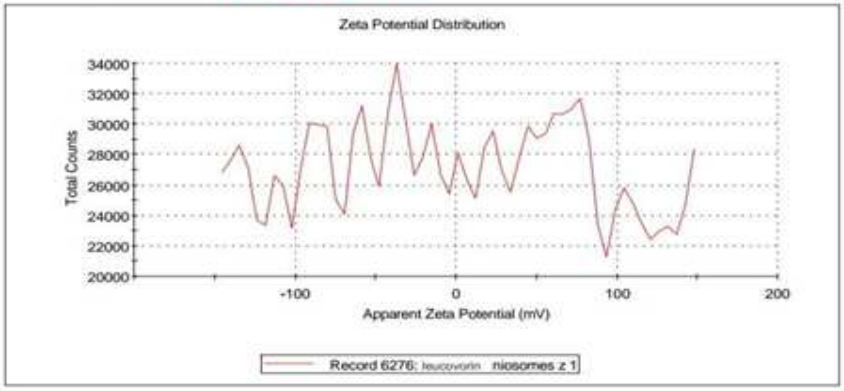
System

Temperature (°C): 25.0
Count Rate (kcps): 17.8
Cell Description: Zeta dip cell
Zeta Runs: 26
Measurement Position (mm): 4.50
Attenuator: 11

Results

		Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -1.05	Peak 1:	-85.8	10.4	10.4
Zeta Deviation (mV): 196	Peak 2:	78.6	9.2	8.96
Conductivity (mS/cm): 3.83	Peak 3:	-132	8.7	9.10

Result quality : See result quality report



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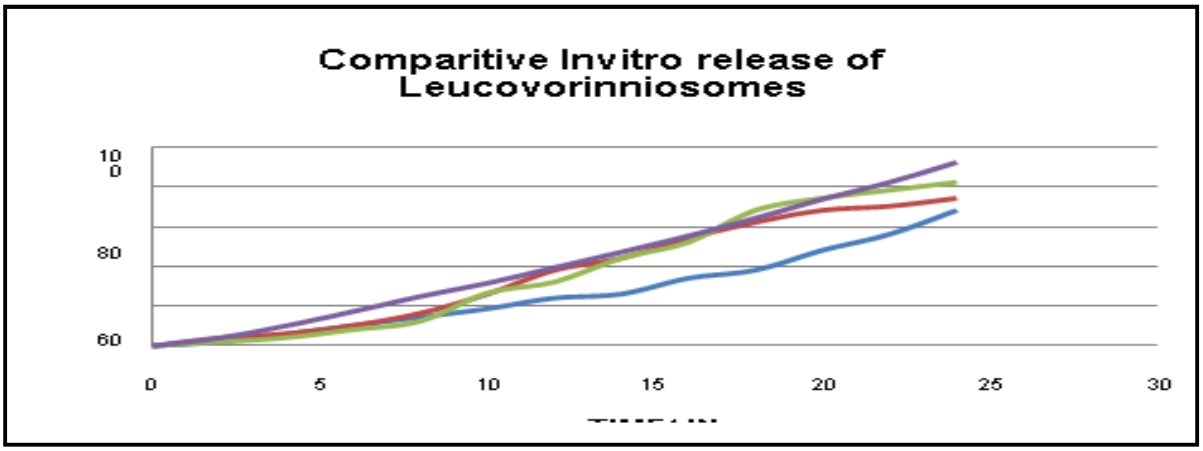
Zetasizer Ver. 7.11
Serial Number : MAL1063652

File name: F1 S
Record Number: 6276
11 Mar 2019 11:21:10 AM



CONCLUSION

In this study we have taken effort to prepare niosomal formulation of Leucovorin, the vesicles formed quitestable. From the results of the present experimental investigation, it may be concluded that formulation LNF_4 containing drug with polymer ratio was showing small vesicle size, high percentage of entrapment with the desired release of Leucovorin. Hence LNF_4 formulation were the optimized formulation. In vitro release from niosomal formulation showed extended release of drug for 24 hours. The optimized formulation was found to follow zero order pattern. So, we can conclude that niosomes could be used as drug carriers for Leucovorin and also to sustain the effect of drug for a longer duration.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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