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FORMULATION AND EVALUATION OF HESPERIDIN ENCAPSULATED NIOSOMES

Kehungteung Nriame*[1], Barish[1], E. Abraham Theodore[1]

[1*]Department of Pharmaceutics, RVS College of Pharmaceutical Sciences, Sulur, Coimbatore-641402, Tamil Nadu, India.

ABSTRACT

Advancement in drug delivery systems with different techniques have developed which controlled the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of a drug to a cell/tissue. Niosomes are multilamellar or unilamellar vesicles capable of entrapping hydrophilic and hydrophobic solutes either in the aqueous layer or in vesicular membrane made of lipid materials. They are osmotically active, stable, biodegradable, biocompatible and non-immunogenic. Niosomes of Hesperidin were prepared by hand shaking method and ether injection method using cholesterol and various ratios of Span 80. The newly prepared Niosomes were evaluated for morphology, vesicle size determination, and percentage of drug encapsulation, drug leakage studies from vesicles, osmotic shock and in vitro release profile and came to conclusion to the point that Niosomes enhance the therapeutic effectiveness of Hesperidin, producing prolonged activity and simultaneously minimizing the side effects.

KEYWORDS

Hespiridin, Niosomes, Encapsulation, Evaluation, Therapeutic effectiveness and Prolonged activity.

Author for Correspondence:

Kehungteung Nriame,
Department of Pharmaceutics,
RVS College of Pharmaceutical Sciences,
Sulur, Coimbatore-641402, Tamil Nadu, India.

Email: nriamekehungteung@yahoo.com

INTRODUCTION

In the past three decades several advancement in drug delivery systems have been made. As the result new techniques have developed in drug delivery systems. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and targeting the delivery of a drug to a cell/tissue.

This advancement led to the development of several novel drug delivery systems of medication and provides a number of therapeutic benefits by encapsulation of different drug in niosomes.

Therefore to formulate Hesperidin niosomes by two different methods in various ratios of surfactant span 80. Since Hesperidin has a short biological half life of 5 h, which necessitates multiple daily dosing and hence a novel delivery system such as niosomes, can be used to encapsulate the drug so that it maintain a therapeutic plasma concentration for a longer period of time, thereby increasing the bioavailability of the drug. Hence this niosomal delivery may reduce the frequency of dosing intervals and may improve patient compliance. More over Hesperidin is used in the treatment blood vessels including hemorrhoids, varicose veins, poor circulation in the legs (venous stasis), and bleeding (hemorrhage) in the eye or gums which may induce toxic side effects. Therefore it is desirable to deliver them to target tissue in the right manner at the right time, by encapsulating in niosomes, we can minimize the drug dose, which in turn can reduce the toxic side effects and a sustained and controlled release rate of Hesperidin can be achieved. The prepared niosomes are to be characterized of their size, shape, entrapment efficiency, leakage studies, osmotic shock and *in vitro* drug release. The best formulation is to be selected on the basis of evaluation characteristics.

MATERIAL AND METHODS

Pure drug and other surfactants

Pure Hesperidin have been used from Sigma Aldrich Private Limited, India, Cholesterol were bought from Loba chemicals, Span 80 and Diethyl ether manufactured by SD fine Chemiclas and CP laboratories respectively. Sodium Lauryl Sulphate, Potassium Dihydrogen Phosphate, Glycerin IP have been used from Nice chemicals.

Instruments and their supplier

Vortex mixer by Science house VM 11, Chennai, FT-IR spectrophotometer by Perkin elmer Rx I, Scanning electron microscope by Hitachi S-150, UV-Visible Spectrophotometer double beam by Schimadzu pharmaspec 1700, Single pan digital balance by Afcoset, Fluorescence optical microscope by Olympus BX 51, Microscope by Unilab, Digital pH meter by Hanna instruments, Italy, Magnetic stirrer by Eltek MS 2012, Mumbai, Dialysis membrane 110 by Himedia.

Preparation of Standard drug solution

Stock solution

100mg of Hesperidin was dissolved in 100ml of Phosphate buffer saline at pH 7.4 so as to get a stock solution of 1000µg/ml concentration

Standard Solution

2ml of stock solution was made to 100ml with phosphate buffer saline pH 7.4 thus giving a concentration of 20µg/ml. Aliquot of standard drug solution ranging from 1ml to 9ml were transferred in to 10ml volumetric flask and were diluted up to the mark with pH 7.4 phosphate buffer. Thus the final concentration ranges from 2-18µg/ml. Absorbance of each solution was measured at 253.0nm against phosphate buffer saline pH 7.4 as a blank. A plot of concentrations of drug vs absorbance was plotted.

METHODOLOGY

Preparation of Hesperidin Niosomes by Hand Shaking Method[1,2]

Cholesterol and span 80 were taken in specified ratios of (1:1, 1:2 and 1:3) and transferred in to a clean round bottom flask. Then the lipid mixture was dissolved in 10 ml of diethyl ether. The flask was continuously vortexed to form a thin film along the sides of the flask. An appropriate amount of Hesperidin was dissolved in phosphate buffer saline (PBS) pH.7.4. This was poured into added to the thin film and vortexed continuously for a period of 30 min at room temperature.

Preparation of Hesperidin Niosomes by Ether Injection Method[3,4]

Cholesterol and span 80 were taken in prescribed ratio (1:1, 1:2 and 1:3) in a 50ml beaker. The mixture was dissolved in diethyl ether and the solution was slowly injected into a beaker containing Hesperidin in phosphate buffer saline (PBS) pH 7.4. The temperature maintained during the injection was 40-60ºC. The difference in temperature between phases causes rapid

vaporization of ether resulting in spontaneous vesiculation.

Evaluation of different batches of prepared Hespiridin niosomes formulation for their

Morphology, Vesicles size determination by Optical Microscopy and Scanning Electron Microscopy, Percentage of drug encapsulation, Drug leakage studies and *In vitro* release pattern.

Drug leakage studies from vesicles

The drug leakage at elevated temperature may be related to the degradation of lipids in bilayers resulting in defects in membrane packing making them leaky.

Table No.1: Size distribution of niosomes by Hand Shaking Method

S.No	Size range (μm)	Number of Niosomes		
		1:1:1 Ratio	2:1:1 Ratio	3:1:1 Ratio
1	Below 0.1	18-20	16-18	8-9
2	0.1-5	70-72	72-75	85-87
3	Above 5	13-17	6-9	5-9

Table No.2: Size distribution of niosomes by Ether Injection Method

S.No	Size range (μm)	Number of Niosomes		
		1:1:1 Ratio	2:1:1 Ratio	3:1:1 Ratio
1	Below 0.5	18-20	13-15	6-8
2	0.5-2.5	65-70	68-74	82- 86
3	Above 2.5	3-6	4-6	4-5

Table No.3: Average vesicle size for hesperidin encapsulated niosomal formulation

S.No	Formulation Code	Average vesicle size (μm) after incubation with			
		PBS (pH 7.4)	1.5 % w/v NaCl	0.9 % w/v NaCl	0.5 % w/v NaCl
1	HS 1	3.82	Shrinked	3.95	6.22
2	HS 2	4.22	Shrinked	4.33	6.57
3	HS 3	4.53	Shrinked	4.64	6.87
4	ES 1	0.59	Shrinked	0.65	0.91
5	ES 2	0.72	Shrinked	0.73	1.52
6	ES 3	0.74	Shrinked	0.78	1.67

Table No.4: Percentage of drug retention in hesperidin niosomal formulation

S.No	Formulation Code	Percentage of drug retention in niosomes											
		Refrigeration Temp. ($4^o \pm 1$ C)				Room Temp ($25^o \pm 1$ C)				High Temp. ($37^o \pm 1$ C)			
		Days				Days				Days			
		7	14	21	28	7	14	21	28	7	14	21	28
1	HS1	100	95	90	86	99	88	84	77	94	85	73	69
2	HS2	100	96	95	87	99	90	84	79	95	84	75	70
3	HS3	100	97	95	90	100	94	87	80	95	85	76	70
4	ES1	100	95	93	85	98	93	84	77	93	84	73	67
5	ES2	100	97	94	86	99	95	85	78	94	85	74	69
6	ES3	100	98	95	90	100	95	88	80	96	86	75	73

Table No.5: Encapsulation efficiency for hesperidin niosomal formulations

S.No	Formulation Code	Amount of drug used in (mgs)	Percentage of drug encapsulated
1	HS 1	50mg	55
2	HS 2	50mg	64
3	HS 3	50mg	83
4	ES 1	50mg	46
5	ES 2	50mg	55
6	ES 3	50mg	71

Table No.6: *In vitro* release data for hesperidin niosomes for formulation HS-1

Time	Absorbance (253nm)	Concentration in mcg/ml	Amount release in mg/ml	Cumulative amount released in mg	Cumulative % drug release
0	0.0000	0.000	0.0000	0.00	0.00
0.5	0.0599	1.219	0.012	3.05	6.11
1	0.0896	1.824	0.018	4.57	9.15
2	0.1510	3.074	0.031	7.71	15.44
4	0.2010	4.091	0.041	10.29	20.59
6	0.2650	5.394	0.054	13.59	27.19
8	0.3110	6.330	0.063	15.98	31.97
10	0.3690	7.511	0.075	19.00	37.97
12	0.4110	8.366	0.084	21.21	42.44
16	0.4960	10.096	0.101	25.62	51.26
20	0.5610	11.419	0.114	29.03	58.07
24	0.6320	12.865	0.129	32.75	65.54

Table No.7: *In vitro* release data of hesperidin niosomes for formulation HS-2

Time	Absorbance (253nm)	Concentration in mcg/ml	Amount release in mg/ml	Cumulative amount released in mg	Cumulative % drug release
0	0.0000	0.000	0.000	0.00	0.00
0.5	0.0539	1.097	0.011	2.74	5.47
1	0.0796	1.620	0.016	4.06	8.13
2	0.1478	3.009	0.030	7.55	15.11
4	0.1896	3.859	0.039	9.71	19.42
6	0.2310	4.702	0.047	11.85	23.71
8	0.3578	7.283	0.073	18.35	36.72
10	0.3640	7.409	0.074	18.74	37.47
12	0.4760	9.689	0.097	24.51	49.04
16	0.5570	11.338	0.113	28.73	57.43
20	0.6250	12.722	0.127	32.31	64.63
24	0.7240	14.737	0.147	37.47	74.92

Table No.8: *In vitro* release data of hesperidin niosomes for formulation HS-3

Time	Absorbance (253nm)	Concentration in mcg/ml	Amount release in mg/ml	Cumulative amount released in mg	Cumulative % drug release
0	0.0000	0.000	0.000	0.00	0.00
0.5	0.0620	1.262	0.013	3.16	6.34
1	0.0790	1.608	0.016	4.03	8.05
2	0.1190	2.422	0.024	6.08	12.16
4	0.2110	4.295	0.043	10.79	21.60
6	0.2398	4.882	0.049	12.30	24.59
8	0.3410	6.941	0.069	17.50	35.01
10	0.4546	9.254	0.093	23.35	46.72
12	0.5413	11.017	0.110	27.85	55.73
16	0.5963	12.138	0.121	30.76	61.54
20	0.7409	15.082	0.151	38.24	76.47
24	0.8170	16.630	0.166	42.26	84.54

Table No.9: *In vitro* release data of hesperidin niosomes for formulation ES-1

Time	Absorbance (253nm)	Concentration in mcg/ml	Amount release in mg/ml	Cumulative amount released in mg	Cumulative % drug release
0	0.000	0.000	0.000	0.00	0.00
0.5	0.039	0.786	0.008	0.79	7.87
1	0.058	1.168	0.012	1.18	11.78
2	0.076	1.531	0.015	1.55	15.52
4	0.086	1.732	0.017	1.77	17.68
6	0.108	2.176	0.022	2.23	22.29
8	0.131	2.639	0.026	2.71	27.15
10	0.169	3.404	0.034	3.50	35.07
12	0.191	3.847	0.039	3.98	39.83
16	0.226	4.553	0.046	4.73	47.26
20	0.246	4.955	0.050	5.17	51.75
24	0.268	5.399	0.054	5.67	56.68

Table No.10: *In vitro* release data of hesperidin niosomes for formulation ES-2

Time	Absorbance (253nm)	Concentration in mcg/ml	Amount release in mg/ml	Cumulative amount released in mg	Cumulative % drug release
0	0.0000	0.000	0.000	0.00	0.00
0.5	0.0510	1.038	0.010	2.60	5.19
1	0.0789	1.606	0.016	4.03	8.03
2	0.1170	2.382	0.024	5.98	11.95
4	0.1990	4.051	0.041	10.18	20.37
6	0.2850	5.801	0.058	14.59	29.14
8	0.3210	6.534	0.065	16.48	32.99
10	0.4210	8.570	0.086	21.64	43.29
12	0.4650	9.465	0.095	23.96	47.94
16	0.5090	10.361	0.104	26.30	52.60
20	0.5460	11.114	0.111	28.28	56.59
24	0.5980	12.172	0.122	31.04	62.47

Table No.11: *In vitro* release data of hesperidin niosomes for formulation ES-3

Time	Absorbance (253nm)	Concentration in mcg/ml	Amount release in mg/ml	Cumulative amount released in mg	Cumulative % drug release
0	0.0000	0.000	0.000	0.00	0.00
0.5	0.0545	1.109	0.011	2.77	5.56
1	0.0694	1.413	0.014	3.54	7.08
2	0.1080	2.198	0.022	5.52	11.08
4	0.1870	3.806	0.038	9.56	19.15
6	0.2931	5.966	0.060	15.00	30.10
8	0.3607	7.342	0.073	18.50	37.05
10	0.4331	8.817	0.088	22.26	44.12
12	0.4690	9.547	0.095	24.17	48.37
16	0.5350	10.890	0.109	27.63	55.26
20	0.5910	12.030	0.120	30.59	61.19
24	0.6760	13.760	0.138	35.03	70.08

Table No.12: *In vitro* release data of pure hespseridin drug

Time	Absorbance (253nm)	Concentration in mcg/ml	Amount release in mg/ml	Cumulative amount released in mg	Cumulative % drug release
0	0.0000	0.000	0.000	0.00	0.00
0.15	0.2320	4.722	0.047	11.81	23.62
0.3	0.3370	6.860	0.069	17.20	34.36
1	0.4230	8.610	0.086	21.64	43.27
1.3	0.5620	11.440	0.114	28.80	57.61
2	0.6990	14.228	0.142	35.89	71.75
2.3	0.8010	16.305	0.163	41.22	82.42
3	0.9200	18.727	0.187	47.44	94.86

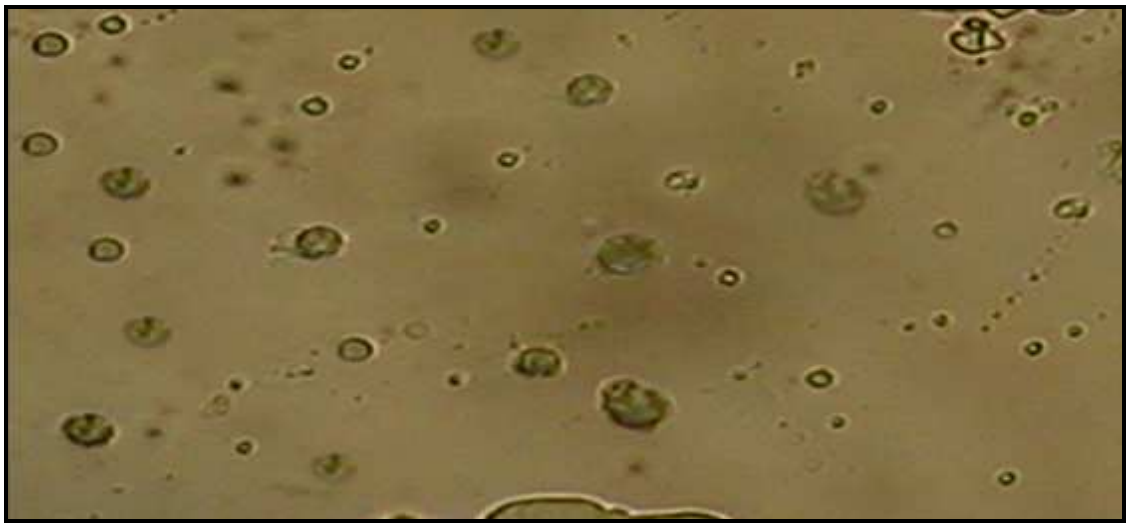

Figure No.1: Optical microscopic view of hesperidin loaded niosomes prepared by Hand Shaking Method for formulation HS 3 (20 x 40X)

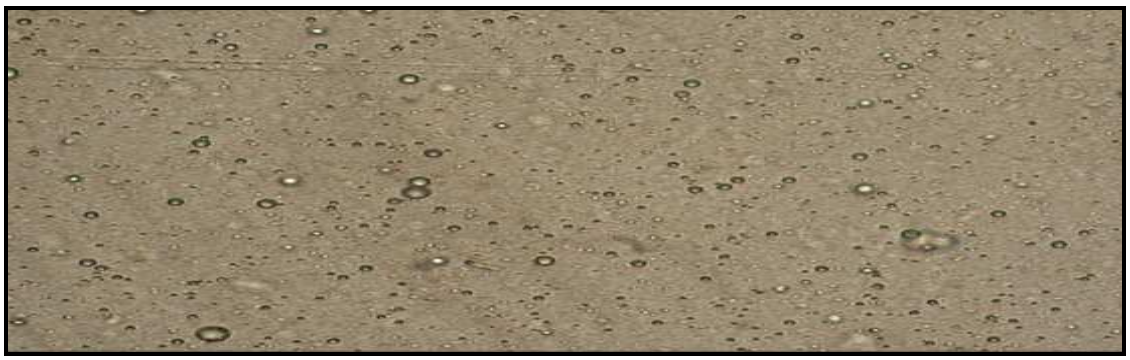

Figure No.2: Optical microscopic view of hesperidin loaded niosomes prepared by Ether Injection Method for formulation ES 3 (20 x 40X)

Figure No.3: Scanning electron microscopic view of hesperidin loaded niosomes by Hand Shaking Method for formulation HS 3 (400 X)

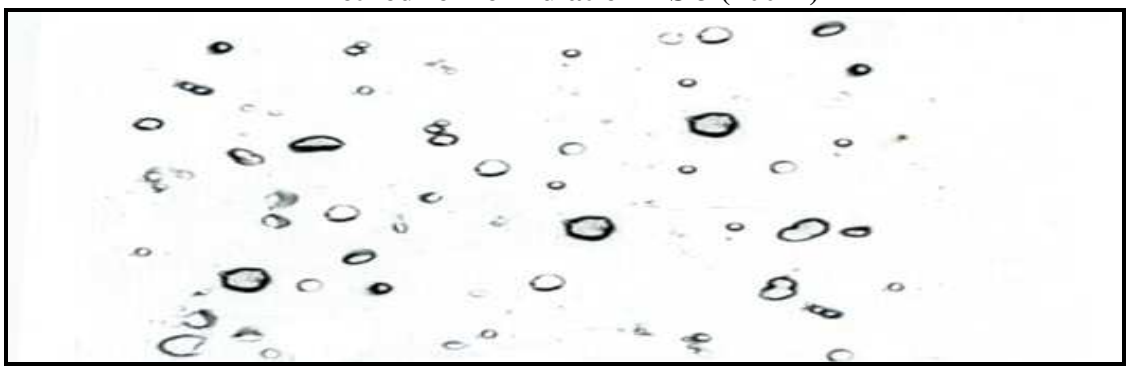

Figure No.4: Scanning electron microscopic view of hesperidin loaded niosomes by Ether Injection Method for formulation ES 3 (300 X)

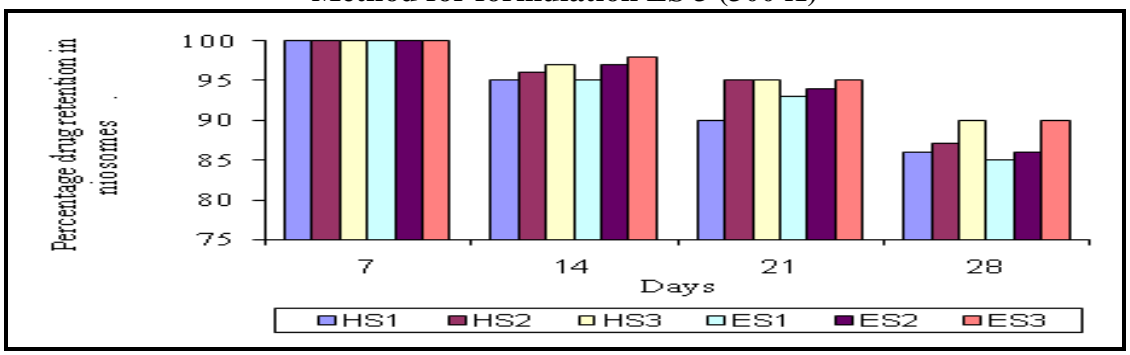

Figure No.5: Comparative bar diagram for drug leakage studies at refrigeration temperature

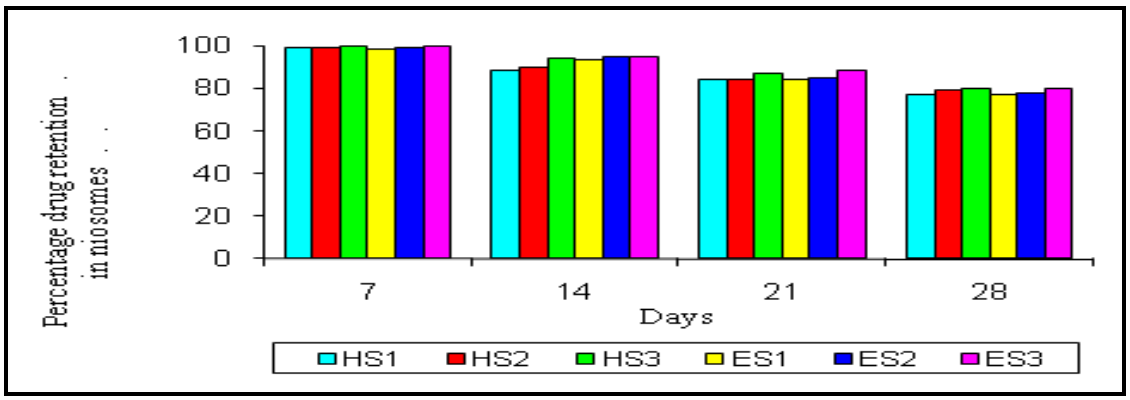

Figure No.6: Comparative bar diagram for drug leakage studies at room temperature

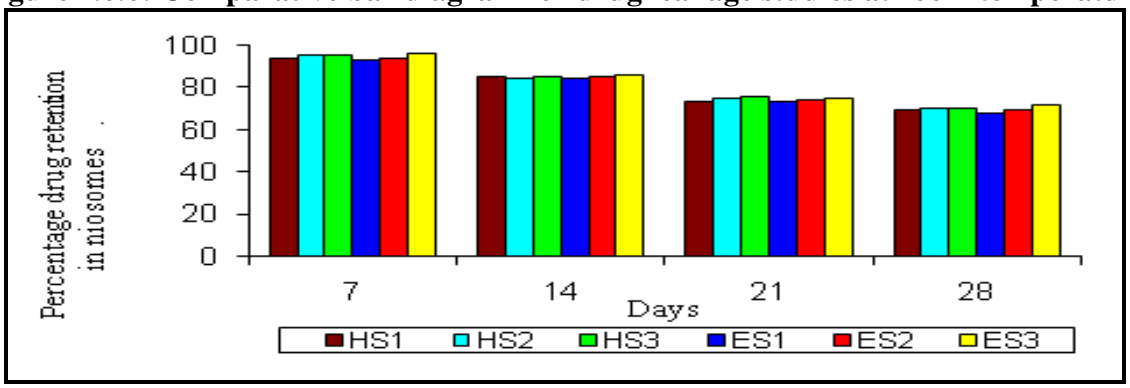

Figure No.7: Comparative bar diagram for drug leakage studies at high temperature

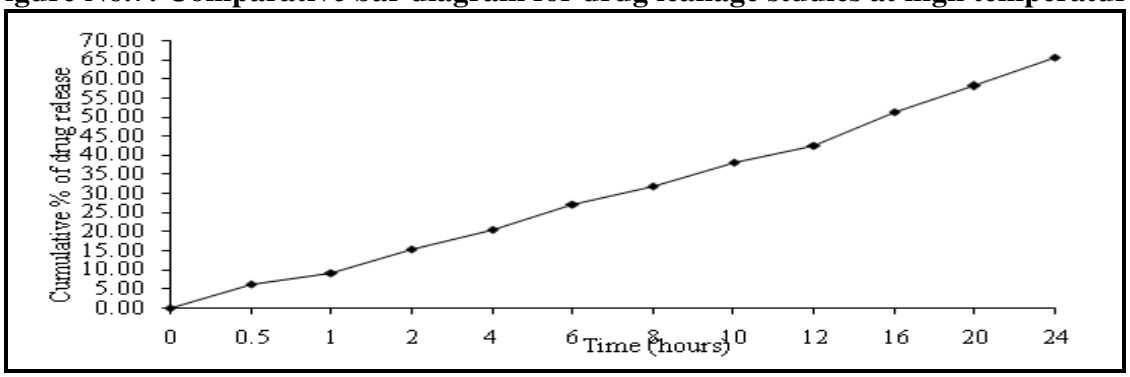

Figure No.8: *In vitro* release profile for hesperidin niosomes for formulation HS-1

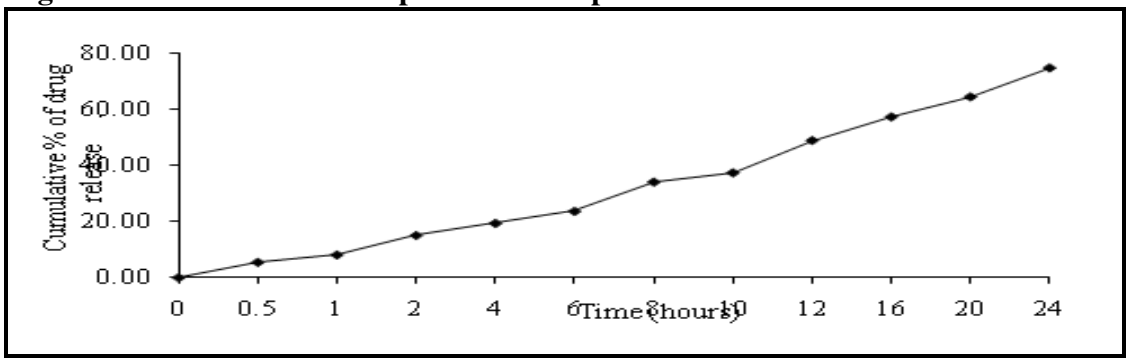

Figure No.9: *In vitro* release profile of hesperidin niosomes for formulation HS-2

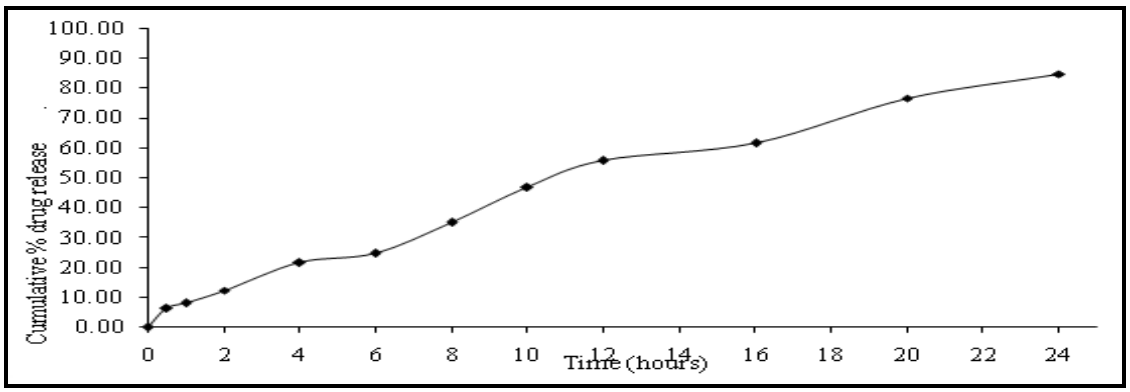

Figure No.10: *In vitro* release profile of hesperidin niosomes for formulation HS-3

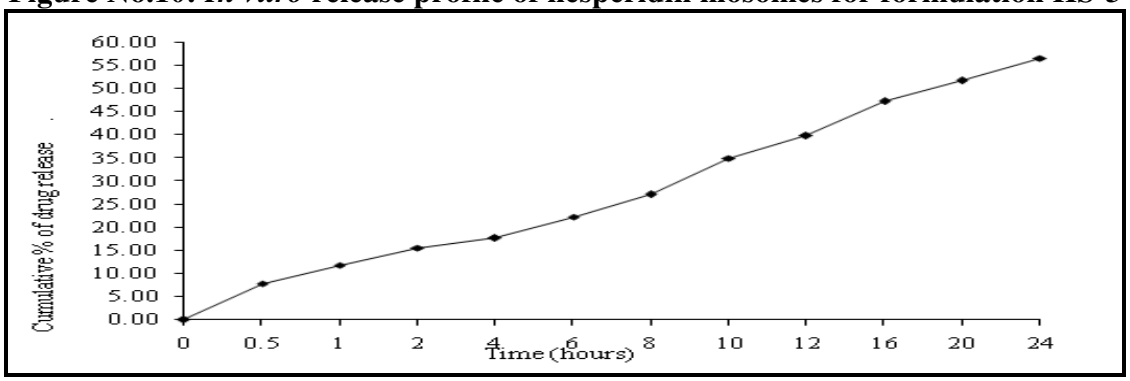

Figure No.11: *In vitro* release profile of hesperidin niosomes for formulation ES-1

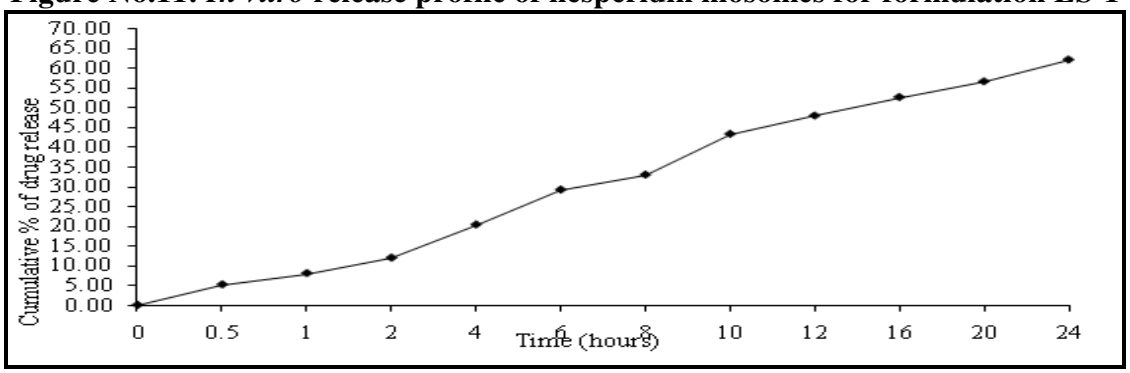

Figure No.12: *In vitro* release profile of hesperidin niosomes for formulation ES-2

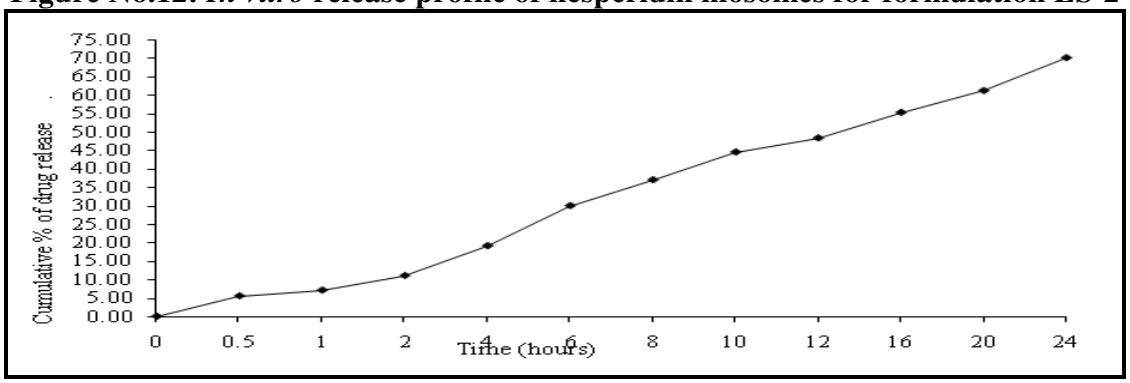

Figure No.13: *In vitro* release profile of hesperidin niosomes for formulation ES-3

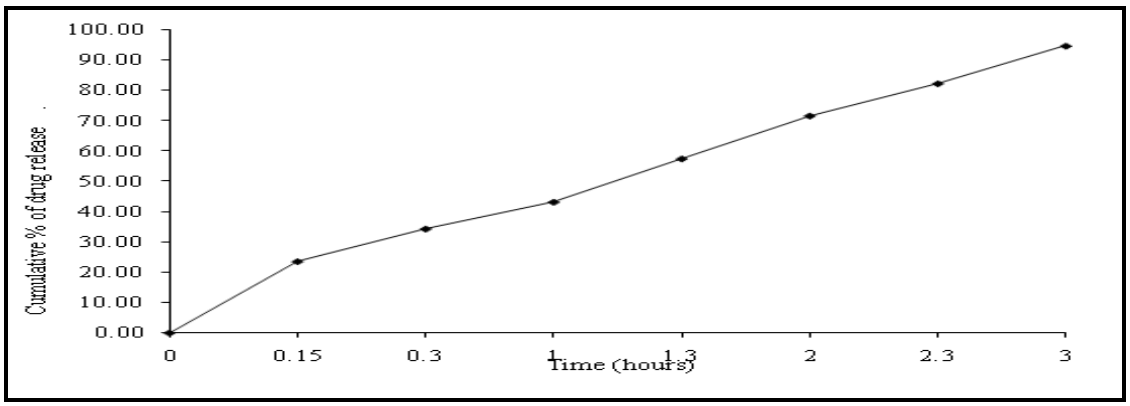

Figure No.14: *In vitro* release profile of pure hesperidin drug

SUMMARY AND CONCLUSION

- Stable Hesperidin loaded Niosomes can be prepared by hand shaking method and ether injection method with Span 80 and cholesterol in the ratio of 1:1, 2:1, and 3:1.
- Preformulation study and drug excipients compatibility study was done initially and results directed the further course of formulation.
- Most of the vesicles are spherical in shape, the size range of the vesicles, fall in the narrow size range of 0.5-5µ and 0.5-2.5µ by hand shaking method and ether injection method respectively.
- A high % of Hesperidin can be encapsulated in the vesicles (75-84%) prepared by hand shaking method.
- Concentration of non-ionic surfactant such as Span 80 might influence the drug release pattern of all formulation.
- In vitro release of Hesperidin from niosomes was very slow when compared to the release from pure Hesperidin solution.
- Drug release studies showed that the niosomal preparation was stable at refrigeration temperature (4^{o}C).
- The vesicles prepared by hand shaking method were found to be larger in size as compared to vesicles prepared by ether injection method.
- From above these studies it was concluded that Hesperidin was successfully encapsulated into niosomes, Span 80 (1:1:3) vesicles prepared by hand shaking method showed best result in terms of encapsulation efficiency, in vitro drug release and to enhance the therapeutic effectiveness of Hesperidin, producing prolonged activity and simultaneously minimizing the side effects.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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