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FORMULATION AND COMPARITIVE OPTIMIZATION OF MULTIPLE LIPID DRUG CARRIERS OF VALSARTAN FOR ORAL CONTROLLED RELEASE

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ABSTRACT

In the present study, an attempt was made to formulate, comparatively evaluate and optimize multiple lipid drug carriers of valsartan for oral controlled release. Two lipid drug delivery systems i.e. Niosomes and Liposomes were studied for the delivery of the anti-hypertensive drug valsartan. They were formulated as suspensions.Ether injection and rotary evaporator method were chosen for the formulation of physically and chemically stable niosomes and liposomes of valsartan.In-vitro evaluation studies for the prepared multiple drug delivery carriers of valsartan were performed. The in-vitro evaluation studies performed were evaluation for physical appearance, particle size by scanning electron microscopy (SEM), drug content , entrapment efficiency and in-vitro drug release. Optimization of the best lipid drug delivery system and the best method of preparation was done based on the results of *In-Vitro* drug release and entrapment efficiency values. Finally, an attempt was made to improve the bioavailabilty of the administered drug, reduce side effects and improve patient compliance by optimizing the best formulation through lipid drug delivery technology.

Keywords: Niosomes, Liposomes, Valsartan, Cholesterol, Lecithin.

INTRODUCTION

Valsartan belongs to angiotensin II antagonist category and is used as a choice for patients with heart failure who are unable to tolerate angiotensin converting enzyme (ACE) inhibitors in the management of hypertension.Valsartan undergoes extensive first-pass metabolism. To enhance the aqueous solubility, dissolution rate, to by-pass hepatic first-pass metabolism and to improve the oral bioavailability of valsartan there is a need to develop lipid drug delivery system for valsartan like liposomes, niosomes, solid lipid nanoparticles etc.

Niosomes aremicroscopic lamellar structures of size range between 10 to1000 nm and consists of biodegradable, non-immunogenic and biocompatible surfactants[1]. Niosomes or nonionic surfactant vesicles are microscopic lamellar structures formed on admixture of nonionic surfactant of the alkyl or dialkylpolyglycerol ether class and cholesterol with subsequent hydration in aqueous media[2]. Structurally, liposomes are concentricbleedervesiclesin which an aqueous volumeis entirely enclosed by a membraneous lipid bilayer.Membranes are usually made of phospholipids,which are molecules that have a hydrophilic headgroup and a hydrophobic tail group[3].

MATERIALS AND METHODS

Materials Used: Valsartan was obtained from Dr. Reddy Laboratories Ltd Hyderabad. Cholesterol was purchased from SD Fine Chemicals Ltd Mumbai. Span 20 was obtained from Rolex Chemical Industries Mumbai. Methanol, Diethyl ether, Chloroform, Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Sodium Chloride were obtained from SD Fine Chemicals Ltd Mumbai. Lecithin was purchased from Hi Media Lab Pvt Ltd Mumbai.

Instruments Used: Rotary Evaporator, Magnetic Stirrers, Electronic Balance, Cooling Centrifuge, UV

Visible Spectrophotometer, Fourier Transform Infra Red, Scanning Electron Microscope

METHODOLOGY OF THE CURRENT STUDY

Formulation of Niosomes by Ether Injection Method: Niosomes containing valsartan were prepared by modified ether injection technique using nonionic surfactant (span 20) and cholesterol at different concentrations. Cholesterol and surfactant were dissolved in diethyl ether mixed with methanol containing weighed quantity of valsartan. The resulting solution was slowly injected using a 24 gauge needle at a rate of 1ml/min into of hydrating solution phosphate buffer (pH 7.4).The solution was stirred continuously on magnetic stirrer and temperature was maintained at 60-65ºC. As the lipid solution was injected slowly into aqueous phase, the differences in temperature between phases cause rapid vaporization of ether, resulting in spontaneous vesiculation and formation of niosomes. Different batches of niosomes were prepared[4].

Formulation of Niosomes by Rotary Evaporator Method: In this method cholesterol and span 20 were dissolved in chloroform, and the drug valsartan was dissolved in methanol. Both drug and cholesterol mixture were mixed to obtain the lipid phase. The lipid phase was taken in the round bottom flask of rotary evaporator and the organic solvent was evaporated at room temperature, using rotary vacuum evaporator at room temperature under application of vaccum. The thin layer of lipid phase was formed on the round bottom flask. The aqueous phase phosphate buffer solution pH 7.4 was added to the round bottom flask at 60-65^{0}Cand shaken till suspension of niosomes is obtained.

Formulation of Liposomes by Ether Injection Method: The same procedure was employed as used in niosomes but lecithin was used instead of surfactant. Different batches of liposomes were prepared.

Formulation of Liposomes by Rotary Evaporator Method: The same procedure was employed as used in niosomes but lecithin was used instead of surfactant. Different batches of liposomes were prepared.

EVALUATION OF FORMULATIONS

Physical Appearance: The formulations were analysed for their physical appearance.

Particle Size &Shape Analysis By SEM: The surface morphology of the prepared formulations was examined under by using Scanning electron microscope (SEM) (Jeol, JSM-6360, Japan)[5].

Estimation of Drug Content: Formulation equivalent to 40mg of valsartan was taken into a standard volumetric flask. Then they were lysed with100ml of propane-1-ol by shaking. Then 5ml of this was subsequently diluted with phosphate buffer (pH 7.4). The absorbance was measured at 250 nm and drug content was calculated from the calibration curve .

Determination of Entrapment Efficiency: Percent entrapment efficiency (EE) was determined by centrifugal method. formulation was centrifuged (18000 rpm) for 40 min at 5^{o}C in order to separate unentrapped drug. The supernatant was taken and diluted with PBS P^{H}7.4. The drug concentration in the resulting solution was assayed spectrophotometrically at 250 nm.

In-Vitro **Drug Release Studies:** The release of valsartan from niosomal formulations was determined by using membrane diffusion technique. The niosomal formulation equivalent to 40 mg of valsartan was placed in a glass tube of diameter 2.5 cm with an effective length of 8 cm which was tied with previously soaked cellulose membrane, which acts as a donor compartment. The glass tube was placed in a beaker containing 100 ml of phosphate buffer (pH 7.4), acting as a receptor compartment. The whole assembly was fixed in such a way that the lower end of tube containing suspension was just touching (1-2 mm depth) the surface of diffusion medium. The temperature of receptor medium was maintained at 37 ± 5^{0}C and was agitated at the speed of 100 rpm using magnetic stirrer. Aliquots of 5ml sample were withdrawn periodically and after each withdrawal same volume of medium was replaced. The collected samples were analyzed at 250 nm in double beam UV-VIS spectrophotometer using Phosphate Buffer (pH 7.4) as blank [6].

Optimization Procedure: Among the 20 formulations prepared optimization of the best formulation and the best method of preparation was done based on the *In-vitro* drug release and EE % results.

Drug Release Kinetic Studies: To study the release kinetics, data obtained from *in-vitro* release were plotted in various kinetic models.

RESULTS

CHARACTERIZATION STUDIES FOR PREPARED FORMULATIONS

Physical Appearance: The Niosomal and Liposomal formulations prepared were evaluated for physical appearance and found to be white suspensions in appearance.

Particle Size and Shape Analysis by SEM: The particle sizes of prepared formulations were analyzed using Scanning Electron Microscopy (SEM). The particle size was found to be in micrometers range. The SEM image is shown in the below figure.

Estimation of Drug Content: The % drug content values for the prepared formulations were calculated.

Determination of Entrapment Efficiency: The % EE values of the prepared formulations were calculated.

***In-Vitro* Drug Release Studies:** The % CDR values of the prepared formulations were calculated. The zero order plots of the prepared formulations are shown in the below figures.

Drug Release Kinetics: The drug release kinetic data of the formulations were determined.

DISCUSSION

CHARACTERIZATION STUDIES FOR PREPARED FORMULATIONS

Physical Appearance: The Niosomal and Liposomal formulations prepared were white suspensions.

Particle Size and Shape Analysis by SEM: The SEM reports of NE 3 showed uniform size and nearly spherical shape and results of all the other formulations were satisfactory.

Estimation of Drug Content: The results revealed that all the formulations showed satisfactory drug content. Formulation NE3 showed the highest drug content.

Determination of Entrapment Efficiency: The results revealed that all the formulations showed good entrapment efficiency values while % EE values for formulations**NE3, NR3, LE3, LR3** were found to be appreciating than other formulations. NE3 formulation showed the highest % EE value.

***In-Vitro* Drug Release Studies:** For all the 20 formulations there was no initial burst release but the release was constant in a controlled manner for a period of time upto 48 hrs. The best formulation is the one which provides good morphology (size and shape), high drug content, entrapment efficiency and controlled and prolonged drug release. Formulation NE 3 was considered to be the best formulation as the drug content, entrapment efficiency and the percent drug release were high for NE 3. This is the niosomal formulation containing comparatively high amount of surfactant prepared by ether injection method. The results of *in-vitro* drug release revealed that the drug was released in a controlled manner from all the formulations and NE 3 showed maximum drug release at the end of 48^{th} hour. Hence from all the above results of morphology, drug content, entrapment efficiency and *in-vitro* drug release studies, it is proved that formulation NE 3 is the best and optimized formulation.

Drug Release Kinetics: The order of drug release was found to be zero order, in which the regression value was found to be close to 1. From the Higuchi model shows linear regression and it can be found that the release follows diffusion kinetics mechanism. The n value of Peppa's equation was found to be greater than 1. From this it is concluded that the drug release follows super case II transport with zero order release. The results were tabulated below.

CONCLUSION

From all the above parameters and results, it was concluded that niosomes of valsartan prepared by ether injection method, Formulation code **NE 3** has shown a promising formula for delivering the drug by which the bioavailability of the drug can be improved, side effects can be reduced,first pass hepatic metabolism of the drug can be avoided and finally the patient compliance can be improved.

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Table No: 1–Formulation table for Niosomes

S No	Ratio of Drug:Surfactant:Cholesterol	Drug (mg)	Surfactant(mg) Span 20	Cholesterol(mg)
1	1:1:1	40	40	40
2	1:2:1	40	80	40
3	1:3:1	40	120	40
4	1:1:2	40	40	80
5	1:1:3	40	40	120

Table No: 2 Formulation table for Liposomes

S No	Ratio of Drug:Lecithin:Cholesterol	Drug (mg)	Lecithin (mg)	Cholesterol(mg)
1	1:1:1	40	40	40
2	1:2:1	40	80	40
3	1:3:1	40	120	40
4	1:1:2	40	40	80
5	1:1:3	40	40	120

Table No: 3 Results for drug content estimation of niosomes prepared by ether injection method

S No	Amount of Drug (mg)	% Drug Content (±SD) n=3
NE1	39.423	98.557± 0.557
NE2	39.166	97.915± 0.333
NE3	39.935	99.837± 0.0700
NE4	39.358	98.395± 0.079
NE5	39.102	97.755± 0.575

Table No: 4 - Results for drug content estimation of niosomes prepared by rotary evaporator method

S No	Amount of Drug (mg)	% Drug Content (±SD) n=3
NR1	39.743	99.357 ± 0.566
NR2	39.423	98.557± 0.901
NR3	39.935	99.837± 0.747
NR4	39.807	99.517± 0.588
NR5	39.743	99.357± 0.514

Table No: 5 - Results for drug content estimation of liposomes prepared by ether injection method

S No	Amount of Drug (mg)	% Drug Content (±SD)n=3
LE1	39.358	98.395 ± 0.456
LE2	39.166	97.915 ± 0.432
LE3	39.230	98.075 ± 0.243
LE4	39.423	98.5575 ± 0.215
LE5	39.615	99.0375 ± 0.213

Table No: 6 - Results for drug content estimation of liposomes prepared rotary evaporator method

S No	Amount of Drug (mg)	% Drug Content (±SD)n=3
LR1	39.166	97.915 ± 0.245
LR2	39.294	98.235 ± 0.434
LR3	39.743	99.3575 ± 0.342
LR4	39.166	97.915 ± 0.321
LR5	39.230	98.075 ± 0.326

Table No: 7 - Results for entrapment efficiency of niosomes prepared ether injection method

S No	Amount of Drug entrapped(mg) for 10mg equivalent preparation	% EE (±SD) n=3
NE1	9.4871	94.871± 0.658
NE2	9.615	96.15± 0.684
NE3	9.7435	97.435± 0.0113
NE4	9.10256	91.0256± 0.349
NE5	9.2307	92.307± 0.0624

Table No: 8 - Results for entrapment efficiency of niosomes prepared rotary evaporator method

S No	Amount of Drug entrapped(mg) for 10mg equivalent preparation	% EE (±SD) n=3
NR1	9.35	93.5± 0.598
NR2	9.48	94.8± 0.4909
NR3	9.615	96.15± 0.167
NR4	8.974	89.74± 0.296
NR5	9.0384	90.384± 0.265

Table No: 9- Results for entrapment efficiency of liposomes prepared ether injection method

S No	Amount of Drug entrapped(mg) for 10mg equivalent preparation	% EE (±SD) n=3
LE1	9.10256	91.0256 ± 0.134
LE2	9.29487	92.9487 ± 0.231
LE3	9.4871	94.871 ± 0.145
LE4	9.1666	91.666 ± 0.121
LE5	9.29487	92.9487 ± 0.132

Table No: 10 - Results for entrapment efficiency of liposomes prepared rotary evaporator method

S No	Amount of Drug entrapped(mg) for 10mg equivalent preparation	% EE (±SD) n=3
LR1	8.9743	89.743 ±0.342
LR2	9.1666	91.666 ±0.254
LR3	9.42307	94.2307 ±0.234
LR4	9.10256	91.0256 ±0.247
LR5	9.23076	92.3076 ±0.143

Table No 11 –Invitro release data for NE1, NE2, NE3, NE4 and NE5

Time (Hr)	**% CDR NE1**	**% CDR NE2**	**% CDR NE3**	**% CDR NE4**	**% CDR NE5**
0	0	0	0	0	0
2	4.45	6.45	6.45	4.22	3.22
4	8.34	9.56	9.56	7.23	6.23
6	10.11	12.11	15.23	10.25	9.25
8	14.43	16.34	18.25	14.43	12.43
12	24.21	24.5	26.95	22.43	19.55
16	32.23	34.16	34.11	30.23	26.65
20	39.55	40.11	42.56	36.55	33.43
24	47.78	48.2	50.34	43.56	42.03
28	53.66	58.11	60.12	51.4	47.85
32	62.45	64.78	68.45	60.44	54.9
36	69.76	72.78	75.64	68.05	60.23
40	77.74	79.5	83.54	74.43	68.43
44	83.5	87.67	92.36	82.54	75.43
48	91.54	94.67	98.55	88.21	82.25

Table 12 –Invitro release data for NR1, NR2, NR3, NR4 and NR5

Time (Hr)	% CDR NR1	% CDR NR2	% CDR NR3	% CDR NR4	% CDR NR5
0	0	0	0	0	0
2	3.45	5.25	3.45	4.45	4.22
4	7.55	8.25	8.64	7.54	7.23
6	9.95	11.54	11.43	9.65	10.25
8	13.56	15.11	14.56	12.53	13.43
12	21.11	23.34	23.43	19.96	20.55
16	28.24	29.45	30.54	26.74	27.65
20	34.44	37.26	37.78	33.63	33.43
24	42.05	43.65	47.53	40.84	42.03
28	49.45	52.25	53.42	48.52	48.85
32	56.67	58.52	62.65	54.75	55.9
36	63.45	66.75	69.56	61.74	61.23
40	71.65	73.67	77.87	68.54	68.43
44	78.55	80.65	86.56	76.14	75.43
48	85.65	89.67	94.55	86.21	83.25

Table 13 –Invitro release data for LE1, LE2, LE3, LE4 and LE5

Time (Hr)	% CDR LE1	% CDR LE2	% CDR LE3	% CDR LE4	% CDR LE5
0	0	0	0	0	0
2	4.25	3.45	4.24	4.24	3.73
4	6.46	6.15	8.54	6.99	6.87
6	9.65	9.54	12.86	11.25	9.94
8	13.53	12.45	16.55	15.63	13.83
12	20.84	20.46	22.45	21.79	20.84
16	27.82	27.85	29.45	28.57	27.85
20	35.15	33.84	36.83	35.78	33.93
24	42.94	42.54	45.43	42.69	42.03
28	49.64	48.95	52.55	49.34	47.75
32	56.15	55.74	59.63	57.28	55.99
36	62.26	64.54	68.64	63.15	61.93
40	69.56	71.65	75.22	69.96	69.45
44	76.65	78.64	83.34	76.64	75.83
48	84.54	86.77	89.65	85.11	82.25

Table 14–Invitro release data for LR1, LR2, LR3, LR4 and LR5

Time (Hr)	% CDR LR1	% CDR LR2	% CDR LR3	% CDR LR4	% CDR LR5
0	0	0	0	0	0
2	2.43	4.44	4.45	4.34	4.32
4	6.35	6.94	8.94	6.96	7.63
6	10.42	9.95	11.43	10.16	10.28
8	13.46	12.94	14.56	13.72	13.33
12	19.64	20.89	22.23	20.88	20.35
16	26.75	29.54	31.54	27.46	27.35
20	32.83	35.44	38.98	33.42	33.23
24	39.94	41.34	46.93	40.88	42.03
28	46.93	49.26	54.82	49.24	48.45
32	54.98	55.83	62.95	54.82	55.9
36	60.93	63.45	70.26	62.34	61.23
40	68.84	70.34	77.97	69.34	68.23
44	75.76	77.57	85.96	75.75	75.73
48	83.74	87.97	90.55	84.11	82.15

Table No: 15 - Regression Coefficient values for Niosomal formulations prepared by Ether Injection Method

S No	Zero Order	Higuchi	Crossmeyer	Peppas	Peppas slope value
CODE	R^2	R^2	R^2		**n value**
NE1	0.9989	0.8698	0.9231		1.2037
NE2	0.9985	0.8903	0.8362		1.2213
NE3	0.9982	0.8903	0.8155		1.2383
NE4	0.9994	0.8745	0.9387		1.1895
NE5	0.9993	0.8671	0.9731		1.1589

Table No: 16 - Regression Coefficient values for Niosomal formulations prepared by Rotary Evaporator Method

S No	Zero Order	Higuchi	Crossmeyer Peppas	Peppas slope value
CODE	R^2	R^2	R^2	**n value**
NR1	0.9997	0.8489	0.9555	1.1743
NR2	0.9994	0.8818	0.8875	1.1938
NR3	0.9994	0.8717	0.9433	1.2036
NR4	0.9984	0.8649	0.9325	1.1661
NR5	0.9996	0.8784	0.9357	1.1684

Table No: 17- Regression Coefficient values for Liposomal formulations prepared by Ether Injection Method

S No	Zero Order	Higuchi	Crossmeyer Peppas	Peppas slope value
CODE	R^2	R^2	R^2	**n value**
LE1	0.9996	0.8595	0.9448	1.171
LE2	0.9988	0.8616	0.9697	1.1692
LE3	0.9993	0.8834	0.9076	1.199
LE4	0.9994	0.8855	0.6442	1.1375
LE5	0.9998	0.8754	0.9518	1.168

Table No: 18- Regression Coefficient values for Liposomal formulations prepared by Rotary Evaporator Method

S No	Zero Order	Higuchi	Crossmeyer Peppas	Peppas slope value
CODE	R^2	R^2	R^2	**n value**
LR1	0.9991	0.848	0.9781	1.1597
LR2	0.9986	0.8418	0.9365	1.1744
LR3	0.9991	0.8807	0.9147	1.205
LR4	0.9995	0.8746	0.935	1.1688
LR5	0.9995	0.8795	0.9288	1.1679

Fig No: 1 Photographs of the Prepared Formulations

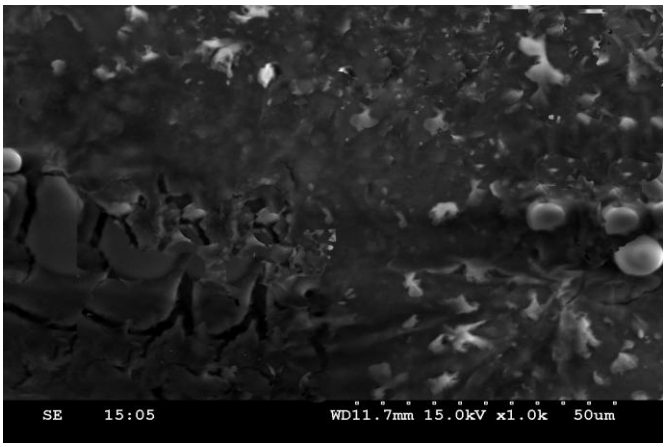

Fig No 2: SEM Reports for NE 3 formulation

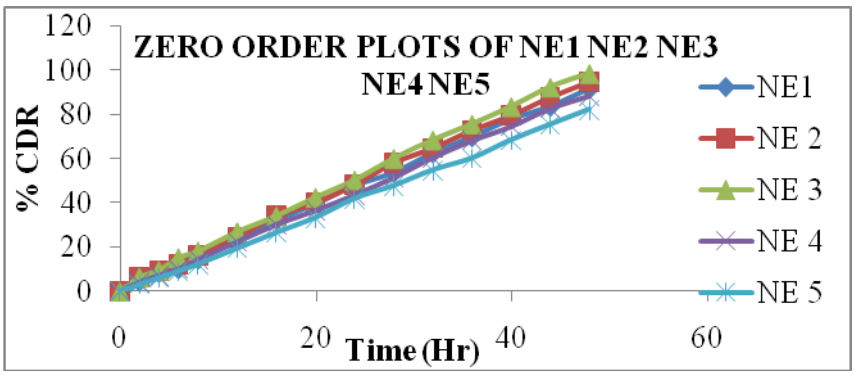

Fig No 3: Zero order plots of NE 1, NE 2, NE 3, NE 4 and NE 5

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