ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
www.ijptonline.com
Research Article

DEVELOPMENT AND CHARACTERIZATION OF PROLIPOSOMES OF PRAVASTATIN SODIUM

Gitanjali Parmar[#1], Rajni Bala[#1], Nimrata Seth[#1], Dr.Angshu Banerjee[#1]
Department of Pharmaceutics, Rayat Institute of Pharmacy, Railmajra, SBS Nagar, Punjab, India.
Email: parmargitanjali@gmail.com

Received on 05-11-2015 *Accepted on 25-11-2015*

Abstract

The present systematic study focused to investigate the advantage of proliposomes for improved orally delivery of pravastatin sodium. Sustained drug delivery of proliposomes Pravastatin sodium was prepared by using Film deposition method using Cholesterol, spray dried mannitol and phospholipon 90H in which different concentration of lipid used. The formulations were characterized for micromeritics properties, particle size analysis, % drug entrapment efficiency and % percentage yield, surface morphology (scanning electron microscopy). Further invitro release and dissolution study carried out to form sustained delivery system. The formulation prepared by using cholesterol, phospholipon 90H and mannitol emerged as the overall best formulation based upon their drug release characteristics (in phosphate buffer 6.8).

Key words: Pravastatin sodium, phospholipon 90H, spray dried mannitol, film deposition method.

Introduction

Oral drug delivery continues to be the preferred route of administration. The objective of this research is to develop new oral drug delivery system utilizing the concept of sustained release drug. In order to maintain the release of a drug at predetermined rate to maintain it's a constant concentration for specific period of time with minimum side effect. Proliposomes are defined as dry, free-flowing particles with a dispersed system that can immediately form a liposomal suspension when in contact with water. Compared with conventional liposomes, proliposomes exhibit more advantages in promoting drug absorption. Because of their solid properties, the physical stability of liposomes can be improved upon without influencing their intrinsic characteristics. Therefore, proliposomes would be a potential vehicle to help improve the oral absorption of hydrophilic[1, 2]. Pravastatin sodium is a hydrophilic drug clinically used to lower cholesterol and triglycerides in blood. The biological half life of pravastatin sodium is 1-2 hrs

and eliminated rapidly and its activity is lost in few hours. Due to its large systemic clearance in circulating blood because of enzymatic degradation in gastrointestinal (GIT) tract. Therefore, sustained release is needed for pravastatin sodium to give prolonged action and reduction of usage frequency[3]. Keeping this in view, the present systematic study was focused to combine the advantages of proliposomes and surface charge for improved oral delivery of pravastatin sodium. The pravastatin sodium loaded proliposomes (neutral, negative and positive) were prepared by film deposition method and characterized[4]. Subsequently the solid state characterization was done to ascertain the morphology, physical state and possible interaction between the formulations and ingredients.

Materials and Method

Pravastatin sodium was gift sample from Biomax laboratories, Karnal, India. Cholesterol and Mannitol were purchased from Central drug house Ltd. Phospholipon 90H, 90G, S100 were purchased from Finar Ltd. All reagents used were of analytical-reagent grade.

Preparation of Proliposomes

The film deposition method was used for the preparation of proliposome powders and the composition was represented table 1. In brief, accurately weighed amounts of lipid mixture at various molar ratios and drug were dissolved in solvent mixture containing chloroform and methanol (9:1). The resultant solution was transferred into a round bottomed flask, and different type of carrier was added to form slurry. The flask was attached to a rotary flash evaporator and the organic solvent was evaporated under reduced pressure at a temperature of 45 ± 2 ^{o}C. After ensuring the complete removal of solvent, the resultant powders were further dried overnight in a vacuum oven at room temperature so as to obtain dry, free-flowing product[5]. The obtained proliposome powders were sieved with a US 60 mesh screen and stored in a tightly closed container at 4^{0}C for further evaluation.

Table-1: Formulation of pravastatin sodium proliposome with different phospholipids and their concentrations

Formulation code	Drug (mg)	Phospholipids(mg)			Cholesterol (mg)	Mannitol (mg)
		90H	90G	S100		
F1	20	125			50	500
F2	20	250			50	500
F3	20	375			50	500

F4	20	500			50	500
F5	20		125		50	500
F6	20		250		50	500
F7	20		375		50	500
F8	20		500		50	500
F9	20			125	50	500
F10	20			250	50	500
F11	20			375	50	500
F12	20			500	50	500

Result and Discussion

Proliposomes were prepared and evaluated certain parameters to optimize the polymer and its concentration.

1. Micrometric properties with different phospholipids

On the basis of micromeritics properties of all the twelve formulations are shown in Table no. 2, which are evaluated for variables parameters such as angle of repose, Carr's compressibility index and Hausner's ratio. The Carr's compressibility index for formulation F1, F2, F3, F4, F5, F6, F7, F8 was found in the range of which indicates the excellent flow properties, formulations F9, F10, F11, F12 was found in the range of 12-16 which indicates good flow properties, all the formulation shown excellent to good flow properties, No single formulation shown poor flow properties[6-8]. So we optimized a formulation F2 for further parameters.

Table-2: Micrometric properties of Pravastatin sodium with different phospholipids concentration.

Formulation Code	Angle of Repose (θ) ±SD	Carr's Index (%)±SD	Hausner's ratio ±SD
F1	27±1.2	8.1±1.1	1.1±0.01
F2	22.2±0.4	6.3±0.9	1.02±0.02

F3	26.8±0.4	7.2±0.5	1.06±0.05
F4	29±0.1	9.5±0.6	1.11±0.02
F5	27.6±0.5	11.1±1.21	1.15±0.1
F6	28.4±0.9	9.5±1.11	1.13±0.04
F7	29.9±0.6	11.3±0.91	1.16±0.05
F8	26.5±0.4	12±0.53	1.17±0.1
F9	27±0.2	13.1±0.79	1.19±0.11
F10	25±0.7	12.9±1.31	1.18±0.13
F11	27.1±0.2	14.1±0.52	1.19±0.01
F12	25.6±0.4	15.2±0.67	1.2±0.05

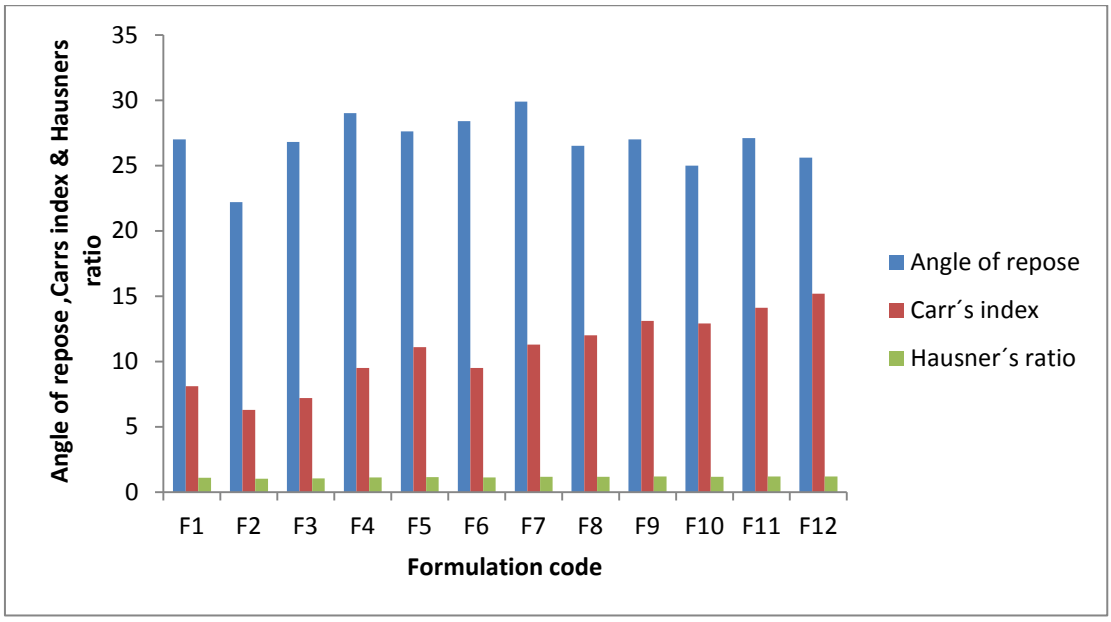

Fig. 1: Micrometric properties of Pravastatin sodium with different phospholipids concentration.

2. Percentage yield and % Drug Entrapment

On the basis of % Drug entrapment and % Yield, from the three phospholipids i.e. phopsholipon 90H, phopsholipon 90G and Lipoid S100, Phopsholipon 90H in different concentrations shown better result than other two phospholipids[9]. From the 4 concentrations of Phopsholipon 90H, F2 formulation with 250mg 90H, shows maximum percentage yield as well as percent entrapment i.e. 76.36 ±1.6 and 80.33 ± 1.3 respectively that shown in table 3 and in fig 2. This F2 formulation is further compared with the other optimization parameters like different cholesterol concentration, different carriers.

The selection of phospholipid is important because it dictate the stability of the liposomes formed. Since the risk of oxidation is high in phosphatidylcholine due to the presence of unsaturated bonds in the fatty acid tails, hydrogenated soyphosphatidylcholine which is in powder form was used in the formulations. The high phase transition temperature and solid state render more stability in GI fluids and augment the flow characteristics of the proliposomes, respectively, which is an important prerequisite for solid dosage forms.

Table-3: Percentage yield and % Drug Entrapment of Pravastatin sodium with different phospholipids concentration.

Formulation Code	% yield	% entrapment
F1	62.21 ±0.5	71.34 ± 0.2
F2	76.36 ±1.6	80.33 ± 1.3
F3	65.71 ± 1.74	76.39 ± 2.57
F4	60.18 ± 1.39	69.1 ± 1.94
F5	53.15 ± 0.83	56.13 ± 0.78
F6	51.27 ± 0.52	58.42 ± 0.83
F7	49.13 ± 0.91	57.23 ± 0.75
F8	48.02 ± 1.01	55.31 ± 0.82
F9	36.43 ± 1.32	47.2 ± 1.04
F10	37.29 ± 0.81	49.13 ± 0.59
F11	34.91 ± 0.49	48.39 ± 0.39
F12	35.18 ± 0.1	44.92 ± 1.23

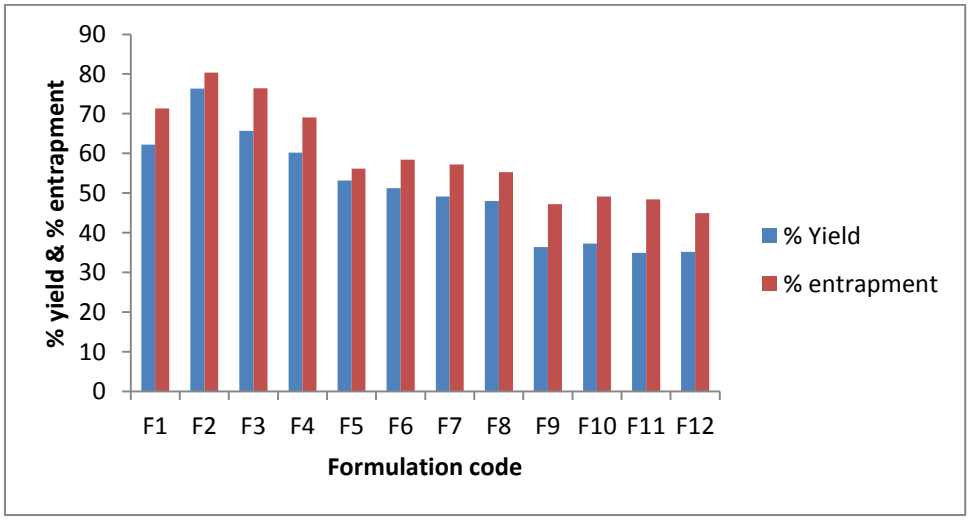

Fig. 2: Percentage yield and % Drug Entrapment of Pravastatin sodium with different phospholipids concentration.

3. Particle size analysis (Zeta sizer)

Based on the results of the practical yield and entrapment efficiency, the formulation F2 was selected and evaluated for particle size analysis[9]. The particle size was determined by using Malvern zetasizer. It was found that the particle size of drug was reduced by formulating the proliposomes. The results are shown in table 4.

Table-4: Average particle size of proliposomes.

Formulation code	Average particle size
F2	1257

The particle size distribution of formulation F2 was shown in figure 3.

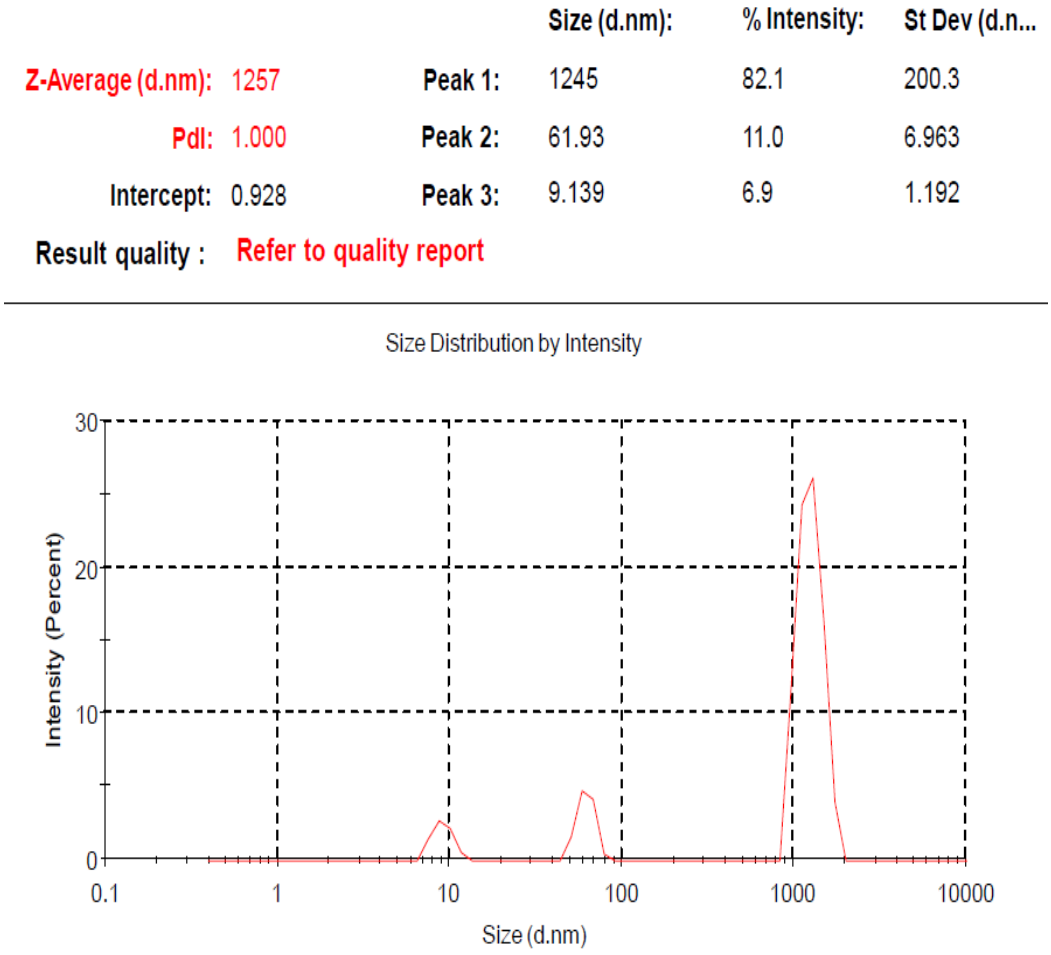

Fig. 3: Particle size of Formulation F2 proliposomes.

4. Scanning electron microscopy (SEM)

The Scanning electron microscopy (SEM) was used to determine the shape and surface morphology of microspheres[9]. The surface morphology of the pure drug, mannitol, and proliposome powders was investigated by scanning electron microscope (SEM). The surface morphology was performed by SEM analysis which showed the spherical shaped, discrete particles without aggregation with smooth texture in surface and the results are shown in figure 4.

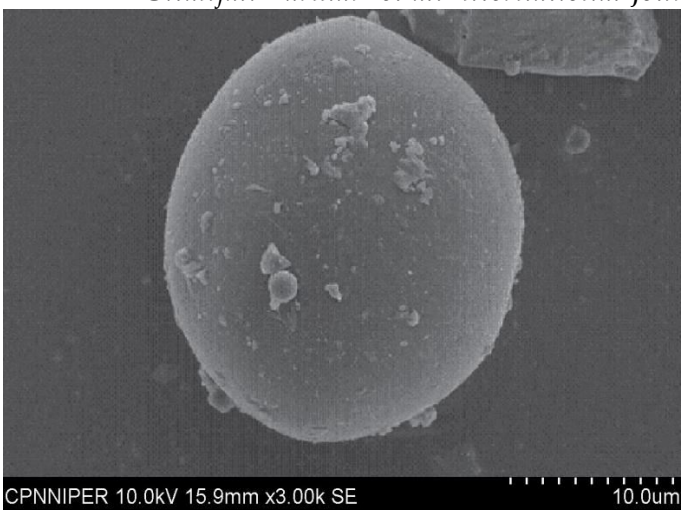

Fig. 4: Scanning Electron Microscopy of Pravastatin sodium proliposome (F2).

5. In vitro drug release study

The *in-vitro* drug release study of Pravastatin sodium proliposomes was performed for F2. The release behavior of drug from the polymer followed sustained release. The formulation F2 showed the release of 80.09% respectively in pH 6.8 at the end of 10th hr. From this release profile, it was observed that the formulation F2 gave maximum drug release. The cumulative % drug released from formulation F2 is shown in the table 5 and figure 5.

Table-5: *In-vitro* Drug Release Data of proliposome.

Time (hrs)	Cumulative % drug release (Drug)	Cumulative % drug release (F2)
0	0	0
1	25.47±0.02	16.39±0.02
2	48.93±0.02	34.63±0.04
3	76.32±0.03	41.03±0.03
4	95.59±0.04	46.84±0.01
5	100.02±0.01	52.96±0.02
6	99.87±0.03	55.35±0.01
7	99.75±0.04	60.08±0.04
8	99.64±0.02	66.37±0.02
9	99.32±0.02	72.84±0.03
10	99.03±0.03	80.09±0.01

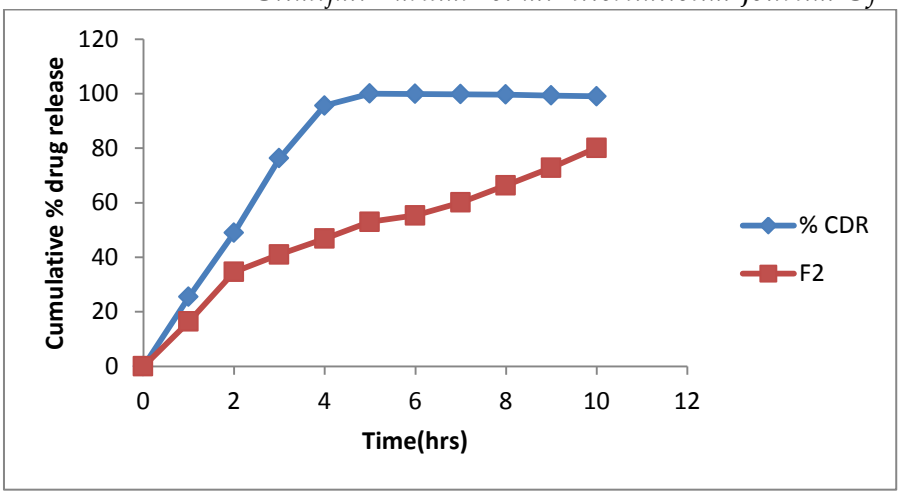

Fig 5: Drug Release of Formulation

6. Drug release kinetics

The release kinetic was studied by various kinetic models as zero order plot, first order plot, higuchi plot and Kosmeyer- Peppas. In order to identify a particular release mechanism, experimental data of statistical significance are compared to a solution of the theoretical model. It is therefore clear that only a combination of accurate and precise data with models accurately depicting the physical situation will provide an insight into the actual mechanism of release. To analyses the mechanism for the drug release and drug release rate kinetics of the dosage form, the data obtained was fitted into zero order, first order, higuchi matrix, and kosmeyer- Peppas. By comparing the R2-values obtained from the above equations, the best-fit model was selected.

1. Zero order model

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Q_0 - Q_t = K_0 t \quad (1)$$

Rearrangement of equation (1) yields:

$$Q_t = Q_0 + K_0 t \quad (2)$$

Where Q_t is the amount of drug dissolved in time t,

Q_0 is the initial amount of drug in the solution (most times, $Q_0 = 0$) and K_0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

2. First order model

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation:

$$\log C = \log C_0 - Kt / 2.303$$

Where, C_0 is the initial concentration of drug, k is the first order rate constant, and t is the time. The data obtained are plotted as log cumulative percentage of drug remaining vs. time which would yield a straight line with a slope of -K/2.303.

3. Higuchi's Model

Graph was plotted between cumulative percentages of drug released Vs square root of time.

$$Q=Kt^{1/2}$$

Where K is the constant reflecting the design variables of the system and t is the time in hours. Hence drug release rate is proportional to the reciprocal of the square root of time.

4. Korsmeyer – Peppas model

Korsmeyer et al. (1983) derived a simple relationship which described drug release from a polymeric system equation. To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model.

$$M_t / M_\infty = Kt^n$$

Where, M_t / M_∞ are a fraction of drug released at time t, k is the release rate constant and n is the release exponent. The n value is used to characterize different release for cylindrical shaped matrices. For the case of cylindrical tablets, $0.45 \leq n$ corresponds to a fickian diffusion mechanism, $0.45 < n < 0.89$ to non-fickian transport, $n = 0.89$ to Case II (relaxation) transport, and $n > 0.89$ to super case II transport. To find out the exponent of n the portion of the release curve, where $Mt / M\infty < 0.6$ should only be used. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time.

The release kinetic of formulation no.3 was studied by various kinetic models as zero order plot, first order plot, higuchi plot and korsmeyer-peppas.

Table-6: *In-vitro* release profile of formulation F2.

Time (hrs)	**Log time**	**S.R. of time**	**% cumulative release of (F2)**	**log % cumulative release**	**%cumulative remaining**	**log % cumulative remaining**
0	0	0	0	0	100	2
1	0.00	1.00	16.39	1.21	83.61	1.92

2	0.30	1.41	34.63	1.54	65.37	1.82
3	0.48	1.73	41.03	1.61	58.97	1.77
4	0.60	2.00	46.84	1.67	53.16	1.73
5	0.70	2.24	52.96	1.72	47.04	1.67
6	0.78	2.45	55.35	1.74	44.65	1.65
7	0.85	2.65	60.08	1.78	39.92	1.60
8	0.90	2.83	66.37	1.82	33.63	1.53
9	0.95	3.00	72.84	1.86	27.16	1.43
10	1.00	3.16	80.09	1.90	19.91	1.30

Zero Order Model

For zero order kinetics, data was plotted with time on x-axis and percent cumulative drug released (%CDR) on y-axis as shown in fig. 6

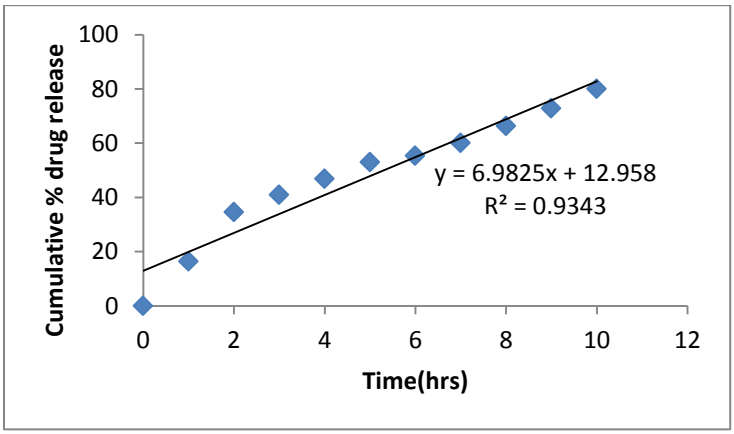

Fig. 6: Zero order release profile of Formulation F2

First Order Model

For first order kinetics, data was plotted with time on x-axis and log cumulative percent remaining on y-axis as shown in figure 7

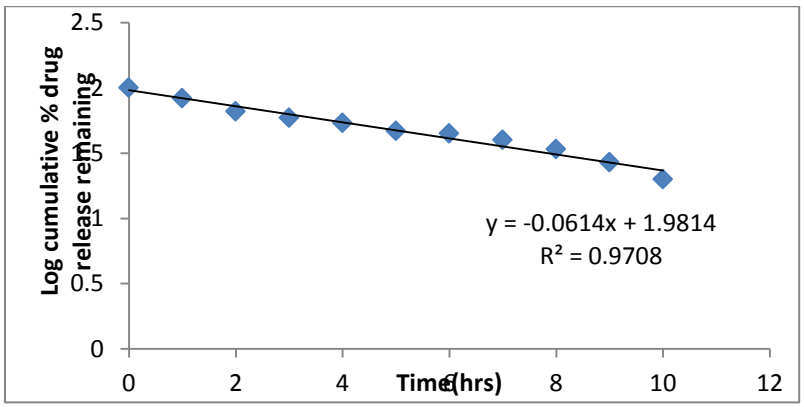

Fig. 7: First order release profile of Formulation F2

Higuchi's Model

Graph was with plotted square root of time on x-axis and cumulative percent drug released (%CDR) on y-axis as shown in fig. 8

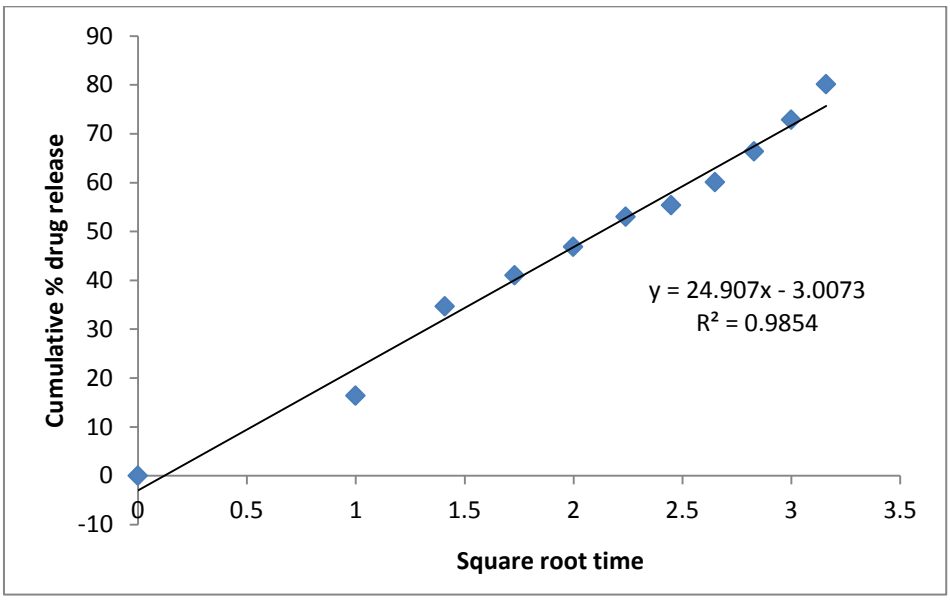

Fig. 8: Higuchi release profile of Formulation F2

KORSMEYER-PEPPAS MODEL

Graph was with plotted log time on x-axis and log cumulative percentage drug release on y-axis as shown in figure 9

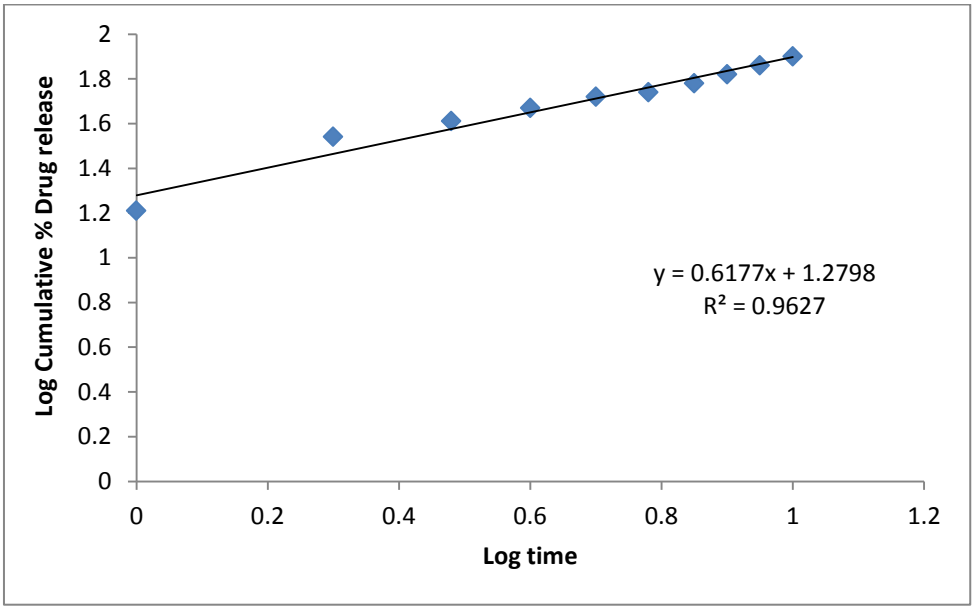

Fig. 9: Korsmeyer-peppas release profile of Formulation F2

The data obtained for in vitro release shown in table no. were fitted into equation for the zero order, first order and higuchi and Korsmeyer peppas release models. The interpretation of data was based on the value of the resulting regression coefficients.

Table-7: Kinetic equation parameter of proliposomes (F2).

Formulation name	Zero order		First order		Higuchi		Peppas	
	R^2	K_O	R^2	K_f	R^2	K_h	R^2	N
F 2 (Pravastatin sodium Proliposome)	0.934	6.982	0.970	-0.0614	0.9854	24.90	0.962	0.6177

The zero order rates describes the system where the drug release independent of its concentration shows the cumulative amount of drug release Vs time for zero order kinetics. The first order rate describes the release from systems where the release of drugs from a matrix as a square root of a time- dependent process based on Fickian diffusion. The calculated regression coefficients for zero order, first order and higuchi models and Korsmeyer were shown in table no. It was found that the in vitro drug release of Pravastatin sodium proliposome was best explained by Peppas equation as the plot showed the highest linearity which followed by the zero order model. Therefore the release pattern seems to fit the Korsmeyer peppas model. The in-vitro data were fit to the different models. The value of R^2 found to be highest for the peppas model. The value of slope and intercept obtained from equation of peppas model were found to be 0.688 and 1.243. This value of N showed that drug release occurred through an anomalous, non-fickian drug diffusion pattern. This pattern was may be due to swallow property of the matrix. This pattern may also due to closure of microcavities during the swollen state of the polymer.

5.4 Stability study

The stability study of proliposomes of Pravastatin sodium was shown in the table 8.The drug content of final drug of the final formulation (F2) was analyzed at interval of 6,12,30,42,48 and 60 days. The drug content lost about 3-17% was observed in proliposomes which are stored at accelerated conditions 40°C±2°C and the formulation which was stored in refrigerator condition it was found that the drug content was about 1-5%.Hence the formulation stored at 4°C±1°C was more stable as compared to other temperatures.

Table-8: Observations of drug content during stability study.

Time (days)	Accelerated Conditions (40˚C)	
	Physical appearance	Drug content
6	+	97.74±0.81

12	+	95.43±0.75
24	+	92.23±0.82
30	+	89.13±0.77
42	+	87.54±0.68
48	+	85.83±0.78
60	+	83.56±0.92

The values were mean ± SD (n =3)

Time (days)	**Refrigerated Conditions (4˚C)**	
	Physical appearance	**Drug content**
6	+	98.78±0.56
12	+	97.14±0.43
24	+	96.95±0.48
30	+	96.24±0.55
42	+	95.82±0.63
48	+	95.12±0.59
60	+	94.76±0.66

The values were mean ± SD (n =3)

Conclusion

The present study has been an attempt to prepare proliposomes of Pravastatin sodium by Film Deposition Method by using polymers like cholesterol, spray dried mannitol and phopsholipon 90H. Pravastatin sodium is a reversible inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the enzyme that catalyses the conversion of HMG-CoA to mevalonate. Pravastatin sodium is a hydrophilic drug clinically used to lower cholesterol and triglycerides in blood. Because of short half life it has been selected as ideal candidates for the design of oral controlled release dosage form.

Preformulation studies like pH, melting point, and solubility and UV analysis of it were complied with USP standards. The FTIR Spectra's revealed that, there was no interaction between polymers and drug. Both the polymers were compatible with the drug. Twelve formulations were formulated with different proportions of phospholipids. All the formulations were evaluated for various parameters. The micromeritic properties were evaluated of all the twelve formulation, all the formulations shown excellent to good flow properties. The practical yield and % drug entrapment efficiency of all formulations (F1-F12) were evaluated. On the basis of % Drug entrapment and % Yield, from the three phospholipids i.e. phospholipon 90H, phospholipon 90G and Lipoid S100, Phospholipon 90H in different

concentrations shown better result than other two phospholipids. From the four concentrations of Phospholipon 90H, F2 formulation with 250mg 90H, shows maximum percentage yield as well as percent entrapment i.e. 76.36 ±1.6 and 80.33 ± 1.3 respectively. These were further evaluated for particle size, morphological character and in-vitro drug release study.

The results of particle size analysis indicated that best formulation F2 exhibited particle size 1257 nm.The SEM analysis of formulation indicated that the proliposomes were of small spherical shaped. The *in-vitro* drug release study of Pravastatin sodium proliposomes was performed for F2. The release behavior of drug from the polymer followed sustained release. The formulation F2 showed the release of 80.09% respectively in pH 6.8 at the end of 10 hours. From this release profile, it was observed that the formulation F2 gave maximum drug release. The data obtained from in vitro drug release were fitted into zero order, first order, higuchi and korsmeyer peppas release models. Further, the stability of formulation F2 at different storage conditions indicates that the drug content loss was less in refrigerated condition (4°C) as compared to accelerated condition.

Hence it can conclude that the formulated proliposomes of Pravastatin sodium may be used as sustained drug.

References

1. Sunesen VH, Vedesdal R, Kristensen HG, Christrup, Mullertz, Effect of liquid volume and food intake on the absolute bioavailability of danazol a poorly soluble drug. Eur J Pharm Sci, 2005, vol-24, pp297-303.
2. Sentjurc M, Vrhovnik K, Liposome as a topical delivery system: the role of the size on transport by the EPR imaging method. J control release, 1999, vol- 59, pp87-97.
3. Tripathi KD., Hypolipidaemic drugs and plasma expanders. In Essentials of Medical Pharmacology. 6th (ed.) Japee Brothers Medical Publishers, New Delhi, 2010.
4. Charman WN, Porter CJH, Mithani S, Dressman JB., Physicochemical and physiological mechanisms for the effects of food on drug absorption: the role of lipids and pH. J Pharm Sci, 1997, vol- 86, pp269-282.
5. Janga KY, Jukanti R, Vepula A, Bioavailability enhancement of zaleplon via proliposomes: role of surface charge. Euro J Pharm and Biopharm, 2012, vol-80(2), pp347-357.
6. Martin A. Diffusion and dissolution. In: Physical pharmacy. 3rd (ed.) Lea and Fibiger, Philadelphia, 1983.
7. Fiese EF, Hagen TA., Lachman L, Lieberman HA, Kanig JL, The theory and practice of industrial pharmacy. 3rd (ed.) Varghese publishing house, 1987.

8. Pinte D, Kumar R, Kumar M, Formulation and evaluation of pravastatin sodium immediate release tablets. IRJP, 2012, vol- 3(5), pp311-313.

9. Singh A, Sharma PK, Malviya R, Sustained drug delivery using mucoadhesive microspheres: the basic concept, Preparation methods and recent patents on Nanomedicine, 2012, vol-2, pp62-77.

Corresponding Author:

Gitanjali Parmar[*],

Email: *parmargitanjali@gmail.com*