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Research Article

Formulation and Evaluation Tolbutamide Liposomes for a Sustained Drug Delivery System

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Abstract:
Novel Drug Delivery system (NDDS) refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects. Applications of liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic applications of liposomes containing various markers or drugs. The aim of the study was to formulate and evaluate Tolbutamide liposomes for a sustained drug delivery system. The tolbutamide was prepared as a liposomal drug delivery system by using two different techniques such as physical dispersion method and ether injection method. The morphological characters of prepared liposomes were determined with the help of optical microscope. The results of the particle size showed, when the concentration of soya lecithin was increased the size of the particle was reduced. The in vitro release showed that as the concentration of soya lecithin was increased the release rate of drug was retarded. Among the two methods ether injection method showed prolonged action when compared to physical dispersion method. The stability studies for all the formulations were performed by keeping the formulations at two different temperatures 4ºC±2ºC and 25ºC±2ºC for a period of 30 days. There was no change in morphological characters at 4ºC±2ºC, but there was a slight reduced in particles size at 25ºC±2ºC. The percentage drug entrapment was reduced in all the formulations at both the conditions.
Keywords: Sustained drug delivery system, physical dispersion method , ether injection method, soya lecithin , stability studies

Introduction

Novel Drug Delivery system (NDDS) refers to the approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effects [1-3]. Liposomes are colloidal, vesicular structure composed of one or more bilayers surrounding an equal number of aqueous compartment. Liposomes are small artificial vesicles of spherical shape that can be created from cholesterol and natural nontoxic phospholipids.

Due to their size and hydrophobic and hydrophilic character (besides biocompatibility), liposomes are promising systems for drug delivery. The sphere likeshell encapsulated a liquid interior which contain substances such as peptides, protein, hormones, enzymes, antibiotics, anti-fungal and anti-cancer agents [4-9]. Liposomes particle sizes ranges from 30 nm to several micrometers. They consist of one or more lipid bilayer surrounding aqueous units, where the polar head groups are oriented in the pathway of the interior and exterior aqueous phases [10].

Applications of liposomes in medicine and pharmacology can be divided into diagnostic and therapeutic applications of liposomes containing various markers or drugs , and their use as a tool, a model, or reagent in the basic studies of cell interactions, recognition processes,and mode of action of certain substances .

Advances in liposome design are leading to new applications for the delivery of new biotechnology products, for example antisense oligonucleotides, cloned genes, and recombinant proteins. Recent improvements include liposomal formulations of all-trans-retinoic acid and daunorubicin, which has received. Sulfonylureas increase both basal insulin secretion and meal-stimulated insulin release. Medications in this class differ in their dose, rate of absorption, duration of action, route of elimination and binding site on their target pancreatic β cell receptor [11-15]. The aim of the study was to formulate and evaluate Tolbutamide liposomes for a sustained drug delivery system.

Material and methodology

Tolbutamide was obtained from Yarrow chem products, Mumbai. Chloroform and ether obtained from Rankem laboraties, Haryana. Soya lecithin received from the urban platter food co., Mumbai. Potassium di hydrogen phosphate purchased from Merck specialities pvt. Ltd, Mumbai. All other chemicals used were of analytical grade and were used without further purification.

Methodology

Preparation of standard curve of tolbutamide using pH 6.8 phosphate buffer

Accurately weighed 100 mg tolbutamide was dissolved in water and the volume was make up to 100 ml using distilled water in a volumetric flask to obtain a solution of 1000 µg/ml. From the above solution 10 ml was pipetted out into a 100 ml volumetric flask and made up to 100 ml using phosphate buffer pH 6.8 to get a stock solution of 100 µg/ml. From this stock solution, aliquots of 0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml, 1.2ml, 1.4ml, 1.6ml, 1.8 ml and 2.0ml were pipetted out into a series of 10 ml volumetric flask and made up to mark with phosphate buffer pH 6.8 to get a concentration in the range of 2 to 20 µg/ml. The absorbance of the resulting solution was then measured at 233 nm using UV Double beam spectrophotometer against phosphate buffer pH 6.8 as blank. The standard curve was obtained by plotting concentration (µg/ml) values in X- axis and absorbance values in Y – axis [16-18].

Preformulation studies

The objective of preformulation testing is to generate information useful to the formulation in developing stable and bioavailable dosage forms. The use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacious and stable product.

a) Solubility

Solubility of Tolbutamide in water, methanol, phosphate buffer pH 6.8 was determined at room temperature with the help of magnetic stirrer.

b) Melting Point

Melting point determination was done by using melting point apparatus. Small amount of pure drug of Tolbutamide was taken in a capillary tube and it was kept in the melting point apparatus and the melting point was noted [19-22].

c) Compatibility (Drug – excipients interaction) studies:

FT-IR spectra were taken for the dried samples using FT-IR 8400S (Shimadzu, Japan) to determine the possible interactions between the drug and polymers. The plain drug, lecithin, cholesterol and combination of drug with cholesterol and lecithin in three different ratios (1:1, 1:2 and 1:3) were taken and mixed with KBr.

The samples were compressed to form a pellet using a hydraulic press. The prepared pellets were transformed into disk. The disk was applied to the centre of the sample holding device and scanned from 4,500 to 400 cm-1 using FT-IR spectrophotometer [23,24].

Formulation of liposomes loaded with Tolbutamide

The formulation of liposomes loaded with Tolbutamide was prepared by two different techniques namely, physical dispersion method and ether injection method. In both the techniques ratio of cholesterol was kept as same and the lecithin concentration was increased as 1:1, 1:2 and 1:3 [25-27].

Physical dispersion method

Liposomes were prepared by physical dispersion method using different ratio of soya lecithin and cholesterol was kept as constant. In this method the soya lecithin and cholesterol were dissolved in chloroform. Then it was spread over flat bottom conical flask and allowed to evaporate at room temperature for overnight without disturbing the solution for a formation of lipid film. The drug was dissolved in phosphate buffer pH 6.8. It acts as an aqueous medium. Then the aqueous medium was added to the lipid film for hydration. For this the flask was inclined to one side and aqueous medium was introduced down the side of flask and flask was slowly returned to upright orientation. Then the conical flask was kept on water bath and the temperature was maintained at 37± 2ºC for 2 hours for the completion of hydration. The conical flask was gently shaken until the lipid layer was removed from wall of conical flask and formation a liposomes suspension. Then the formed liposomes suspension was stored at 4ºC for one day for the maturation of liposomes. The prepared liposome suspension was centrifuged at 15,000 rpm for 20 mins. Then the precipitate was collected and diluted with distilled water for further studies12. Different batches of liposomes were prepared as per the general method described above and composition for the preparation of liposomes is given in Table No. 1 [28,29].

Ether injection method

Liposomes were prepared by ether injection method using different ratio of soya lecithin and cholesterol was kept as constant. In this method the cholesterol and soya lecithin were dissolved in ether and methanol. The drug was dissolved in phosphate buffer pH 6.8. It act as an aqueous medium. The aqueous medium was heated to 60°C. The method involves injecting drop by drop of ether-lipid solutions into the above warmed aqueous medium. The ether vaporizes upon contacting the aqueous phase, and the dispersed lipid forms primarily unilamellar liposomes. Then the product was collected and it was stored at 4°C for maturation of liposome. Then prepared liposomal suspension was centrifuged at 15,000 rpm for 20 mins. The precipitate was diluted with distilled water for evaluation studies. Different batches of liposomes were prepared as per the general method described above and composition for the preparation of liposomes is given in Table No. 1 [30-32].

Table 1: Formulation of Tolbutamide liposomes

S. No.	Ingredients	Physical dispersion method			Ether injection method		
		F1	F2	F3	F4	F5	F6
1.	Cholesterol	100 mg	100 mg	100 mg	100 mg	100 mg	100 mg
2.	Lecithin	100 mg	200 mg	300 mg	100 mg	200 mg	300 mg
3.	Tolbutamide	10 gm	10 gm	10 gm	10 gm	10 gm	10 gm
4.	Ether	-	-	-	7 ml	7 ml	7 ml
5.	Methanol	-	-	-	3 ml	3 ml	3 ml
6	Chloroform	5 ml	5 ml	5 ml	-	-	-
7.	Phosphate buffer pH 6.8	50 ml	50 ml	50 ml	50 ml	50 ml	50 ml

Evaluation of liposomes:

1. Determination of percentage drug entrapment efficiency:

Drug entrapment efficiency was calculated by using centrifugation method. 10 ml of liposome suspension was taken and centrifuged at 15,000 rpm for 20 mins. The supernatant liquid was collected and suitably diluted. Then the absorbance was taken at 284 nm with the help of UV double beam spectrophotometer using pH 6.8 as a blank. The drug entrapment efficiency was calculated from the following formula.

$$\textbf{Total entrapment efficiency} = \frac{\text{Amount of drug in supernatant liquid}}{\text{Amount of drug}} \times 100$$

2. Morphology analysis:

The prepared Tolbutamide liposomes for all the formulations were viewed under for observing the vesicle formation and discreteness of dispersed vesicles. A slide was prepared by placing a drop of liposome dispersion on a glass slide and cover slip was placed over it and this slide was viewed under optical microscope at 40X magnification. Photographs were taken to prepared slides using digital camera.

3. In vitro drug release study:

The in vitro release for all the formulated Tolbutamide liposomes were carried out for 8 hours in phosphate buffer pH 6.8. The studies were carried in USP dissolution apparatus II (Paddle) at 37ºC ± 0.5ºC and 50 rpm speed. 900 ml of phosphate buffer pH 6.8 was used as a dissolution medium. Equivalent to 100 mg of Tolbutamide liposome was taken in a dissolution jar contains dissolution medium and the paddle was rotated at 50 rpm. 1 ml of samples were withdrawn at every 30 minutes up to 480 minutes and make up the sample to 10 ml with pH 7.5 buffer and analyzed for Tolbutamide content at 233 nm with pH 6.8 as blank using double beam UV double beam spectrophotometer.

4. Particle size determination

The particle size determination is done by using Shimadzu SALD – 2300 (WingSALD II: Version 3.1.1). Groups of particles are dispersed in a liquid medium and measured as they are circulated between the flow cell, which is placed in the measurement unit, and a dispersion bath in the sampler. The dispersion bath incorporates a stirrer and an ultrasonic sonicator. A pump delivers the dispersed suspension to the flow cell. The pump is specially designed to ensure both liquid medium and the particles are circulated. It can be controlled from a computer. Organic solvents can be used as dispersion media.

5. Stability studies

The behavior of the liposome to retain the drug was studied by storing the liposome at two different temperature conditions, i.e., 4ºC (refrigerator RF), 25°C±2ºC for a period of 1 month. The liposomal preparations were kept in sealed vials. At 30th day the samples were analyzed for the drug content following the same method described in % drug encapsulation efficiency and in vitro drug release. And also, the liposomes were studied for their morphology.

Results and Discussion

Calibration of Tolbutamide using phosphate buffer pH 6.8:

The Standard Calibration curves of tolbutamide hydrochloride were prepared by using phosphate buffer pH 6.8 and absorbance were analyzed in 248nm. The correlation coefficient was found to be 0.9905. The results indicate Tolbutamide obeys the beer's law within the concentration range of (2-20µg/ml). Calibration plot of tolbutamide was shown in Table No. 2 and Figure 2.

Table 2: Calibration curve of tolbutamide

Sl no.	Concentration (ug/ml)	Absorbance at 248nm
1	2	0.241
2	4	0.482
3	6	0.634
4	8	0.872
5	10	1.055

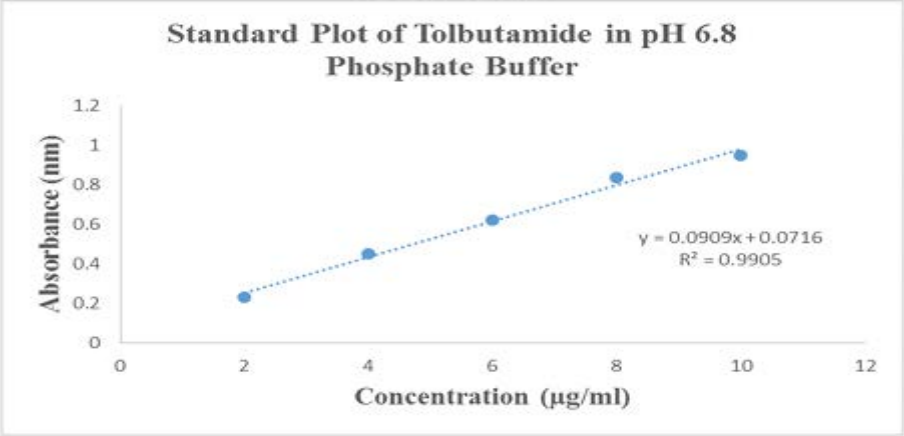

Figure 2: Calibration curve of tolbutamide

Preformulation studies

a) Solubility

The solubility of raw drug was determined by dissolving in distilled water, methanol and phosphate buffer pH 6.8. The drug was practically insoluble in the water, soluble in methanol and phosphate buffer p H 6.8.

b) Melting point

It was found to be 128ºC which was within the specification range of standard. So it confirmed Tolbutamide present in raw material of drug.

c) Compatibility (Drug – excipients) studies

The FT – IR studies of pure Tolbutamide, cholesterol, soya lecithin and combination of Tolbutamide, cholesterol and soya lecithin were conduct to study the interaction between the drug and excipients. FT- IR spectral analysis showed that the fundamental peaks and patterns of the spectra were similar both in pure drug and combination containing drug and highest proportion of excipients. This indicated that there was no chemical interaction between Tolbutamide and the other excipients used in

the formulations. The spectral data's are presented in Table 3-5 and spectral peaks were presented graphically in Figure 3-5.

Figure 3: FTIR of Tolbutamide

Table 3: FTIR of Tolbutamide

Wave length (cm^{-1})	Functional group
3400	N-H stretching
2950	C-H stretching
1610	Amino N-H bending
1540	CH_3 bending alkanes
1100	C-N stretching

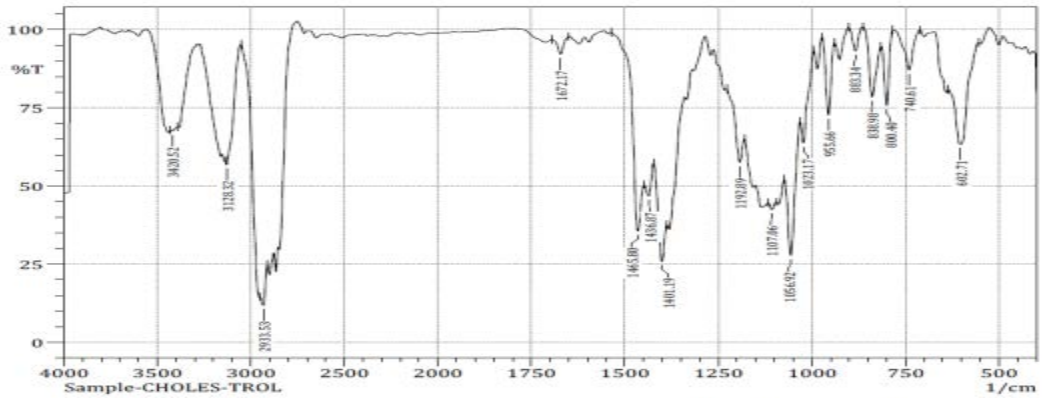

Figure 4: FTIR of cholesterol

Table 4: FTIR of cholesterol

Wave length (cm^{-1})	Functional group
3420	N-H stretching
1466	CH_3 bending alkanes
1057	C-N stretching
956	Alkene C-H bending

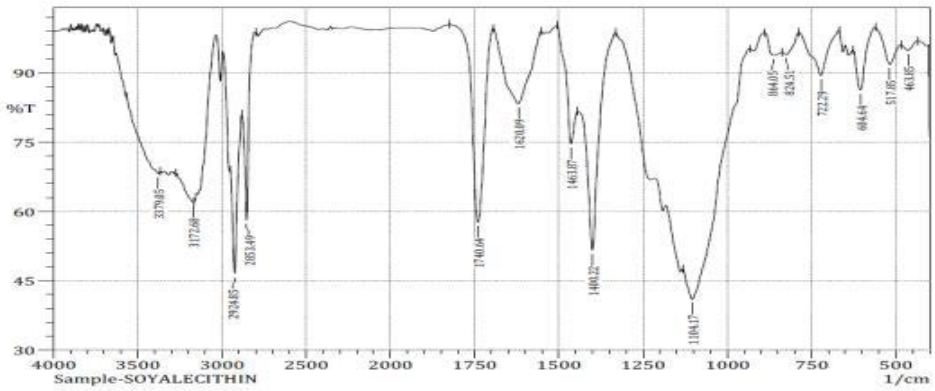

Figure 5: FTIR of Lecithin

Table 5: FTIR of Lecithin

Wave length (cm^{-1})	Functional group
3379	N-H stretching
1621	Amino N-H bending
1464	CH_3 bending alkanes
1105	C-N stretching
863	Alkene C-H bending

Formulation of Tolbutamide Liposomes

The tolbutamide liposome was prepared by physical dispersion method and ether injection method using Soya lecithin and cholesterol in different ratios as per formula designed in Table 1. The F1 to F3 formulation prepared by physical dispersion method, F4 to F6 formulation prepared by ether injection method.

Evaluation of tolbutamide Liposomes

Percentage drug entrapment efficiency

The percentage drug entrapment efficiency of formulations F 1, F 2 and F 3 were 85.61 %, 79.90 % and 73.21 % respectively and formulations F 4, F 5 and F 6 were 31.48%, 39.68% and 39.71% respectively. The results may specify physical dispersion method have better drug entrapment efficiency than ether injection method.

Morphology analysis

The morphology characters of liposomes were analyzed by optical microscopy (Olympus Opto System, India) and the images were taken using digital camera. The formulation F 1 - F 6 microscopic images were showed in Figure No. 6 -11.

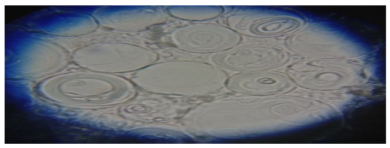

Figure 6: microscopic image (45 X) of F1 formulation

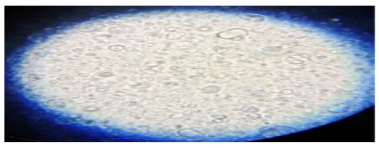

Figure 7: microscopic image (45 X) of F2 formulation

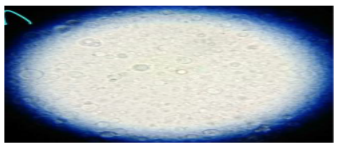

Figure 8: microscopic image (45 X) of F3 formulation

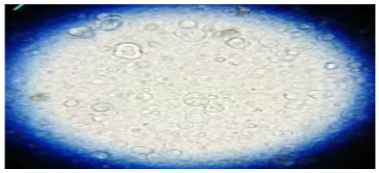

Figure 9: microscopic image (45 X) of F4 formulation

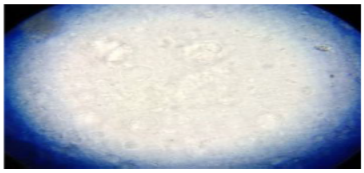

Figure 10: microscopic image (45 X) of F5 formulation

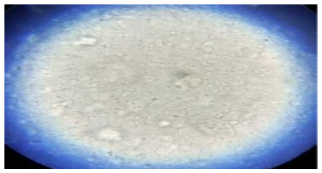

Figure 11: microscopic image (45 X) of F6 formulation

Particle size

The particle size analysis was carried out by malven particle size analyzer for all the prepared liposome formulations. The particle size for all the formulated liposomes were found be in the range of 30.618 µm to 0.035 µm as shown in Table No. 6. The particle size data showed that when the concentration of soya lecithin was increased the particle size was decreased invariably the tolbutamide liposomes in prepared by both methods. The particle size of tolbutamide liposomes of F 3 and F 6 were found to be lower when compared with other formulations this may be due to higher concentration of soya lecithin.

Table 6: Particle size range

Sl. no	Formulations	Particle size range
1	F1	30.618-1.553
2	F2	19.023-1.589
3	F3	0.072-0.021
4	F4	24.133-1.566
5	F5	0.082-0.032
6	F6	0.072-0.035

Partition coefficient determination

The partition coefficient is defined as the ratio of unionized drug distributed between the organic and aqueous phase at equilibrium. Po/w = (Coil/Cwater) equilibrium Partition coefficient is a measure of drugs lipophilicity and an indication of its ability to cross biomembrane. The partition coefficient of tolbutamide was determined in n-octanol: water system. Accurately weighed tolbutamide (10 mg) was added to 10.0 ml each of n-octanol and aqueous phase. The mixture was put on mechanical shaker for 24 hours until equilibrium was reached. Phases were separated in a separating funnel and the aqueous phase was analyzed for amount of drug after appropriate dilution by UV spectrophotometer.

Procedure

10 ml of n-octanol and 10mg of the tolbutamide with 10 ml of water was taken in a separating funnel and allowed to stand for 24 hrs on mechanical shaker. After 24 hrs, the aqueous layer was separated out and measured absorbance after appropriate dilution by UV spectroscopy. 10 ml of noctanol and 10mg of the tolbutamide with 10 ml of Phosphate buffer saline (pH6.8) was taken in a separating funnel and allowed to stand for 24 hrs on mechanical shaker. After 24 hrs the aqueous layer was separated out and measured absorbance by UV spectroscopy.

Table 7: Partition coeffiecient values of tobutamide

S.No.	Solvent system	Partition coefficient
1.	n-Octanol/Distilled water	0.0622± 0.0023
2.	n-Octanol/PBS (pH 7.4)	0.057± 0.0018

In vitro drug release studies

The cumulative percentage drug release of formulations F 1, F 2 and F 3 were found to be 103.03±2.50, 91.94±2.72 and 82.12±2.51 respectively in 8 hours. The formulation F 1 shows faster release than formulations F 2 and F 3 due to the lower concentration of soya lecithin. The cumulative percentage drug release of formulations F 4 was found to be 100.58 ± 1.12 at the end of 7 hours. And the cumulative percentage drug release of formulations F 5 and F 6 were found to 85.06±1.73 and 80.10±1.03 respectively in 8 hours. The formulation F 4 show faster release than formulations F 5 and F 6. While the concentration of soya lecithin was increasing it decrease the release of drug.

The prepared liposomes F 1 to F 6 showed sustained release of drug. When increased ratios of soya lecithin also sustain the release of drug was increased in both methods of preparations. The Figure No. 12 and 13 shows the formulation F 1, F 2 and F 3 and F 4, F 5 and F 6 respectively in 8 hours.

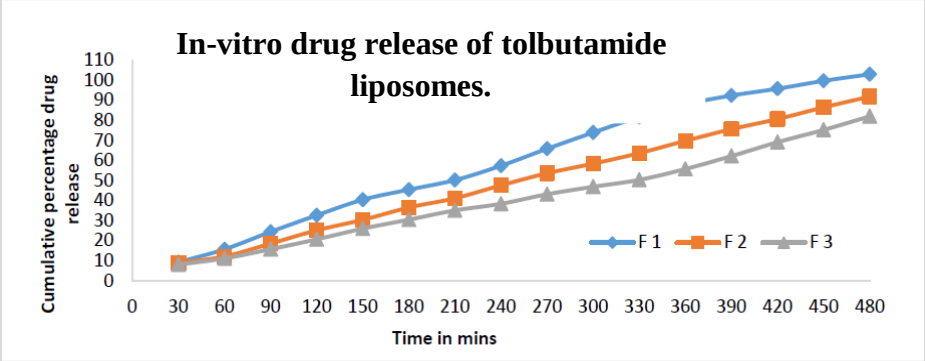

Figure 12: Comparative cumulative percentage drug release of tolbutamide liposome formulation of F1, F2 and F3

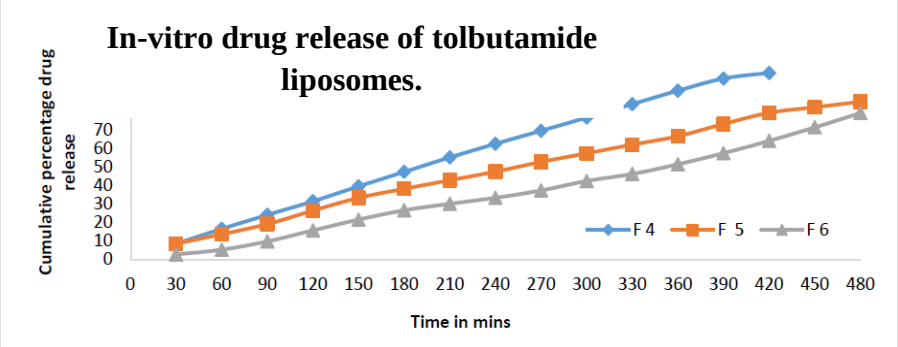

Figure 13: Comparative cumulative percentage drug release of tolbutamide liposome formulation of F4, F5 and F6

Stability Studies

All the formulations of Tolbutamide liposomes were relatively stable at 4ºC storage condition. The drug leakage percent amounts of original entrapped in liposomes were very small and the amount retained in vesicle had no significant difference after one month as compared to the amount immediately after preparation. But at the storage condition of 25°C±2°C, all the formulations of Tolbutamide liposomes were unstable. In addition, the result of drug entrapment studies showed higher leakage at higher temperature. This may be due the higher fluidity of lipid bilayer at higher temperature, resulting into higher drug leakage. The drug entrapment results were shown in table no. 8.

Table 8: Stability study of percentage drug entrapment of tolbutamide liposomes compared with percentage drug entrapment of immediately after preparation.

Sl no.	Formulation code	Immediately after preparation (%)	After one month	
			At 4^0C	At 25^0C ±2^0C
1	F1	85.61	85.91%	76.87%
2	F2	79.90%	77.98%	70.99%
3	F3	73.21%	71.12%	66.88%
4	F4	31.48%	29.34%	24.91%
5	F5	39.68%	38.45%	35.41%
6	F6	39.71%	38.22%	36.70%

The morphological characters of Tolbutamide liposomes for F 1 – F 4 didn't show any characteristic changes after it was stored at 4ºC and 25ºC±2ºC for a period of one month. F 5 and F 6 formulations were showed slightly reduced in the size after it was stored at 25ºC±2ºC for a period of one month but there was no changes for the same formulation when it was stored at 4ºC. Microscopic images of all the formulations (F 1 – F 6) of Tolbutamide liposomes were compared with before and after stability studies. The results show there no significant changes.

After one month, Tolbutamide liposomes formulations F 1 to F 6 were showed difference in in vitro drug release profile. Dissolution rate was decreased in all Tolbutamide liposomes formulations at both storage conditions like 4°C and 25ºC±2ºC. The results of in vitro drug release of all the formulations at both storage conditions were compared with before and after stability studies and the results were shown in Table No. 9.

Table 9: Invitro drug release of tolbutamide formulations after stability study, compared with before stability

Sl no.	Formulation code	Immediately after preparation	After stability study	
			At 4^OC	At 25^OC± 2^OC
1	F1	103.03±2.50	92.81	73.41
2	F2	91.94±2.72	87.67	69.26
3	F3	82.12±2.51	77.90	64.37
4	F4	100.58±1.12	91.69	87.40
5	F5	85.06±1.73	78.84	61.82
6	F6	80.10±1.03	74.98	61.32

At storage condition 4°C showed better stability than another condition. This may due to their elevated temperature reduce the stability. But in both storage conditions higher proportion of soya lecithin contains formulations like F 3 and F 6 showed better stability than other their formulations.

Summary and Conclusion

This study concluded that tolbutamide was successfully prepared as a liposomal drug delivery system by using two different techniques such as physical dispersion method and ether injection method. The liposomes prepared by physical dispersion method showed better percentage drug entrapment when compared with ether injection method. The morphological characters of prepared liposomes were determined with the help of optical microscope. The results of the particle size showed, when the concentration of soya lecithin was increased the size of the particle was reduced. The in vitro release showed that

as the concentration of soya lecithin was increased the release rate of drug was retarded. Among the two methods ether injection method showed prolonged action when compared to physical dispersion method. The stability studies for all the formulations were performed by keeping the formulations at two different temperatures 4ºC±2ºC and 25ºC±2ºC for a period of 30 days. After the stability period the formulations were tested for morphological analysis, percentage drug entrapment and in vitro drug release and compared with before stability study. There was no change in morphological characters at 4ºC±2ºC, but there was a slight reduced in particles size at 25ºC±2ºC. The percentage drug entrapment was reduced in all the formulations at both the conditions. The in vitro drug release was reduced for all the formulations. Liposomes prepared by physical dispersion method showed better stability compared with ether injection method.

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