FORMULATION AND EVALUATION OF NANOEMULSION LOADED WITH TOLBUTAMIDE

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Abstract

The oil in water nanoemulsion for antidiabetic activity developed by the spontaneous emulsification method by using different ratio of HPMC, glycerine, pluronic 127, lecithin, tween-80, SLS, propylene glycol, isopropyl myristate. The prepared nanoemulsion was subjected to IR spectroscopy and DSC for compatibility study, which was compatible for tests. The nanoemulsion formulations that passed tests were characterized for viscosity, droplet size, zeta potential, scanning electron microscopy and invitro study was carried out in USP dissolution paddle type apparatus. The release kinetics was studied and stability study as per ICH guidelines was also carried out for 3 months. Release kinetics was found to follow zero order kinetics, first order kinetics, Higuchi diffusion model and Korsmeyer-Peppas model. The antidiabetic activity of nanoemulsion showed a significant reduction in glucose level. Amongst the nine formulations F1 shows the best results. These results suggest that formulation F1 is suitable for oral administration as antidiabetic agents.

Keywords : Nanoemulsion, Antidiabetic, IDDM, NIDDM, Tolbutamide.

Introduction

There are an estimated 143 million people worldwide suffering from diabetes[1] almost five times more than the estimates ten years ago. This number may probably double by the year 2030[2]. Therefore the human population worldwide appears to be in the midst of an epidemic of diabetes. Reports from WHO indicates that diabetes mellitus is one of the major killers of our time, with people in South East Asia and Western Pacific being most at risk[3]. Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin.

This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time ,it leads to hyperglycemia that in due course tuns into a syndrome called diabetes mellitus[4]. There are two main categories of this disease. Type 1 diabetes mellitus also called insulin –dependent diabetes mellitus (IDDM) and Type 2 the non-insulin dependent diabetes mellitus (NIDDM).IDDM accounts for 5% to 10% of all cases[5]. NIDDM is far more common and results from a combination of defects in insulin secretion and action. This type of disease accounts for 90 to 95% of all diabetic patients. Diabetes mellitus is a chronic disease characterized by hyperglycemia and by complications that include microvascular disease of the eye and kidney and a variety of clinical neuropathies[6].The specific association of microvascular disease and neuropathy with diabetes and the relation of the two complications to the duration of diabetes suggest that they are linked to hyperglycemia or a concomitant metabolic abnormality. The Diabetes Control and Complications Trial (DCCT), conducted for nearly 10 years with 1441 patients between the ages of 13 and 39 years who had insulin-dependent diabetes mellitus (IDDM), demonstrated that the incidence of retinopathy,nephropathy,and neuropathy could be reduced by intensive treatment[7].Although the metabolic control of hyperglycemia should also limit the incidence and development of retinopathy, nephropathy, and neuropathy in patients with non-insulin-dependent diabetes mellitus (NIDDM), the extent of that effect has yet to be determined[8].The specific strategies for achieving metabolic control of the two types of diabetes also differ; diet, exercise, and oral antidiabetic drugs are the primary means of reducing blood glucose concentrations in patients with NIDDM. Moreover, the risks, for both children and middle-aged adults, of intensive attempts to lower blood glucose to nearly normal levels have not been defined, either for patients with IDDM or for those with NIDDM[9].

The future offers great hope for the treatment of patients with diabetes, with improved forms of insulin and other adjuncts to intensive treatment, pancreatic transplantation, and new drugs that interrupt the pathophysiologic mechanisms of the complications of diabetes[10]. Currently, however, the most successful strategy for preventing complications of diabetes is intensive treatment of hyperglycemia. The DCCT demonstrated the value of this approach. In the DCCT, as in other studies, there was a curvilinear relation between glycosylated hemoglobin and the incidence of retinopathy[11].Thus, lowering the glycosylated hemoglobin 1 percent from markedly abnormal value will be of more benefit than lowering it 1 percent from a slightly abnormal value. Because of the curvilinear relation between glycosylated hemoglobin and retinopathy, it is possible to generate break points by using two straight lines with differing slopes[12].Whereas this may represent a thresh- old below which complications do not occur, it may just be a reflection of the curvilinear relation between glycosylated hemoglobin and retinopathy. Recent data from the feasibility trial of a multicenter cooperative study by the Department of Veterans Affairs suggest that in- tensive insulin therapy to control hyperglycemia in patients with NIDDM is possible without adverse changes in weight, blood pressure, or plasma lipids and without hypoglycemia[13]. Additional research will be needed to learn how intensive treatment of hyperglycemia can be widely applied and to identify the risks and appropriate strategies of intensive therapy in patients with NIDDM[14].

Material and method

List of Materials used

Table 1 : List of chemicals

Sr. No.	Chemical	Manufacturer
1	Tolbutamide	Loba Chemie, Mumbai
2	HPMC (hydroxypropyl methylcellulose)	Otto Chemie, Mumbai
3	Glycerine	Chong Yu-Tech chemicals,China
4	Pluronic 127 (P-127)	Ottokemi, Mumbai
5	Lecithin	Sonic Biochem, M.P
6	Tween-80	Akshar chemicals, Mumbai
7	SLS (sodium dodecyl sulphate)	Matangi Industries,Gujarat
8	Methyl Paraben	Novaphene Pvt.Ltd, Mumbai
9	Propylene glycol	Antares Chem Pvt.Ltd, Mumbai
10	Isopropyl myristate	Kosha Chemtech Pvt.Ltd, Gujarat

List of Equipments used

Table 2 : List of Eqipment

Sr. No.	Instruments	Manufacturer
1.	Double Beam UV Spectrophotometer	Jasco Corporation Tokyo, Japan
2.	Melting Point Apparatus	Remi Scientific Instruments, Mumbai
3.	Digital pH meter	Eutech Instrument
4.	FTIR	Shimadzu, Japan
5.	Microfluidizers	LM10,India
6.	Homogenizer	ULTRA-TURRAX
7.	Ultrasonicators	Bharat Emporium, Roorke
8.	Dissolution Apparatus	Veego Instruments, Mumbai
9.	Brookfield Viscometer	Brookfield Engineering Lab, Middleboro
10.	Micro Osmometer	KNAUER

Preparation of Nanoemulsion containing Tolbutamide

In order to provide strong disruptive forces for size reduction during high-energy emulsification, mechanical equipment is required. Microfluidizers, homogenizers, and ultrasonicators can provide these forces, but they are expensive and produce high working temperatures, which are inappropriate for drugs that are thermolabile.

Liposomes were prepared by physical dispersion method using different ratio of lecithin and Isopropyl myristate. In this method the lecithin and Isopropyl myristate 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. Pluronic 127 and HPMC(hydroxypropyl methylcellulose) were dissolved in water Then it was spread over flat bottom conical flask and allowed to dissolve at room temperature for overnight without disturbing the solution for a formation of thick translucent mass 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 add thick translucent mass of Pluronic 127 and HPMC added .The prepared was stirred at 15,00 rpm for and 30 mins. Then add other inactive ingredients and make up the volume 100 ml with water and stirrer for 30 minutes for making nano- emulsion.

Evaluation

Evaluation parameters of nanoemulsion formulations are as :

Drug content

An accurately weighed portion of film is dissolved in 100ml of suitable solvent in which drug is soluble and then the solution is shaken continuously for 24 hours in shaker incubator. Then the whole solution is sonicated. After sonication and subsequent filtration, drug in solution is estimated spectrophotometrically by appropriate dilution.

Zeta potential

The nano emulsion sample was screened for zeta potential at 25ºC by Zetasizer (Nano-ZS90, Malvern instrument, UK). The disposable cuvettes were used for sample analysis. The results were reported as the mean standard deviation foe three replicates. Zeta potential of particles represents good stability of nanoemulsion and minimizes chances of flocculation.

Scanning Electron Microscopy (SEM)

SEM is used to attain scanning electron micrographs of nanoemulsion containing Tolbutamide. The instrument used for this purpose is Hitachi S-4800 scanning electron microscope. The microspheres were assembled directly onto the SEM sample stub, using double sided sticking tape, and coated with gold film under reduced pressure.

Differential scanning calorimetric (DSC) analysis

Selected samples were examined for thermotropic properties using DSC instrument. The DSC instrument was calibrated using Indium as standard. Accurately weighed 2 mg sample were sealed in standard aluminum pans and screened between 30-300ºC with a heating rate of 10ºC/min under the nitrogen environment (60ml/min). The empty aluminum pan was used as a reference.

pH

1 % aq. a solution of emulsion formulation was prepared and stored for 2 h and pH was determined using a digital pH meter. The pH of each gel formulation was done in the triplicate, average value and ± standard deviation was calculated.The pH of the emulsion formulations was in the range of 5.5 ± 0.2 to 7.5± 0.2, which lies in the normal pH range of the oral emulsion and therefore it will not induce any irritation orally and suspension's physical stability.

Drug content

An accurately weighed portion of film is dissolved in 100ml of suitable solvent in which drug is soluble and then the solution is shaken continuously for 24 hours in shaker incubator. Then the whole solution is sonicated. After sonication and subsequent filtration, drug in solution is estimated spectrophotometrically by appropriate dilution.

In vitro drug release of tolbutamide nanoemulsion

The in vitro drug release studies were carried out using a modified Franz diffusion (FD) cell. The formulation was applied on dialysis membrane which was placed between donor and receptor compartment of the FD cell. Phosphate buffer ph7.4 was used as a dissolution media. In-vitro drug release study of combination of drug formulation was also studied using dissolution test apparatus. The dissolution medium is phosphate buffer (pH 6.8). The temperature was maintained at 37(+/-)1ºC and 100 rpm. The formulation was added in this dissolution medium, and sample was taken after some intervals of time. Samples were analyzed by UV, whereas absorbance was measured at 370nm. The concentrations of released drugs were determined from calibration curve for individual drug.

Drug release kinetics

These drug kinetics studies are dependent on the various mathematical model applications affecting the rate of drug. Once a suitable function has been selected, the release profiles are evaluated depending on the derived model parameters. The data obtained from ex-vivo permeation studies were plotted in different models of data treatment as follows:

Zero-order kinetic model

This model can be applied to describe the release of the drug from the coated forms of dosing, products with low-solubility in water and the osmotic systems.

Model Expression: $Q_t=K_0t$

Where Q_t = the rate of drug released in time t, K_0 = zero-order model constant unit of inverse time.

First-order kinetic model

This model cannot explain the mechanisms that based on the theories because of its difficulty. Thus, this kinetics model is used to describe the absorption of drugs release and its elimination from the body and also, to describe the drug that contained water soluble in the porous material.

Model Expression: $Q_t= Q\infty(1 – e^{-k_1t})$

Where $Q\infty$ = the total fraction of drug released, Qt = the rate of drug released in time t, and K_1 = the first-order constant.

Higuchi kinetic model

Higuchi proposed his models of water solubility and low solubility in the solid and semi-solid matrix. In this systems there are two mechanisms which responsible for controlling the rate of release of drug: swelling and erosion/degradation, resulting in a layer on the surface of the drug and thus, prevent the entry of more water and prevent the release of more drug, resulting in decline the drug over time.

Model Expression: $Q_t = K_H t^{1/2}$

Where Q_t = the rate of drug released in time t, K_H = Higuchi dissolution constant.

Korsmeyer-Peppas kinetic model

This model describes the mechanism of drug release from the polymer nanoparticles system, which was derived as a simple relationship to detect these mechanisms, where the first 60% of the drug release data were fitted in this model.

Model Expression: $Q_t = At^n$

Where Q_t = the rate of the drug in time t, A = the nanoparticles constant incorporating geometric structure feature, and n = the release exponent that indicates the release rate mechanism.

Stability Studies

The main objective of stability testing is to give evidence on the changes of quality of drug product with respect to time under the influence of various environmental factors such as temperature, humidity and light and enables recommended storage conditions,re-test periods and shelf lives to be accomplished. According to the ICH guidelines the optimized formulation was kept in stability chamber maintained at temperature of 40°C ± 2°C /RH70 % ± 5%. During the study period, the formulation was monitored at prearranged time intervals of 0, 15, 30, 45, 60, 75, 90, 180 days for change in physical appearance.

Result and discussion

Table 3 : Formulation of nanoemulsion containing tolbutamide

Ingredients	**Quantity**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Tolbutamide	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**
HPMC (hydroxypropyl methylcellulose)	0.5%	1.5%	1.5%	2%	0.5%	1%	1.5%	2%	1%
Isopropyl myristate	1%	0.2%	0.3%	0.4%	0.05%	0.5%	0.4%	0.2%	0.3%
Pluronic 127 (P-127)	0.2%	0.2%	0.5%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
Lecithin	0.6%	0.2%	0.5%	0.6%	0.8%	0.4%	0.2%	0.4%	0.6%
Tween-80	0.2%	0.4%	0.6%	0.7%	0.4%	0.2%	0.4%	0.5%	0.4%

Ingredients	**Quantity**								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Tolbutamide	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**	**10gm**
SLS (sodium dodecyl sulphate)	0.3%	0.3%	0.4%	0.3%	0.4%	0.6%	0.7%	0.8%	0.9%
Methyl Paraben	0.4%	0.5%	0.4%	0.2%	0.2%	0.4%	0.5%	0.6%	0.4%
Propylene glycol	0.2%	0.4%	0.4%	0.2%	0.2%	0.6%	0.4%	0.2%	0.2%
Glycerine	1%	1%	1%	1%	1%	1%	1%	1%	1%
Distilled Water	q.s. 100ml	q.s. 100ml	q.s. 100ml	q.s. 100ml	q.s. 100ml	q.s. 100ml	q.s. 100ml	q.s. 100ml	q.s. 100ml

Evaluation

Evaluation of nanoemulsion containing tolbutamide

Drug content

Table 4 : Drug content of formulations

Sr.no	Formulations	Drug content
1	F1	98.18%
2	F2	90.02%
3	F3	87.98%
4	F4	91.71%
5	F5	88.23%
6	F6	82.78%
7	F7	89.09%
8	F8	84.98%
9	F9	82.92%

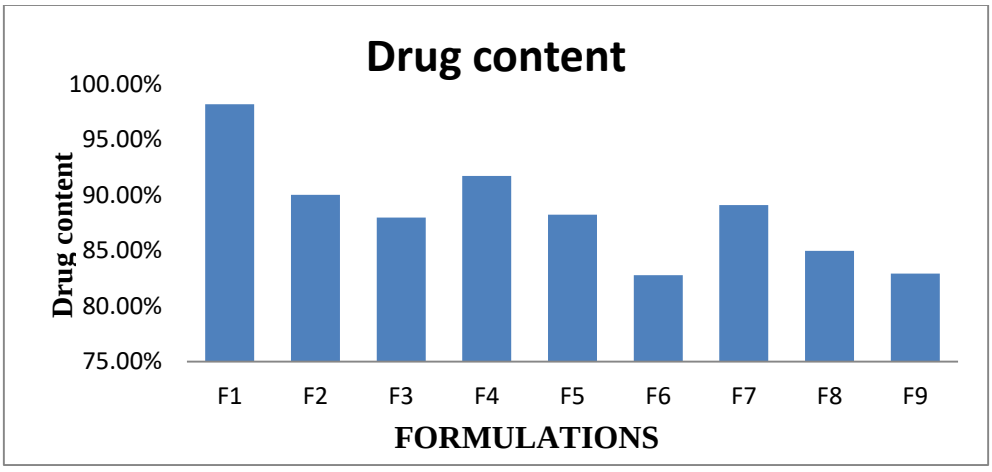

Figure 1 : Drug content of formulations

Discussion:

F1 formulation shows maximum drug content among the nine formulations. The drug content data of tolbutamide nanoemulsion ranges from 82.78% to 98.18% which is shown in table 4 and figure 1. F2 to F9 shows low drug cotent due to factors like inefficient mixing, encapsulation or stabilization. Larger particle or unevenly distributed particles may have smaller surface area. In contrast the F1 shows high drug content due to optimized formulation process include efficient formulation techniques such as proper mixing, encapsulation can lead to high drug content. Smaller and uniformly distributed particles can provide a larger surface area for drug absorption, increasing drug content.

Determination of Solubility:

Table 5 : Solubility of Tolbutamide in different Solvents

Sr. No.	Solvents	Solubility in (mg/ml)
1.	Water	18.65 ± 0.001
2.	Methanol	16.69± 0.004
3.	Phosphate buffer	33.45 ± 0.002
4.	PEG200	14.52 ± 0.001
5.	PEG400	15.34 ± 0.002
6.	Propylene glycol	26.73 ± 0.003

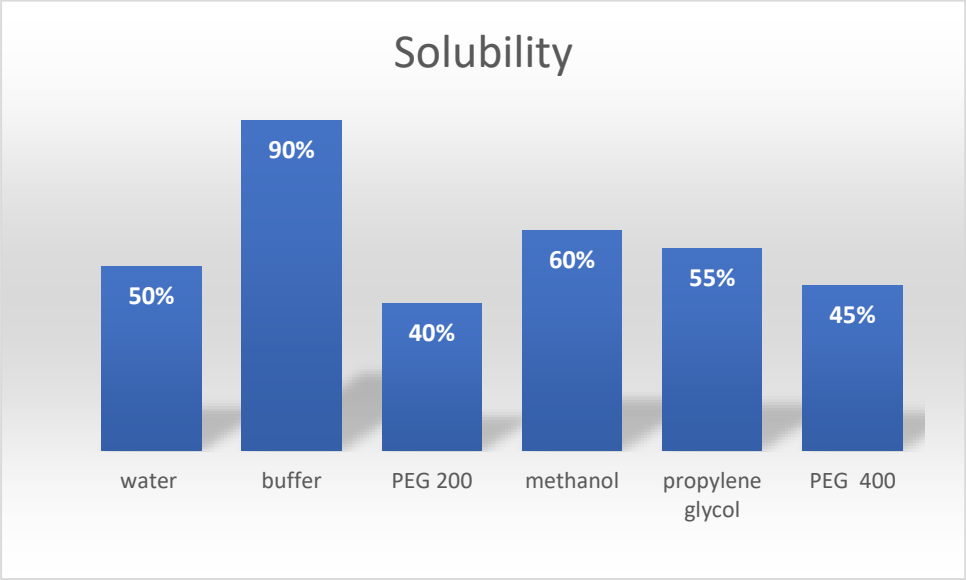

Figure 2 : Solubility studies of drug in different solvents

Discussion:

Tolbutamide exhibits varying solubility across solvents which were shown in figure 2 and values were given in table 5. The graph indicates that Tolbutamide is highly soluble in buffer solution, then methanol and then rest of the solvents. It is least soluble in PEG 200 and PEG 400.

pH :

Table 6 : pH of nanoemulsion containing Tolbutamide

Sr.no	Formulations	pH
1	F1	6.5
2	F2	5.5
3	F3	5.8
4	F4	5.3
5	F5	5.2
6	F6	6.1
7	F7	5.6
8	F8	6.4
9	F9	6.2

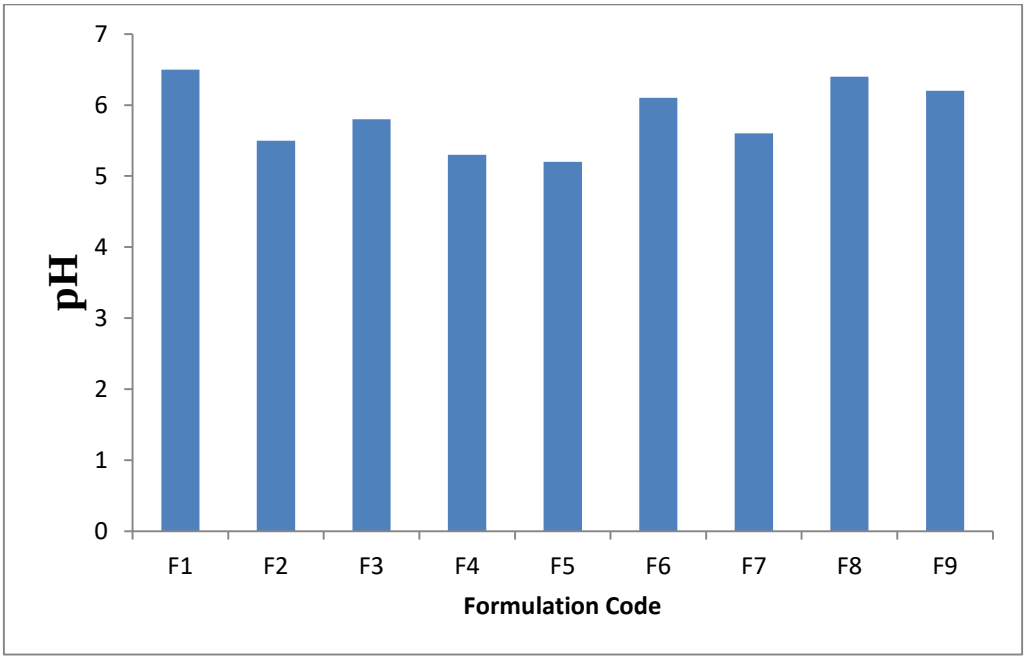

Figure 3 : pH of different formulations

Discussion :

The pH data of Tolbutamide nanoemulsion ranges from 5to7 as shown in table 6 and figure 3. F1 shows a good pH range of 6.5 which can be easily acceptable by GIT. Hence, does not cause any irritation to the mucosal membrane. And active ingredient of nanoemulsion is also more stable in this pH.

Viscosity :

A rotational viscometer of the Brookfield type can be used to measure the viscosity of LBFs with different compositions at different temperatures and shear rates. The samples to be tested must be submerged in it prior to testing, and a thermo bath must be used to keep the sample temperature at 37°C. To ensure reproducibility at a specific temperature, the viscometer used must be properly calibrated to measure the apparent viscosity of the suspension at equilibrium. Abbe's refractometer can be used to calculate the refractive index.

Table 7 : Viscosity of nanoemulsion containing Tolbutamide

Formulation Code	Viscosity(Cps)
F1	23.87±2.22
F2	28.01±2.09
F3	31.12±1.22
F4	45.23±1.45
F5	25.78±1.86
F6	29.32±1.45
F7	35.19±1.34
F8	32.13±0.31
F9	30.12±0.42

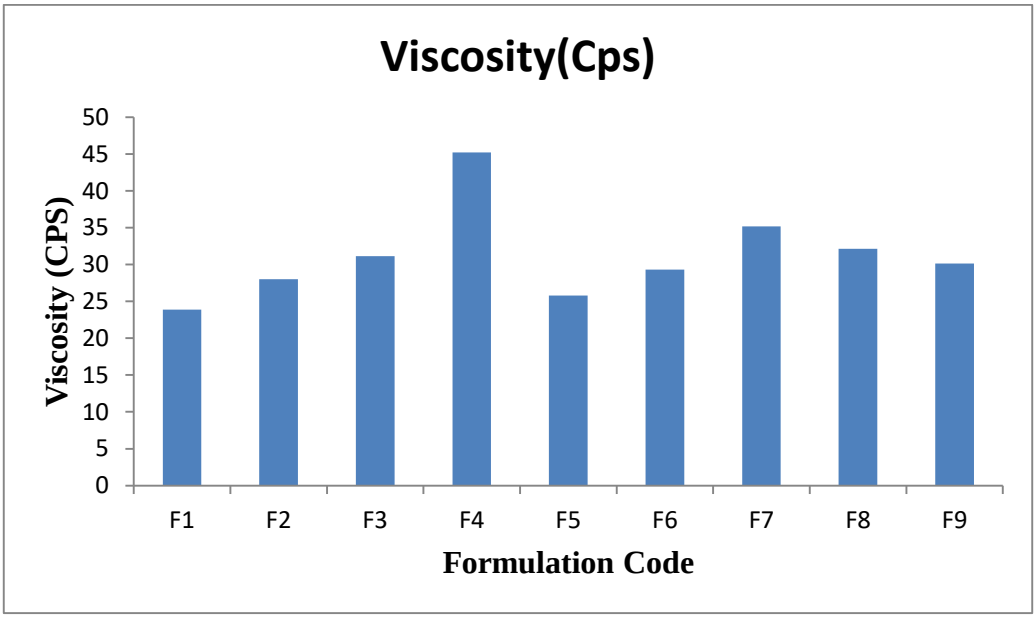

Figure 4 : Viscosity of nanoemulsion formulation

Discussion :

The rheological behaviour of all formulated nanoemulsion was studied using Brookfield viscometer at a speed of 50rpm and spindle number 63 was used. The viscosity of F1, F2, F3, F4, F5, F6, F7, F8, F9 were given in table 7 and their graphical representation in figure 4. The viscosity values indicated that the formulation are efficient to hold the nanoemulsion formulation. F1 formulation has low viscosity showed good flow properties as compared to other formulations.

SEM analysis of formulation : SEM analysis of formulation was performed to determine the surface morphology of drug.

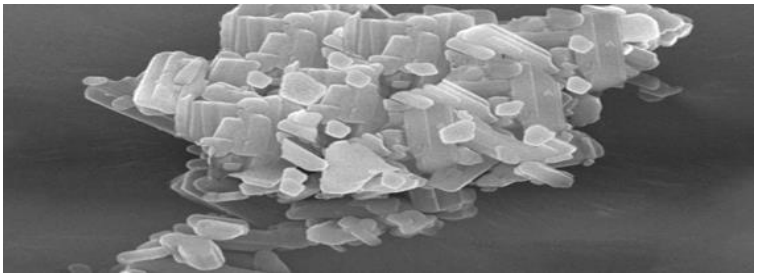

Fig 5 : SEM image of Tolbutamide of F1 formulation

Discussion : The preparation method for formulation 1 is probably designed to produce and maintain crystalline structure as shown in figure 5. The high zeta potential of F1 results in strong repulsive force, reducing aggregation and helping maintain a crystalline structure. While the other formulations likely contain an inferior mix of lipids that favours rod like structure. Variation in preparation conditions such as temperature, hydration rate or lack of sufficient shear forces could lead to rod like structures. Lower zeta potential results in weak repulsive forces, leading to more aggregation and less control over shapes. Hence, the best representation of scanning electron microscopy was found in F1 .

Zeta Potential Determination

Table 8 : zeta potential of formulation

Formulation Code	Zeta Potential(mV)
F1	-35.9±0.52
F2	-19.53±0.91
F3	-22.30±0.42
F4	-21.49±0.43
F5	-20.30±0.33
F6	-23.93±0.31
F7	-24.8±0.23
F8	-25.4±0.31
F9	-27.5±0.42

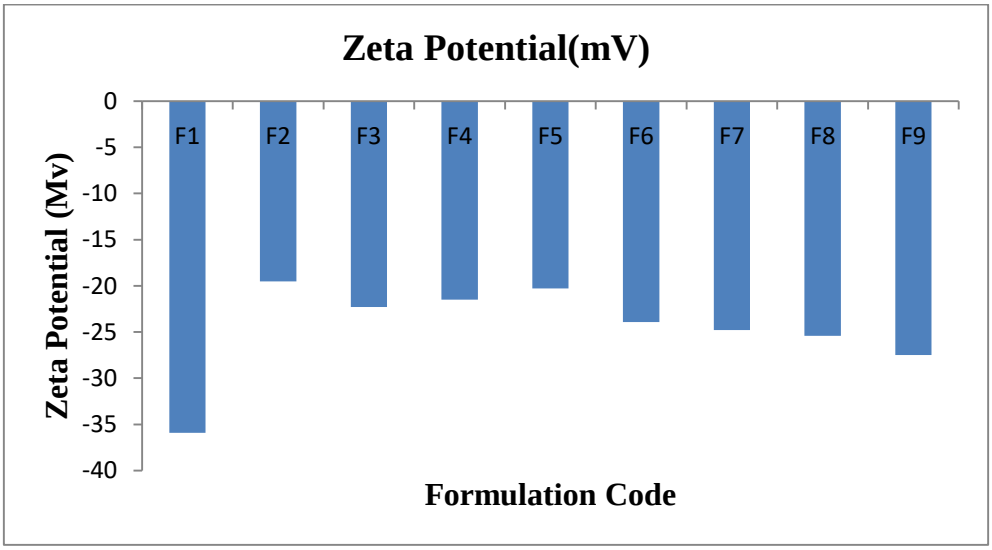

Figure 6 : Zeta potential of formulation

Zetasizer Nano-ZS was used to determine the zeta potential of Tolbutamide nanoemulsion. The developed nanosuspensions samples were diluted further, using the dispersion medium. The original samples of the produced nanosuspension were further diluted with water (1:1) and analysed. All the samples were analyzed in triplicate and measured mean ± S.D. Zeta potential was found to be -35.9 mV Negative value of zeta potential might be due to process of formulation phosphate buffer at pH 6.8 was used as the hydrating medium.Nanoemulsion is diluted for the purpose of evaluating zeta potential value which is determined by the oil globules electrophoretic mobility. Zeta potential should normally increase to a value over 30 mV to establish stable nanoemulsion by preventing the Nanodroplets from coalescing and flocculating.

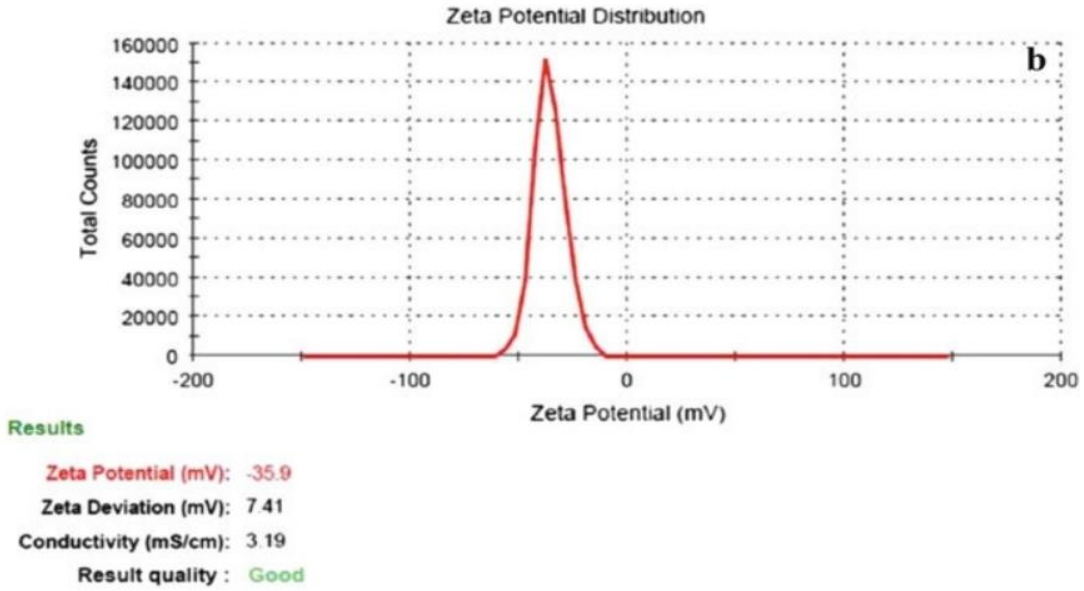

Figure 7 : Zeta potential of F1 formulation

Discussion :

Zeta potential data of nanoemulsion formulations are -35.9±0.52, -19.53±0.91, -22.30±0.42, -21.49±0.43, -20.30±0.33, -23.93±0.31, -24.8±0.23, -25.4±0.31 and -27.5±0.42 were F1, F2, F3, F4, F5, F6, F7, F8&F9 respectively. The high zeta potential of F1 is likely due to optimal values such as lipid composition, inclusion of charged molecules, the pH and ionic strength of the dispersion medium, presence of stabilizing agent and the preparation methods used. The type and concentration of lipids used in F1 might leads to a higher surface charge density. In contrast, while other formulations shows low zeta potential such as in F2, F3, F4, F5, F6, F7, F8 & F9 is likely due to sub optimal values such as lipid composition, presence of impurities, particle aggregation etc.

The best zeta potential was in the T2 (-35.9±0.52mV) ensures better colloidal stability, reduces risk of aggregation, and contributes to improved performance and longer shelf life of the formulation.

In vitro release studies of formulations:

In-vitro drug release studies revealed that the release of Tolbutamide from different formulations varies with characteristics and composition of excipients. All the nine formulations showed a chrono modulated pattern of drug release. The comparison of the drug release profile of all formulations showed that formulation F1 shows maximum drug release of 98.90%. And all the others release studies of formulations showed in Table no.5.13.

Table 9 : Invitro release studies of formulations

Sr.No.	Time	F1	F2	F3	F4	F5	F6	F7	F8	F9
1	10	20.89 ±0.92	18.10 ± 0.89	17.09 ±0.92	19.89 ±0.93	16.15 ±0.91	18.43 ±0.92	17.20 ±0.88	16.7 ±0.98	20.7 ±0.93
2	20	30.9± 0.81	27.90 ± 0.82	26.09 ± 0.81	27.89 ± 0.81	23.05 ± 0.78	24.89 ± 0.90	23.9± 0.82	27.02 ± 0.82	27.04 ± 0.82
3	30	46.89 ± 0.71	40.80 ± 0.71	39.09 ± 0.69	41.82 ± 0.68	40.12 ± 0.71	42.89 ± 0.71	32.4± 0.71	36.98 ± 0.74	41.67 ± 0.71
4	40	54.8± 0.65	50.78 ± 0.71	51.98 ± 0.65	47.01 ± 0.65	45.9± 0.82	58.93 ± 0.81	42.23 ± 0.65	48.01 ± 0.65	49.09 ± 0.71
5	50	60.01 ± 0.81	56.90 ± 0.81	58.09 ± 0.84	55.78 ± 0.71	54.34 ± 0.74	59.34 ± 0.68	58.8± 0.81	55.01 ± 0.81	54.54 ± 0.81
6	60	74.9± 0.51	64.90 ± 0.68	65.34 ± 0.68	65.9± 0.67	67.65 ± 0.74	64.66 ± 0.61	62.08 ± 0.54	62.01 ± 0.51	65.98 ± 0.51
7	70	89.98 ± 0.78	77.90 ± 0.68	74.67 ± 0.73	69.08 ± 0.73	74.67 ± 0.78	69.9± 0.78	67.98 ± 0.78	75.12 ± 0.72	73.09 ± 0.78
8	80	95.99 ± 0.81	89.89 ± 0.81	77.78 ± 0.87	78.54 ± 0.72	82.66 ± 0.87	76.01 ± 0.87	73.09 ± 0.72	79.01 ± 0.87	82.08 ± 0.87
9	90	98.90 ± 0.81	92.76 ± 0.81	84.80 ± 0.91	86.8± 0.91	88.9± 0.91	83.09 ± 0.91	79.03 ± 0.94	87.09 ± 0.91	87.01 ± 0.91

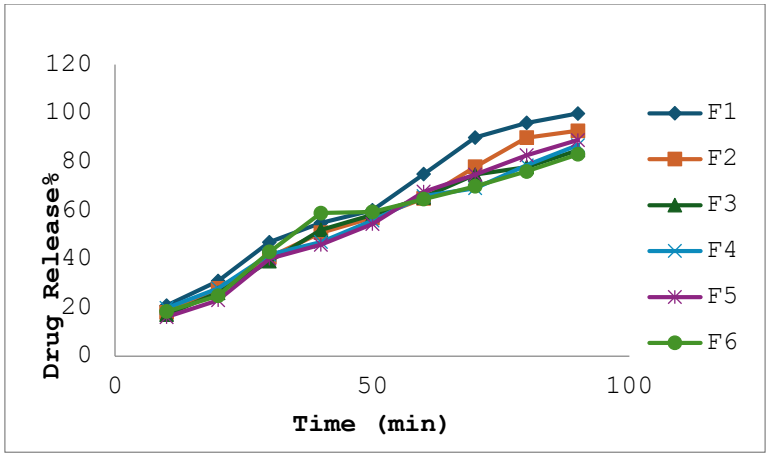

Figure 8 : Dissolution profile of Formulations F1 to F6.

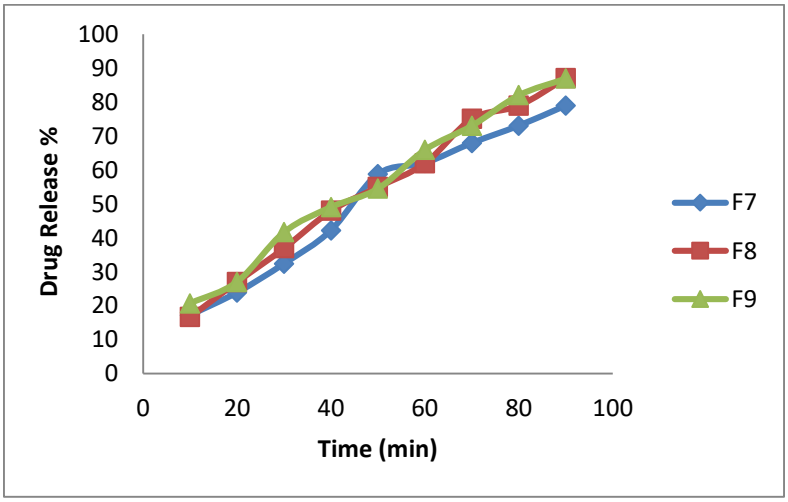

Fig 9 : Dissolution profile of Formulations F7 to F9

Discussion :

From table 9 and figures 8 and 9 the invitro drug release pattern of initial burst release of surface adsorbed drug was observed followed by slow and sustained release of drug from nanoemulsion. The initial burst effect on the surface release of tolbutamide may be due to the loosely associated tolbutamide. The burst release is clinically significant to achieve initial high drug concentration in the target tissue. The slow release of drug is controlled by the speed of degradation of polymer.

In vitro kinetics release:

Table 10 : In vitro kinetic release

Time (min)	Square root of Time (min)	Log Time	Cumulative % drug release	Log Cumulative % drug release	Cumulative % drug remaining	Log cumulative % drug remaining
0	0	0	0	0	0	0
10	3.16	1	20.89	1.31	79.11	1.89
20	4.47	1.30	30.9	1.48	69.10	1.83

30	5.477	1.47	46.89	1.67	53.11	1.75
40	6.324	1.60	54.8	1.73	45.20	1.65
50	7.07	1.69	60.01	1.77	39.99	1.60
60	7.74	1.77	74.9	1.87	25.10	1.39
70	8.366	1.84	89.98	1.95	10.02	1.00
80	8.94	1.90	95.99	1.98	4.01	0.60
90	9.48	1.95	98.9	1.99	1.1	0.04

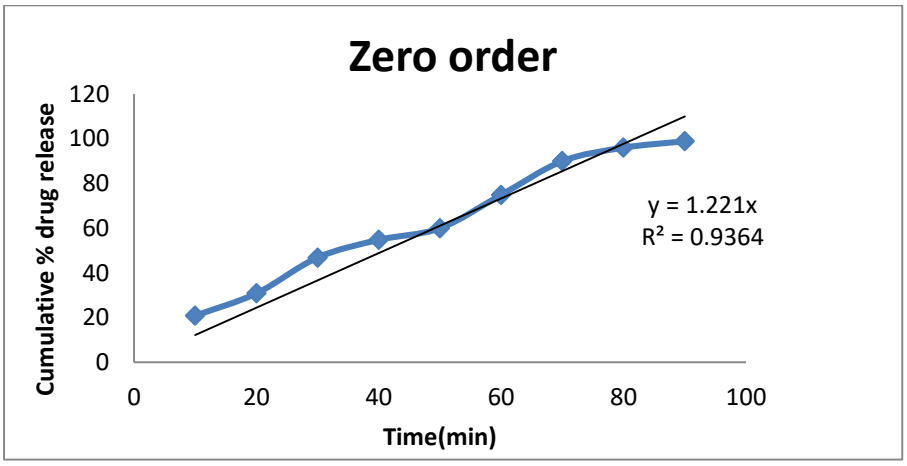

Figure 10 : Zero order release kinetics of formulation F1

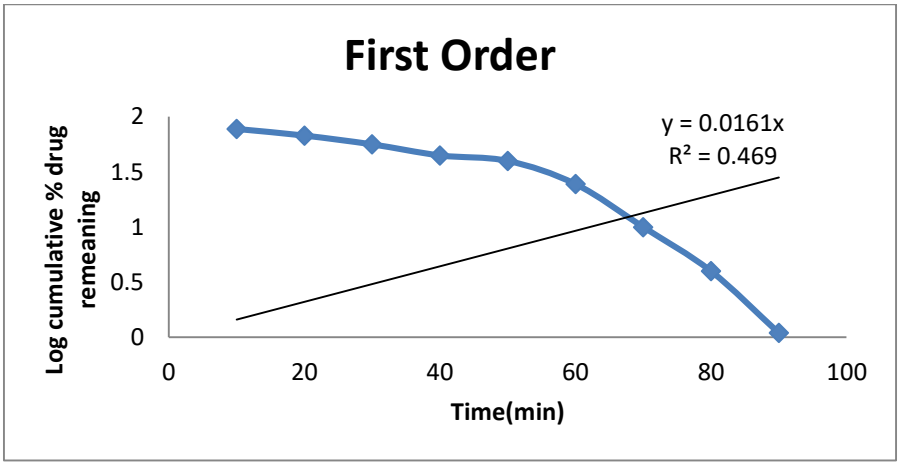

Figure 11 : First order release kinetics of formulation F1

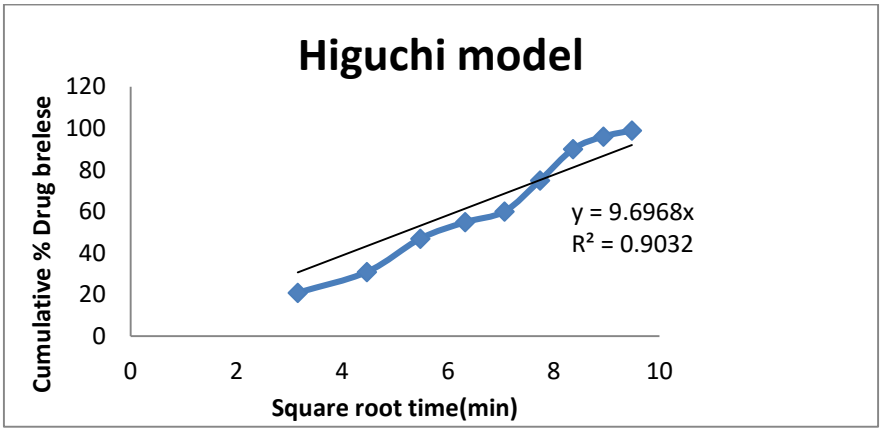

Figure 12 : Higuchi order release kinetics of formulation F1

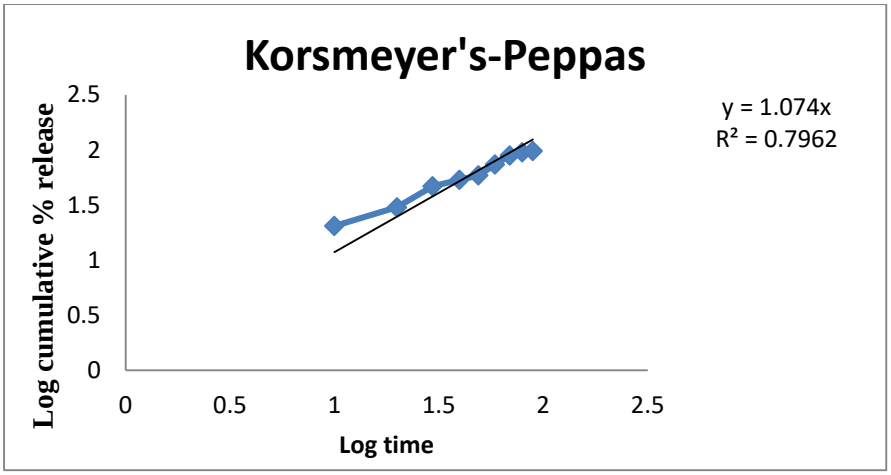

Figure 13 : Korsmeyer's-Peppas order release kinetics of formulation F1

Discussion : In case, R^2 value was calculated from the graph and reported in table 5.23 and figures as such figure 22 representing the zero order release kinetics, figure 23 showing first order release kinetics, figure 24 showing Higuchi order release kinetics and figure 25 showing korsmeyers peppas order release. Considering the determination coefficients, Korsmeyers peppas model was found (R^2 = 0.9968) to best fit the release data.

Stability studies:

Table 11 : Stability study of drug content of formulation F1

Duration(days)	Percentage of drug content at 4±2ºC	Percentage of drug content at 25±2ºC/60%±5%RH	Percentage of drug content at 40±2ºC/75%±5%RH
0	97.18±0.543	97.15±0.545	97.09±0.119
30	97.18±0.528	97.11±0.524	97.03±0.111
60	97.16±0.509	97.08±0.498	97.01±0.108
90	97.13±0.501	97.06±0.477	97.00±0.102
120	97.11±0.490	97.03±0.465	98.70±0.098
150	97.09±0.461	97.01±0.432	98.56±0.083
180	97.05±0.446	97.00±0.405	98.20±0.072

Table 12 : Stability studies of in vitro drug release of Tolbutamide nanoemulsion formulation F1 at 4±2ºC

Time (min)	Cumulative drug release at 4±2ºC						
	0days	30days	60days	90days	120days	150days	180days
0	0	0	0	0	0	0	0
10	20.89 ±0.09	20.63 ±0.10	20.20 ±0.08	20.08 ±0.84	19.87 ±0.89	19.69 ±0.09	19.54 ±0.89
20	30.9± 0.08	30.1± 0.09	29.9± 0.075	29.01± 0.09	28.9± 0.08	28.05± 0.076	28.00± 0.08
30	46.89± 0.071	45.08± 0.081	45.00± 0.071	44.87± 0.071	44.78± 0.071	44.59± 0.071	46.32± 0.071

40	54.8± 0.065	53.30± 0.075	52.88± 0.062	52.65± 0.065	52.43± 0.060	52.18± 0.065	54.01± 0.065
50	60.01± 0.081	60.00± 0.091	59.51± 0.080	59.21± 0.081	59.01± 0.071	58.81± 0.081	58.21± 0.081
60	74.9± 0.051	73.9± 0.061	73.40± 0.051	73.19± 0.051	73.01± 0.051	72.90± 0.051	72.69± 0.051
70	89.98± 0.078	89.12± 0.058	89.00± 0.088	88.98± 0.078	88.68± 0.079	88.41± 0.078	88.24± 0.078
80	95.99± 0.087	95.09± 0.047	95.01± 0.087	94.99± 0.087	94.67± 0.087	94.29± 0.087	94.09± 0.087
90	98.90± 0.091	98.48± 0.061	98.13± 0.081	98.01± 0.090	97.71± 0.087	97.50± 0.079	97.15± 0.082

Table 13 : Stability studies of in vitro drug release of formulation F1 at 25ºC

Time (min)	Cumulative drug release at 25±2ºC/60%±5%RH						
	0days	30days	60days	90days	120days	150days	180days
0	0	0	0	0	0	0	0
10	20.89 ±0.09	20.54 ±0.10	20.35 ±0.08	20.15 ±0.84	19.56 ±0.89	19.29 ±0.09	19.10 ±0.89
20	30.9± 0.08	30.0± 0.09	29.50± 0.075	29.00± 0.09	28.60± 0.08	28.15± 0.076	28.00± 0.08
30	46.89± 0.071	45.34± 0.081	45.07± 0.071	43.87± 0.071	43.78± 0.071	42.59± 0.071	42.32± 0.071
40	54.5± 0.065	53.10± 0.075	52.98± 0.062	52.55± 0.065	52.27± 0.060	52.08± 0.065	51.91± 0.065
50	60.01± 0.081	59.00± 0.091	58.91± 0.080	59.21± 0.081	59.01± 0.071	58.81± 0.081	58.21± 0.081
60	74.9± 0.051	72.90± 0.061	72.40± 0.051	72.19± 0.051	72.01± 0.051	71.90± 0.051	71.69± 0.051
70	89.98± 0.078	88.12± 0.058	88.09± 0.088	87.98± 0.078	87.68± 0.079	87.41± 0.078	87.24± 0.078
80	95.99± 0.087	94.09± 0.047	94.01± 0.087	93.99± 0.087	93.67± 0.087	93.29± 0.087	93.09± 0.087
90	98.70± 0.091	98.48± 0.061	97.53± 0.081	97.01± 0.090	96.71± 0.087	96.50± 0.079	96.15± 0.082

Table 14 : Stability studies of in vitro drug release of formulation F1 40±º2C

Time (min)	Cumulative drug release at 40±2º/75%±5%CRH						
	0days	30days	60days	90days	120days	150days	180days
0	0	0	0	0	0	0	0
10	19.89	19.54	19.35	19.15	19.01	18.89	18.10

	±0.09	±0.10	±0.08	±0.84	±0.89	±0.09	±0.89
20	29.9± 0.08	29.00± 0.09	28.50± 0.075	28.00± 0.09	27.60± 0.08	27.15± 0.076	26.00± 0.08
30	45.89± 0.071	43.34± 0.081	43.07± 0.071	42.87± 0.071	42.78± 0.071	41.59± 0.071	41.32± 0.071
40	54.5± 0.065	53.10± 0.075	52.98± 0.062	52.55± 0.065	51.27± 0.060	51.08± 0.065	50.91± 0.065
50	59.01± 0.081	57.00± 0.091	56.91± 0.080	56.21± 0.081	56.01± 0.071	55.81± 0.081	55.21± 0.081
60	74.9± 0.051	72.90± 0.061	72.40± 0.051	72.19± 0.051	72.01± 0.051	71.90± 0.051	71.69± 0.051
70	88.98± 0.078	86.12± 0.058	86.09± 0.088	85.98± 0.078	84.68± 0.079	83.41± 0.078	82.24± 0.078
80	94.99± 0.087	94.09± 0.047	94.01± 0.087	93.99± 0.087	93.67± 0.087	93.29± 0.087	93.09± 0.087
90	97.70± 0.091	95.48± 0.061	95.53± 0.081	94.01± 0.090	93.71± 0.087	93.50± 0.079	92.15± 0.082

Discussion : Stability of nanoemulsion containing tolbutamide drug was carried out for 180 days at 4±2ºC, 25±2ºC/60%±5%RH, 40±2ºC/75%±5%RH. Responses obtained for different parameters for nanoemulsion during stability period. Nanoemulsion was found to be reasonably stable in terms of aggregation, fusion, shelf life over the studied storage period.
Stability studies for F1 formulation were carried out at 4±2ºC, 25±2ºC/60%±5%RH, 40±2ºC/75%±5%RH for a period of 180 days. Stability studies performed for nanoemulsion indicates that the prepared formulation shows that there was no significant variation found in physical appearance, but slightly decreases % drug content and cumulative % drug release of the nanoemulsion formulation F1 as the temperature increases. The results conclude that the F1 formulation is stable after stability study for 180 days.

DSC thermogram of Tolbutamide:

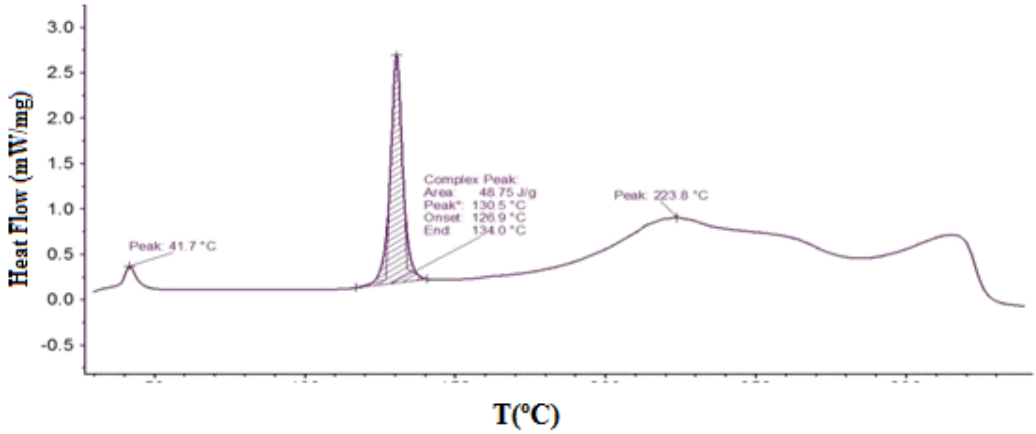

Figure 14 : Differential scanning calorimetry thermogram of Tolbutamide

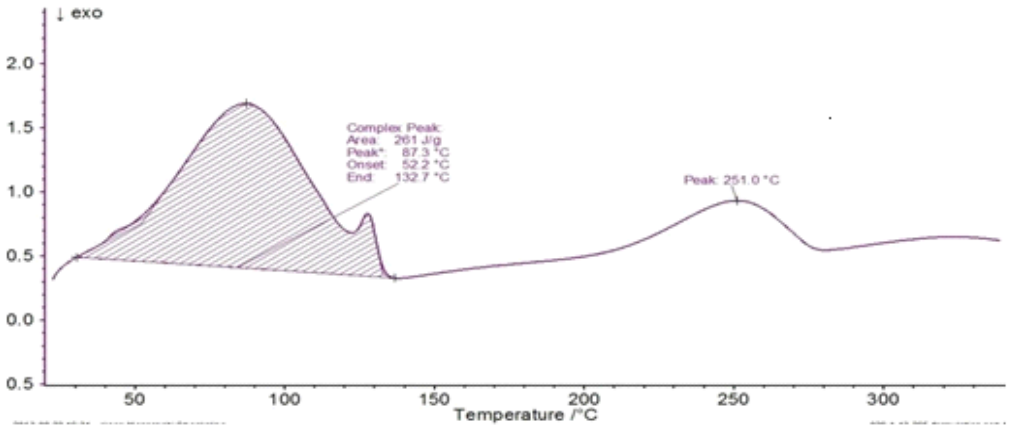

Figure 15 : Differential scanning calorimetry thermogram of Tolbutamide nanoemulsion F1.

Discussion:

In the DSC curve of pure Tolbutamide a sharp endothermic peak was observed at 130.5ºC corresponding to its melting point. The DSC analysis of physical mixtures of drug, surfactant and oil demonstrated negligible change in the melting point of Tolbutamide in presence of any of the surfactant and oil mixture studied. The DSC thermogram are illustrated in figure 14 and 15.

CONCLUSION:

The main objective of this work was to formulate and evaluate of nanoemulsion containing antidiabetic drug Tolbutamide to enhance the solubility, bioavailability and improves the dissolution rate of drug. Tolbutamide belongs to BCS class second i.e., poorly water soluble. The dissolution rate of the drug was increased by formulating the drugs in the form of nanoemulsion. In nut shell, it was concluded that bioavailability of drug Tolbutamide was increased by the formulation of nanoemulsion. F1 formulation has highest drug content with 98.23%. All the nine formulations showed a chronomodulated pattern of drug release. The comparison of the drug release profile of all formulations showed that formulation F1 shows maximum drug release.

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