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COMPOSITION OF LIPID BASED NANOEMULSION FOR ORAL DELIVERY OF ORLISTAT

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ABSTRACT: Aim: The present work focused on to formulate, evaluate and optimize lipid based nanoemulsion of orlistat to enhance drug release. **Materials and Methods:** Nanoemulsion was prepared using Olive oil, Tween 80, and Distilled water as components. Lipid based nanoemulsion was evaluated for its pH, Rheology study, zeta potential, conductivity, particle size analysis, transmission electron microscopy (TEM) and stability. Central composite design was utilized for the optimization purpose. Formulation variables such as the concentration of Oil (ml) (X1) and Water (ml) (X2) were investigated for their effect on viscosity (Y1) and drug content (Y2). Optimized formulation evaluated for the various parameters. **Result and Discussion:** The responses Y1 and Y2 for the optimized formulation were found to be 0.167 cps and 99%. Orlistat release from the optimized formulation was faster than other formulations obtained from DOE. Increased in vitro drug release of the drug from lipid based nanoemulsion suggests that the nanoemulsion could serve as potential formulation strategy for Orlistat. **Conclusion:** The lipid based nanoemulsion can be used as a possible alternative to traditional formulations of orlistat to improve its dissolution rate leading to enhanced bioavailability.

Keywords: Orlistat, lipid based nanoemulsion, central composite design.

Introduction

It is assessed that more than 1 billion adults around the world are overweight and at least one third of this population are classified as obese. While genetic predisposition, age, and environmental factors may contribute to a person's tendency to gain weight, it is accepted that the two primary causes of obesity are increased intake of energy-rich foods and reduced physical activity. Overweight and obesity have been important public health problems throughout the world, affecting both developed societies and developing countries. Orlistat, a potent, specific, long-acting and reversible inhibitor of lipases, is a member of a new

class of drugs available for the treatment of obesity. Orlistat plus diet has frequently demonstrated significantly greater weight loss, when compared to placebo plus diet. Moreover, effects of orlistat are meaningful and meet the FDA standards of efficacy for prescription weight control drugs. Orlistat is a reversible inhibitor of lipases. It exerts its therapeutic activity in the lumen of the stomach and small intestine by forming a covalent bond with the active serine residue site of gastric and pancreatic lipases. The inactivated enzymes are thus unavailable to hydrolyze dietary fat in the form of triglycerides into absorbable free fatty acids and monoglycerides. As undigested triglycerides are not absorbed, the resulting caloric deficit may have a positive effect on weight control. The adverse effects associated with Oily Spotting, Flatus with Discharge, Fecal Urgency, Fatty/Oily Stool, Oily Evacuation, Increased Defecation, Fecal Incontinence These and other commonly observed adverse reactions were generally mild and transient, and they decreased during the second year of treatment. on an oral 14 orlistat mass balance study in obese patients, two metabolites, M1 (4-member lactone ring hydrolyzed) and M3 (M1 with N-Formyl leucine moiety cleaved), accounted for approximately 42% of total radioactivity in plasma. M1 and M3 have an open β-lactone ring and extremely weak lipase inhibitory activity (1000-and 2500-fold less than orlistat, respectively). In view of this low inhibitory activity and the low plasma levels at the therapeutic dose (average of 26 ng/mL and 108 ng/mL for M1 and M3, respectively, 2 to 4 hours after a dose), these metabolites are considered pharmacologically inconsequential. The primary metabolite M1 had a short half-life (approximately 3 hours) whereas the secondary metabolite M3 disappeared at a slower rate (half-life approximately 13.5 hours). In obese patients, steady-state plasma levels of M1, but not M3, increased in proportion to orlistat doses. Nanoemulsions are a colloidal particulate system in the submicron size range acting as carriers of drug molecules. These carriers are solid spheres and their surface is amorphous and lipophilic with a negative charge. Magnetic nanoparticles can be used to enhance site specificity[1]. As a drug delivery system they enhance the therapeutic efficacy of the drug and minimize adverse effect and toxic reactions. Major application includes treatment of infection of the reticuloendothelial system (RES), enzyme replacement therapy in the liver, treatment of cancer, and vaccination[2-3]. The present work attempts to prepare orlistat based nanoemulsion based on several components like oil, surfactants, co surfactants etc. These types of components while enhancing the bioavailability, and gives the composition can be any of these types are more soluble in water absorption characteristics[4-6].

The present work is achieved by nano-emulsion-type compound, is not made of several of these ingredients separately and then simply nanoemulsion mixture, taking into account the stability of the whole system, the best ratio of individual components, the use of Solvent compatibility problems. Poor water solubility of drug is major challenge amongst researchers. Lipid based nanoemulsion have the potential to improve the poor solubility of drugs due to their nanomeric size, large surface area; high drug entrapment efficiency, high drug loading, long term stability and interaction of phase at inter-phase developed them with enhanced solubility and bioavailability.

Material and method

Analytical grade materials were used for study. Orlistat (Supreem pharmaceuticals Mysore PVT LTD , Mysur) were received as gift sample. Ecalyptus oil, Olive oil, Cotton seed oil, Castor oil, Polyethylene glycol 400, 600, Glycerol monostearate(Research lab fine chemical industry, Mumbai). All other chemicals and reagent were of analytical grade and were use without further purification.

Screening and selection of potential oil-in-water nanoemulsion components (oils and surfactant) [9]

Screening of Oil

The solubility of Orlistat in various oils was determined by adding an excess amount of drug in 2 mL of the oils (Ecalyptus oil. Olive oil, Cotton seed oil, Castor oil,) separately in 5-mL-capacity stopper vials, and mixed using a vortex mixer. The mixture vials were then kept at 25 ± 1.0°C in an isothermal shaker (Nirmal International, Delhi, India) for 72 h to reach equilibrium. The equilibrated samples were removed from the shaker and centrifuged at 3,000 rpm for 15 min. The supernatant was taken and filtered through a 0.22-µm membrane filter. The concentration of Orlistat was determined in oils using a HPLC method.

Screening of Surfactants

Five types of surfactants were screened for nanoemulsion formulation, which included Labrasol, Cremophor EL, Tween 20, 80 and Span 20,80. In water, 2.5 mL of 15 wt.% surfactant solution was prepared, and 4 µL of oil was added with vigorous vortexing. If a one-phase clear solution was obtained, the addition of the oil was repeated until the solution became cloudy.

Central composite experimental design[8-11]

The objective of the present study selected as the potential to improve the poor solubility of drugs. Hence, Central composite statistically designed with 2 factors, 2 levels, and 13 run was selected to statistically optimized the formulation parameters and evaluate the main, quadratic, and interaction effects of the preparations on the drug content and entrapment efficiency of Nanoemulsion. 2 factors, 2 level designs were used to explore the quadratic response surface and for constructing the polynomial models thus helping in optimizing a process using a small number of experimental runs. The independent and dependent variables are listed in Table 1. The polynomial equation generated by this experimental design is as follows:

$$Y_i=b_0+b_1X_1+b_2X_2+b_{12}X_1X_2+b_{21}X_2X_1+b_{11}X_1^2+b_{22}X_2^2$$

Where, Y_i is the dependent variables, b_0 is the intercept, b_1 to b_2^2 are the regression coefficients computed from the observed experimental values of Y from experimental runs; X_1 and X_2 are the independent variables that were selected from the preliminary experiments. $X_1 = (A-X_0)/\Delta X$; X_1 = Coded value of the variables A; X_0 =Value of A at the center point; ΔX = Step change and so on where A, B, etc., are the input variables.

Independent variables were Oil (X_1) and Distilled water (X_2). The dependent variables were Viscosity (Y_1) and Drug Content (Y_2). The range of independent variables in study table1 along with their low, medium, and high levels, which were selected based on the result from preliminary experiments. The computation

for optimized formulation was carried using Version 10 software. Optimization was performed to find out the level of independent variables (X1 and X2) that would yield a maximum value of viscosity constraints on drug content.

Statistical analysis

Statistical analysis of the Central Composite design batches was performed by multiple regression analysis using Design-Expert® Version 10 Software. The models were evaluated in terms of R2 values, and statistically significant coefficient and various feasibility and grid searches were conducted to find the optimum parameters. To graphically demonstrate the influence of each factor on the response, the response surface plot was generated using the Design-Expert® Version 10 Software.

Optimization data analysis

The computation for optimized formulation was carried using Design-Expert® Version 10 Software. The optimized formulation was obtained by applying constraints (goals) on dependent and independent variables. After developing the polynomial equation for the response Viscosity and Drug Content with the independent variables, the formulation was optimized for the both responses. Optimization was performed to find out the level of independent variables (X1 and X2) that would yield a maximum value of Viscosity constraints on Drug Content.

FORMULATION OF OPTIMIZED NANOEMLSION[12-14]

An optimized formula was obtained by Design-Expert® Version 10 Software. Each batch contains 300 mg of Orlistat, 25ml of olive oil and 22.5 ml disttiled water. Weighed quantity of Orlistat was added to olive oil and this was transferred to mortar. 2.5 ml polysorbate 80 was dissolved in Olive oil to room temperature. Water was added slowly to the oil with continuous stirring for 15 minut. A homogenizer used for the formulation Sand Panda (Japan) High-pressure Homogenizer. It consists of a blender that raises the speed of blender to a range of 1000 rpm for 5min. Nanoemulsion formulation were prepared by adding 300 mg drug in Olive oil, Surfactant (Tween -80) and Distilled Water and homogenizer it by used of high-pressure homogenizer formulation of lipid based nanoemulsion takes place.

Characterization

Rheology[15,16]

All rheological tests were performed by using Brookfield R/S-CPS + Rheometer. Measurements were carried out by using plate-plate type instrument and by using C75-2 spindle at room temperature. A gap of 0.5 mm was kept between two plates. A fresh sample was loaded at each run. For all rheological tests a common procedure was used. Rheometer was calibrated to give a gap of 0.5 mm between the two plates of Rheometer. About 2 gm of performed by using plate-plate type instrument

Viscosity[18]

Viscosity assessment is an important parameter for physicochemical characterization of nanoemulsion. Various instruments are employed for measuring viscosity such as Ostwald viscometer, Hoeppler falling ball viscometer, Stormer viscometer, Brookfield viscometer and Ferranti-Shirley viscometer. Among all these viscometer, Brookfield is the preferred one for measuring the viscosity of nanoemulsion. Determination of viscosities affirms whether the system is O/W or W/O emulsion. Low viscosity of systems shows that it is O/W type and high viscosity shows that it is water in oil type system. The viscosity of the formulation was determined by using Brookfield viscometer with spindle C75-2 at room temperature. The viscosity of formulation was measured at decreased and increased (100-1.0-100sec-1) shear rates for 150.0 sec.

Drug Content[19-20]

Drug content was determined by1 mL of detailing was broken up in 10 ml of Methanol and volume was made up to 10 ml with Methanol. The arrangement was sifted and from filtrate 1 ml was taken and made up to 10 ml with Methanol and drug content was estimated using ultraviolet (UV)-visible (Cary 60, 2100, Agilent Technology, Germany) spectrophotometric method at 245 nm.

Conductivity[20]

The conductance of nanoemulsion is measured by a conductometer. In this test a pair of electrodes connected to a lamp and an electric source is dipped into an emulsion. If the emulsion is o/w type, water conducts the current and lamp gets lit due to passage of current between the electrodes. The lamp does not glow when the emulsion is w/o: oil being in external phase does not conduct the current.

pH [23]

nanoemulsion formulation was checked using pH meter. The pH meter was calibrated before use in formulations using pH 4 and pH 7 standard buffer solutions. The pH meter electrode was immersed in 10% nanoemulsion and pH (Mettler Toledo MP 220, Greifensee, Switzerland) in triplicate at 25°C.)was recorded.

Zeta potential[24]

Zeta potential was determined by sympatec GmbH zetasizer instrument. The zeta potential of the diluted nanoemulsion formulae was determined using Zetasizer (Malvern Instruments, UK). Samples were placed in clear disposable zeta cells and results were recorde.

Globule size analysis[25]

Globule size determination by digital microscope and Zetasizer. 1 ml nanoemulsion was diluted to 1 ml with distilled water. A sample was placed on glass slide and mean globule size of resulting emulsions determined by digital microscope (LABOMED microscopy). The formulation (0.1 ml) was dispersed in 50

ml of water in a volumetric flask and gently mixed by inverting the flask. Measurement was done using a Zetasizer 1000 HS (Malvern Instrument, UK). Light scattering was monitored at 25°C at a 90° angle

Drug Release study[8]

Release of orlistat from Nanoemulsion was studied using the dialysis (Slide-A-Lyzer, 3500 MWCO, Thermo-Scientific) method at 37 ± 2°C and was compared with the pure drug solution. Briefly, the dialysis bags were first hydrated for 30-60 min with PBS (pH 7.4) and Nanoemulsion (500 µL) were loaded carefully using a syringe without puncturing the dialysis membrane. The prepared dialysis bags were placed in the fourteen-station USP XXVII type II (paddle) apparatus at 37°C ± 0.5°C and 100-rpm speed. The dissolution studies were carried out in 900 ml, 1% SLS & 0.5 % NaCl. From the release medium and the same volume was replaced with fresh medium.[15,33-35] The sample was analyzed at 245 nm using the UV-visible spectrophotometer (Shimadzu Corporation, Japan).

STABILITY STUDIES [21,22]

1. Ageing and Temperature test

A container containing formulation was placed on reciprocating shaker (approximately 60 cycles per minute at room temperature for 24 hrs).

2. Centrifugation test

Those formulations that passed were centrifuged at 3500 rpm for 30 min using centrifuge. The formulations that did not show any phase separation were taken for further tests.

Freeze thaw cycles

Three freeze-thaw cycles were carried out between refrigerator temperature, 25°C and 40^0C with storage of formulations at each temperature for not less than 24 hrs.

1. **Accelerated Stability Study**

Samples of optimized formulation were kept in refrigerator and programmable environmental chamber for three months at 5°C ± 2°C, 25°C and elevated temperature 45°C ± 2°C. Samples were withdrawn at initial/0, 1, 2 and 3 months from the time of placing sample into the chamber.

RESULTS AND DISCUSSION

Screening of Material

From the solubility study, we selected oils and three surfactants for further study for selection of surfactants, it is necessary to have good emulsification ability along with good drug solubility. For this reason, selected three surfactants were screened for their emulsification ability with orlistat. In emulsification study of surfactants, four combinations were evaluated for ease of emulsification and percent transmittance. After the screening for emulsification study, Tween 80 (surfactant) showed maximum transmittance hence selected for further study.

FORMULATION OF NANOEMULSION

Nanoemulsions batches were prepared by homogenization techniques which required two immiscible phases' oil and aqueous phase with an emulsifier helped in the formation of an emulsion by reducing the interfacial tension. Oils used were Castor oil, Cotton seed oil, Olive oil, Eucalyptus oil, Coconut oil and surfactant were span 20, span80, tween20 and tween80 this all excipients were uses in the formulation of nanoemulsion and Aqueous phase was injected to the oily phase with continuous homogenization at 2500 rpm for 6 h.

Design of experiment

The traditional approaches to developing a formulation are to change one variable at a time. By this method, it is difficult to develop an optimized formulation, as the method reveals nothing about the interaction among the variables. The use of experimental design allows for testing a large number of factors simultaneously and precludes the use of a huge number of independent runs when the traditional step-by-step approaches are used. Systematic optimization procedures are carried out by selecting an objective function, finding the most important or contributing factor, and investigating the relationship between response and factors by the so-called surface response methodology. Nanoemulsion were prepared by homogenizing method and optimized the process using Central Composite experimental design. The objective functions for the present study were selected as maximizing the Viscosity and drug content as responses depending on three independent variables concentration of oil and water at three different levels. Hence, Central Composite statistical design with 2 factors, 2 levels, and 13 runs was selected to statistically optimize the formulation parameters and evaluate the main, interaction, and quadratic. Response surface optimization of camouflaged effects of the formulation ingredients on the Viscosity and drug content of nanoemulsion.2-factor, 2-level design was used to explore the quadratic response surfaces and for constructing polynomial models thus helping in optimizing a process using a small number of experimental runs. Statistical analysis of the Central composite design batches was performed by multiple regression analysis using Design-Expert® Version 10 Software. The contribution of each factor with different levels to the response was evaluated with two-way analysis of variance (ANOVA). The models were evaluated in terms of statistically significant coefficients and R2 values.[26] The experimental design consists of a set of points lying at the midpoint of each edge and the replicated center point of the multidimensional cube.

Data analysis

All the batches of prepared within the experimental design yielded Nanoemulsion, and these were evaluated for Viscocity and Drug content. The Central composite experimental design has the advantages of requiring fewer experiments (13 batches) than would a 32 full factorial design. Transformed values of all the batches were shown in Table 1. The all selected dependent variables obtained at various levels of the two independent variables (X1 and X2) were subjected to multiple regression to yield a second polynomial equation

Probability plots

Normal probability graph explains the whether the residuals follow a normal distribution, in which case the points will follow a straight line. Expect some scatter even with normal data. Look only for definite patterns like an "S-shaped" curve, which indicates that a transformation of the response may provide a better analysis, the plot shown by viscosity and drug content [Figures 1]. From this concluded that the normal probability distribution the blue spot indicates the nonsignificant effect on variable distributed around the straight line. [26]

Contour plot

Two-dimensional contour plots [Figures 2] are useful to study the single and interaction effect of the factor on the responses at one time and the third factor was kept at a constant level. All the relationships among the three variables are linear up to certain range the effect of X1 and X2 with their not interaction on viscosity at a fixed or the level X1. The plots were found to be linear up to 95.96% indicating a linear relationship between X1 and X2. Similarly, all values for reminded dependent variables. An optimum value of drug content could be obtained with and X1 level range from 0 to 0.05 and X_2 at 85% to 95%.

Pareto chart

The ANOVA Pareto chart was used to investigate the standardized effect of the independent variables and their interaction on the dependent variables as Viscosity (Y1) and Drug Content (Y2), which depicts the main effect of the independent variables and interactions with their relative nonsignificance on the Y1 and Y2 . The length of each bar below significance or critical line detonated by blue in the chart indicates the standardized effect of that factor in the responses. [26] Factor remains inside the reference line indicate that these terms contribute the least in prediction of responses so form the Pareto chart concluded that for linear, interaction, and quadratic effect showed nonsignificance effect on Viscosity (Y1) and Drug Content (Y2) response [Figures 4]

ANOVA, pure error, and lack of fit

The results of ANOVA demonstrate that the model was nonsignificant for all dependent variables [Tables 3 and 4]. Regression analysis was carried out to determine the regression coefficient, and all the independent variables were found to be nonsignificant for all response variables. The linear as well as quadratic model was found to be not significant for Y1 and linear model for Y2 . So, above result indicates that both the factors not play an important role in the formulation of nanoemulsion containing orlistat. The pure error and lack of fit [Tables 3and 4] can provide a mean response and an estimate of pure experimental uncertainty. The residual is the difference between observed and predicted values. The ANOVA for the dependent variables demonstrates that the model was not significant for all response variables. The effects are like the concentrations of oil and water were found to be not significant along with its quadratic and

interaction terms for all the dependent variables. Hence, the above results lead us to believe that the all independent variables are not play important role and optimal concentration in Nanoemulsion gives rise to optimum Viscosity and drug content. The data for pure error and lack of fit provide a mean response and estimate of pure experimental uncertainty. The residual value represents the difference between observed and predicted value respectively. The computed F-value was lower than critical F-values, which denotes non significance with regard to lack of fit [Tables 3 and]. The three replicated center point in Box-BehnkeCentral composite experimental design made it possible to assess the pure error of the experiments and enabled the models lack of fit to be checked. In this study, the model was checked for lack of fit for the all the responses. For lack of fit P values, we obtained are not showed for response Y1 and Y2 , and hence, the current model provided a satisfactory fit to the data and had no lack of fit.[11-15] The statistical nonsignificance of each effect was tested by comparing the mean square against and estimates of the experimental error. It was noted X1 and X2 with their interaction effect other than X1 X2 and quadratic effect had P value more than 0.05, indicating nonsignificance effect of this variables in prediction of X, whereas linear effect X1 interaction effect X1 X3 and quadratic effect of X22, X33 indicating nonsignificance effect of this variables in prediction of response Y2 [Tables 2 and 3] because of having P value is also more than 0.05. Standard error indicates the standard deviation of the coefficient

The results of ANOVA demonstrate that the model was nonsignificant for all dependent variables [Tables 4 and 4]. The linear as well as quadratic model was found to be not significant for Y1 and linear model for Y2. So, above result indicates that both the factors not play an important role in the formulation of nanoemulsion. The residual is the difference between observed and predicted values. Hence, the above results lead us to believe that the all independent variables are not play important role and optimal concentration in Nanoemulsion gives rise to optimum Viscosity and drug content. Computed F-value was lower than critical F-values, which denotes non significance with regard to lack of fit [Tables 3 and 4]. For lack of fit P values, we obtained are not showed for response Y1 and Y2 , and hence, the current model provided a satisfactory fit to the data and had no lack of fit.[11-15] The statistical nonsignificance of each effect was tested by comparing the mean square against and estimates of the experimental error. It was noted X1 , X2 , and X3 with their interaction effect other than X1 X2 and quadratic effect had P value more than 0.05, indicating nonsignificance effect of this variables in prediction of X, whereas linear effect X1 interaction effect X1 X2 and quadratic effect of X11, X22 indicating nonsignificance effect of this variables in prediction of response Y2 [Tables 3 and 4] because of having P value is also more than 0.05. Standard error indicates the standard deviation of the coefficient.

Polynomial equation

The negative coefficient of X1 suggests decrease in sonication time and has inversely proportional relationship with drug content, and same coefficient is observed with X2 , clearly indicates that individual effect of X1 and X2 have negative coefficients. Likewise interaction effect and quadratic effect of X1 and on drug content showed the positive coefficient except the interaction effect of X1 X3 and quadratic effect of X3 .

Viscosity $Y1=0.017+0.0241X^{1}+0.0134X_{2}+0.0237X_{12}+0.0141x21-4.652X_{2}^{2}$

The positive coefficient of X1 suggests increase in Conc of water and has directly proportional relationship with Drug content, and the same coefficient is observed with X2 , clearly indicates that individual effect of X1 and X2 have positive coefficients. Likewise interaction effect and quadratic effect of X1 on drug content showed the negative coefficient except the interaction effect of X1 and quadratic effect of X

Drug content $Y2=99.00+2.505X_{1}+1.409X_{2}+2.00X_{12}-6.125X^{2}-6.125X_{2}^{2}$

The three replicated center point in Central Composite experimental design made it possible to assess the pure error of the experiments and enabled the models lack of fit to be checked. In this study, the model was checked for lack of fit for the all the responses.[14] For lack of fit P values, we obtained are not showed for response Y1 and Y2 , and hence, the current model provided a satisfactory fit to the data and had no lack of fit

Optimal solution

After using the desirability approach, optimal solution suggested by version 10 was used for further study Figure 8: Interaction plot showing effect of conc. of oil on viscosity (Y1) Figure 9: Interaction plot showing effect of conc of water on drug content (Y2) . Coded and actual values of independent variable clearly state that when concentration of oil increases viscosity of system increases. In the preparation, the concentration of oil is 20ml to 30 ml showed better viscosity, and 22.5 ml showed excellent drug content. The optimized solution [Table 5,7] predicts that 25ml concentration of oil and 22.5 ml is concentration of water as independent variables and 0.255 viscosity and 98.67 % drug content for preparation of nanoemulsion

Characterization of Nanoemulsion

pH

The pH of human stomach typically ranges from 1 to 3 therefore, the formulations intended for oral application to stomach should have pH close to this range. pH of the freshly prepared nanoemulsion was found to be in the range 2.3-2.8 which is similar to normal stomach pH.

Viscosity

Depending upon the surfactant concentration and oil concentration ratio of oil-in-water nanoemulsion was varied from 0.0252.Pa.S. the viscosity optimized of oil in water nanoemulsion formulation was found to be 0.0167Pa.S. Viscosity of the optimized formulation was found to be 0.0167c.Pa.S

Drug Content

he loaded amount, the Viscosity, and the percent cell recovery were determined. The UV method was used to estimate the Orlistat content nanoemulsion. The obtained data indicate that 70 µg of orlistat was loaded with an nanoemulsion. The observed cell recovery of approximately 99.21 % is comparable to the recovery results for various drugs reported in other studies.

Conductivity

The upgraded Lipid based nanoemulsion was described for conductivity and Conductivity of enhanced nanoemulsion detailing was estimated utilizing conduct meter and current stream was noticed. Conductivity of advanced nanoemulsion discovered to be 0.0584mS/cm

Partical size and Zeta potential

We used different sizing methods to assess their influence on the homogeneity and Drug Content of the formulations. Sizing with homoginzation produced polydispersed particles (PI >0.5) and drastically reduced the drug content. We used different sizing methods to assess their influence on the drug content of the formulations. Sizing with homopginization produced polydispersed particles (PI >0.5) and drastically reduced the drug content. The particle size of orlistat loading nanoemulsion were found to be 413 nm and PI was found to be -31.28mV [Figure 9]. The PI of orlistat loaded Nanoemulsion was less than one and concludes that Nanoemulsion formed are monodispersed or of uniform size. The sizes of nanoemulsion were found in nanometer; therefore, we could expect better accumulation at tumor by enhanced permeability and retention effect. Further, concentration of water had minimal effect on drug content, suggesting little or no disruption of stomuch. Thus, nanoemulsion are expected to be optimal for in-vivo efficacy and are likely to avoid clearance by alveolar macrophages.

Drug Release study

The in vitro drug release from orlistat solution, optimized orlistat loaded Nanoemulsion were studied at 37°C ± 2 in PBS buffer [Figure 15]. When the release of plain orlistat was evaluated using dialysis bag as barriers, ~100% drug was available in the receiver chamber only after 1 h, suggesting that bags were not controlling the passage of drug molecules from donor to receiver chambers.[21] No degradation in the release media is expected since orlistat remained stable over an extended period (~24 weeks) at various storage conditions. [15,33,38] The percent cumulative drug release from the orlistat solution was found 80.14±1.24% to be after 4 h. The release was better faster release from orlistat loading nanoemulsion compared to standard drug solution. From the above results, it is very clear that the drug-loaded nanoemulsion would faster release of orlistat in biological system.

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Table1: Central Composite experimental design for preparation of nanoemulsion

Run order	Independent variables		Dependent variables	
	(X1) Oil(mL)	(X2) Distilled Water (mL)	Y1 (Viscosity)	Y2 (drug content) (%)
1	1	-1	0.0056	94
2	-1	1	0.1042	88
3	0	0	0.0739	97
4	-1	-1	1.0167	99
5	1	1	0.0167	99
6	0	-1	0.0096	80
7	-1	0	0.0167	99
8	0	0	0.0062	84
9	0	0	0.0167	99
10	0	0	0.0167	99
11	1	0	0.00252	78
12	0	0	0.00582	80
13	0	1	0.00207	93

Independent variables	Levels			Dependent variables
Oil (X_1)	20	25	30	Y_1=Viscosity
Water (X_2)	0.250	0.255	0.260	Y_2=Drug content

Table 2: Polynomial equation values in terms of actual values (coefficients)

Sr. No	Term	Viscosity	Drug content
1	*Intercept	0.017	99.00
2	*A:Oil	0.0241	2.505
3	*B:Distilled Water	0.0134	1.409
4	*AB Interaction	0.0237	2.00
5	*A:Oil ratio(r^2)	0.014	-6.125
6	*B:Distilled Water(r^2)	-4.65	-6.125

Table 3: Analysis of Variance (ANOVA) for Viscosity

Source of variation	F valve	P Valve
Model	11.26	0.301
A:Oil	26.09	0.0014
B:Distilled water	8.10	0.0248
AB	12.64	0.0093
A2	7.80	0.0268
B2	0.84	0.3891
Residual		
Lack of fit	-	-
Pure Error	-	-
Cor total		
S= -	PRESS=8.893	
R-sq= -	R-sq(adj)=0%	
R-sq(pred)=0%		

Table 4: Analysis of variance (ANOVA)for Drug Content

Source of variation	F valve	P Valve
Model	2.61	0.121
A:Oil	1.21	0.3084
B:Distilled water	0.38	0.5563
AB	0.38	0.5549
A2	6.27	0.0408
B2	6.27	0.0408
Residual		
Lack of fit	-	-
Pure Error	-	-
Cor total		
S= -	PRESS=4.144	
R-sq= -	R-sq(adj)=0%	
R-sq(pred)=0%		

Table 5 : Optimized nanoemulsion for independent value

Sr. no.	Factor	Actual value
1.	A:Oil(mL)	25
2.	B:Distiled Water	22.5

Table 6 : Optimized Nanoemulsion for dependent value

Sr.no.	Response	Actual value
1.	Viscosity	0.255
2.	Drug Content	98.67

Table 7. Stability Study analysis

Test	Conditions	Duration	Observation				
			Phase Separation	Creaming	Cracking	Phase Inversion	Stability
Agitation	On rotary shaker 60 cycles/min	24 hrs	No	No	No	No	Yes
Centrifuga-tion	3500 rpm	2hrs	No	No	No	No	Yes
Freeze-thaw cycles	Three cycles between refrigerator temp .-RT-45°C	6 days	No	No	No	No	Yes

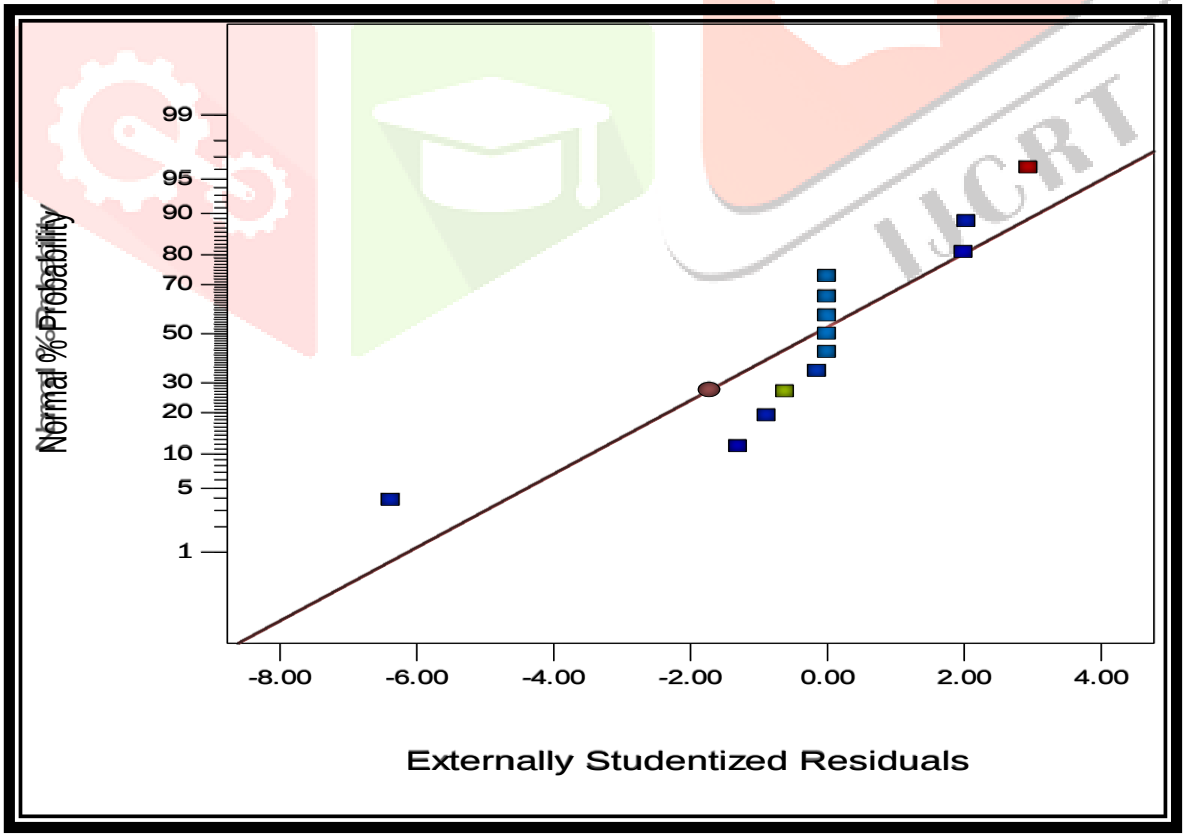

Figure 1:

Normal probability plot for residual of viscosity

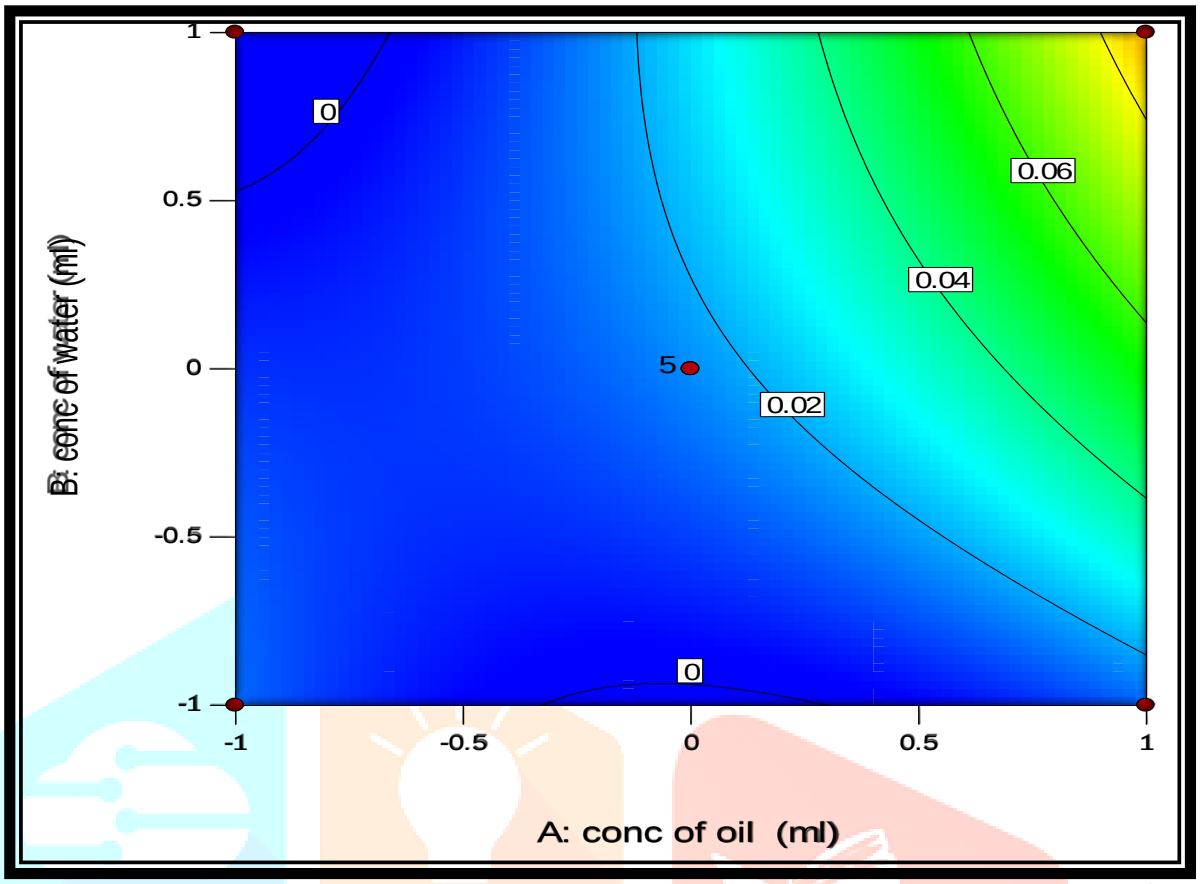

Figure 2: Contour plot showing effect of phase volume ratio (X1) and drug concentration (X2) on response X

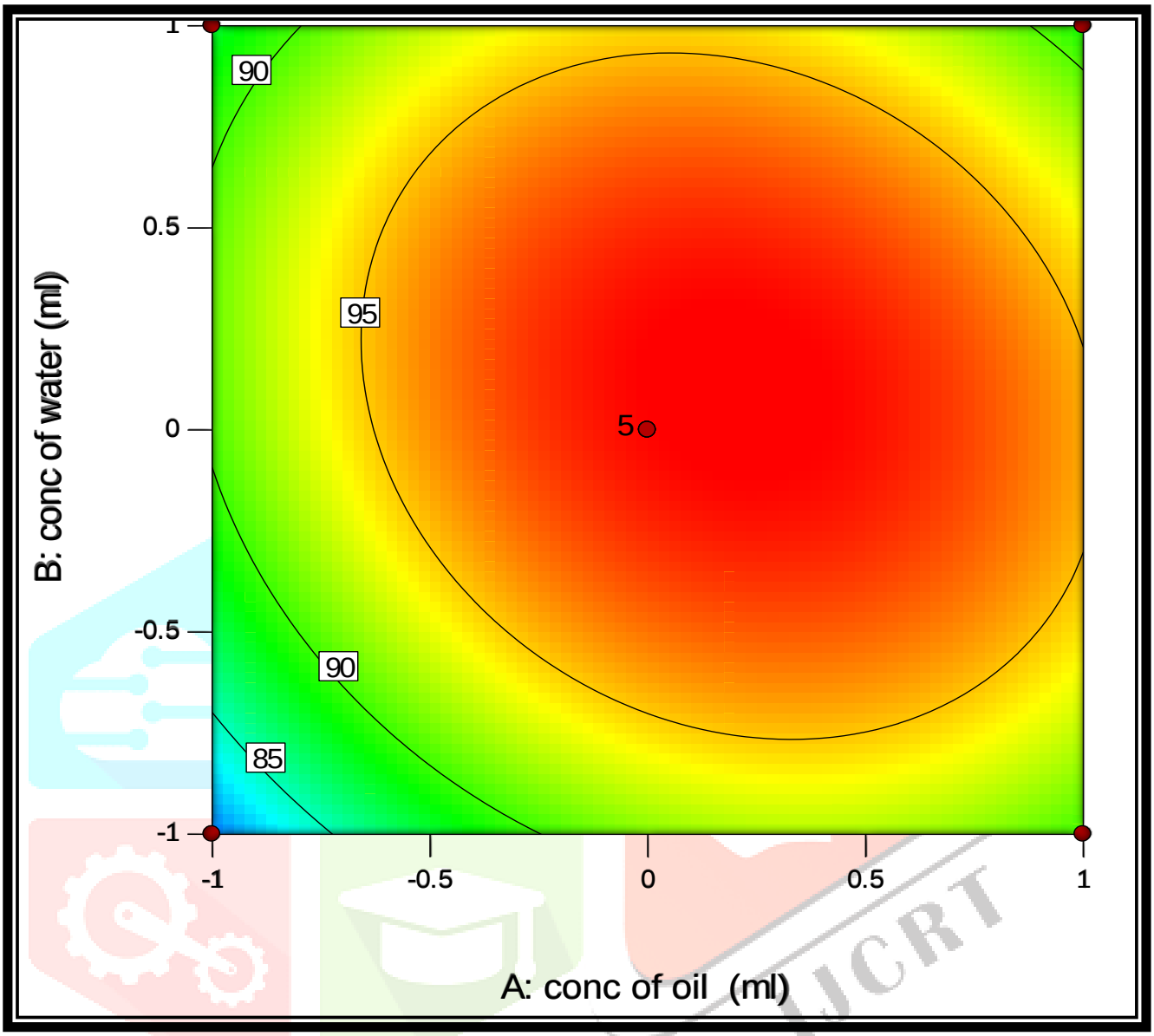

Figure 3: Contour plot showing effect of phase volume ratio (X1) and drug concentration (X2) on response Y

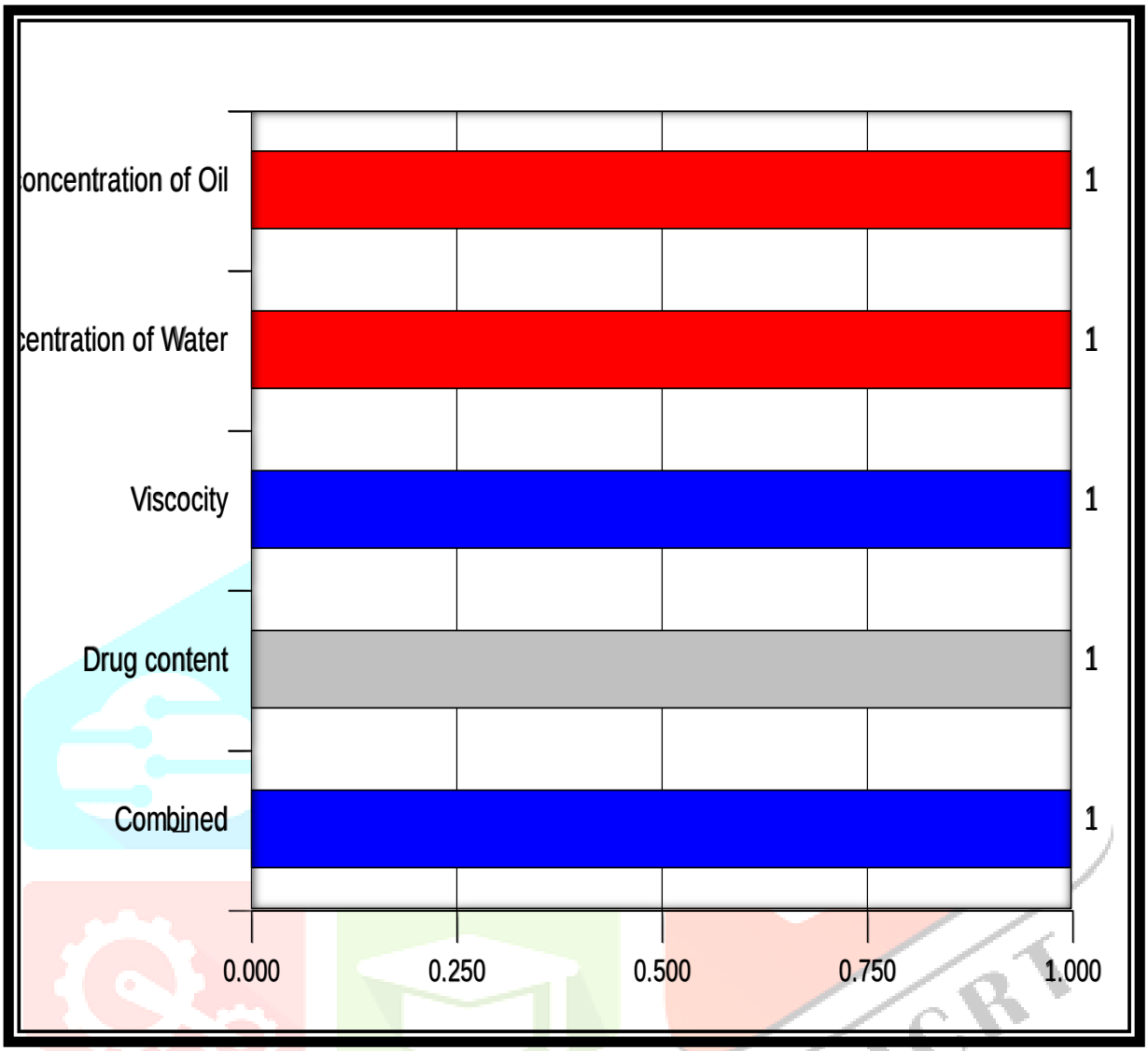

Figure 4: Puerto chart of effect showing the response Viscosity and Drug Content

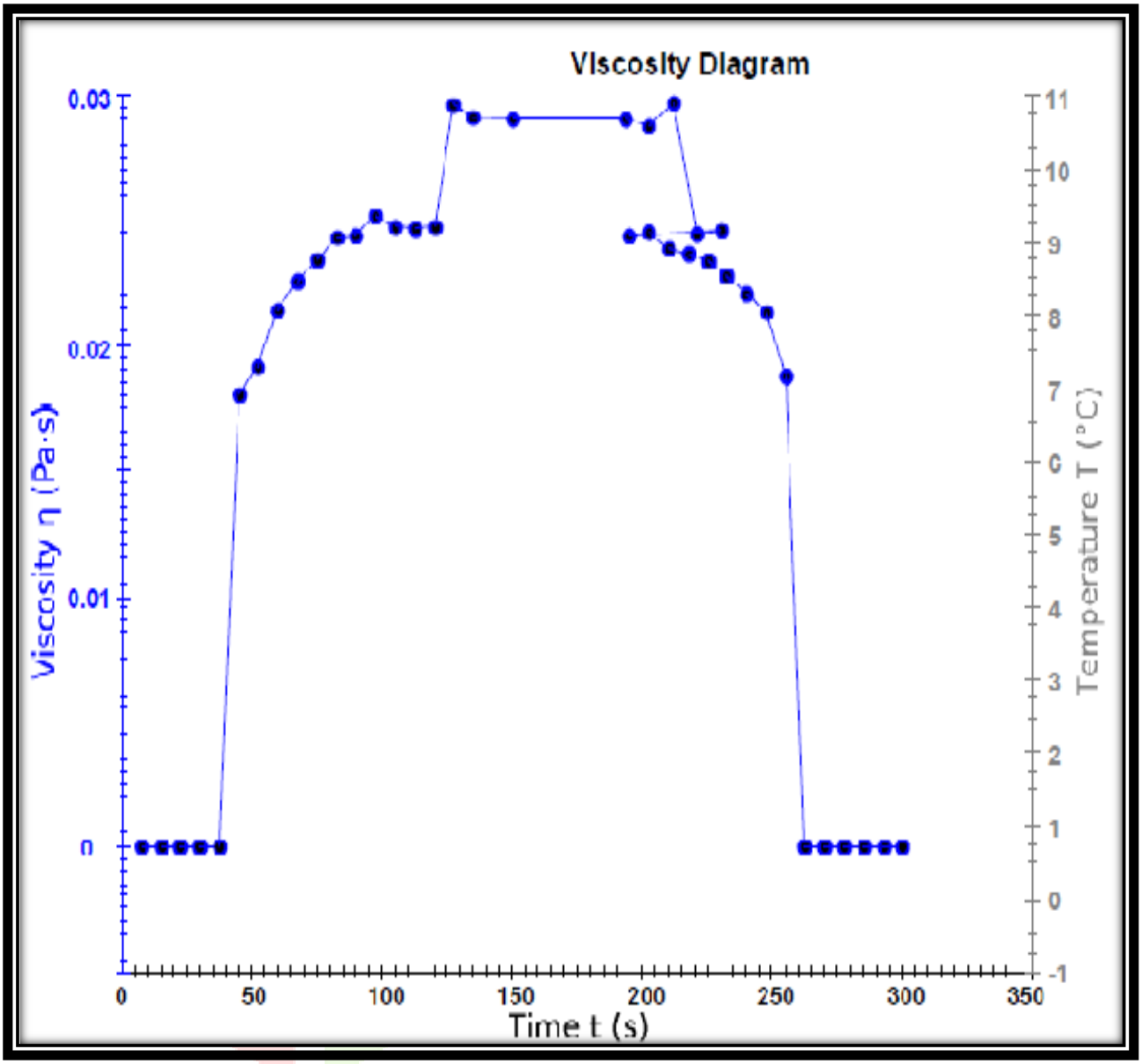

Figure 5: Viscosity of optimized nanoemulsion

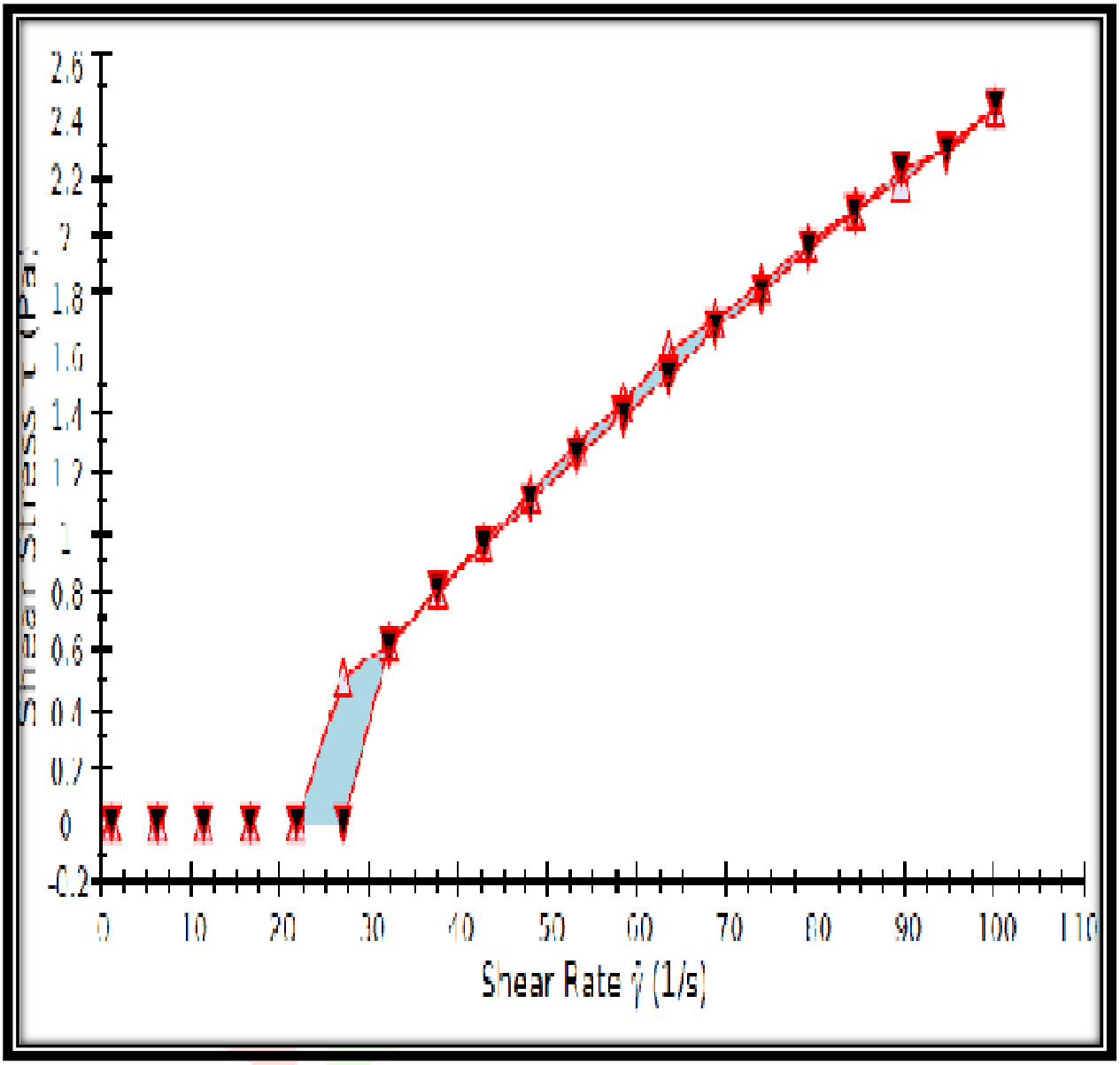

Figure 6: Flow curve of nanoemulsion showing thixotropy

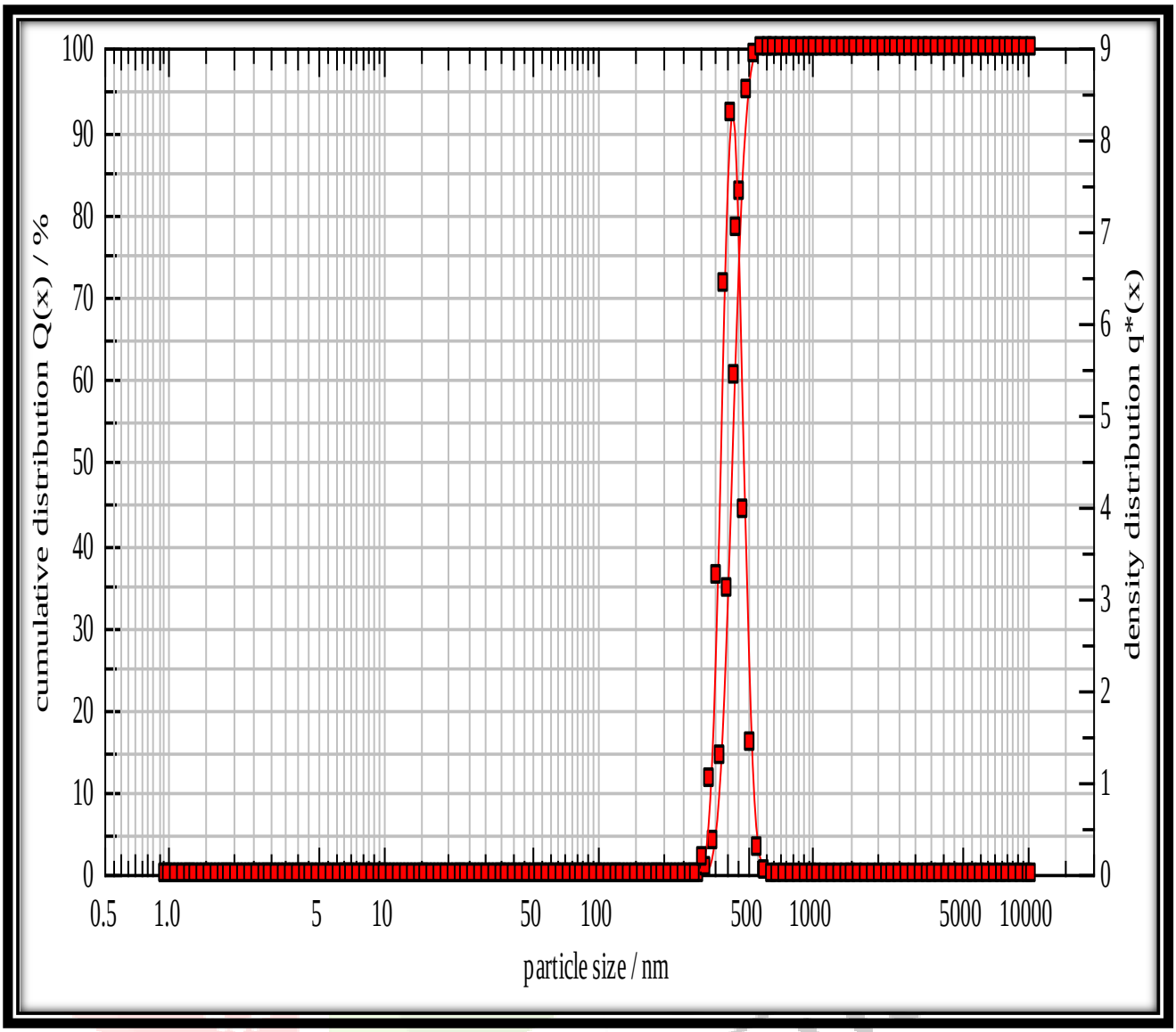

Figure 7 : Particle size analysis of orlistat loaded nanoemulsion

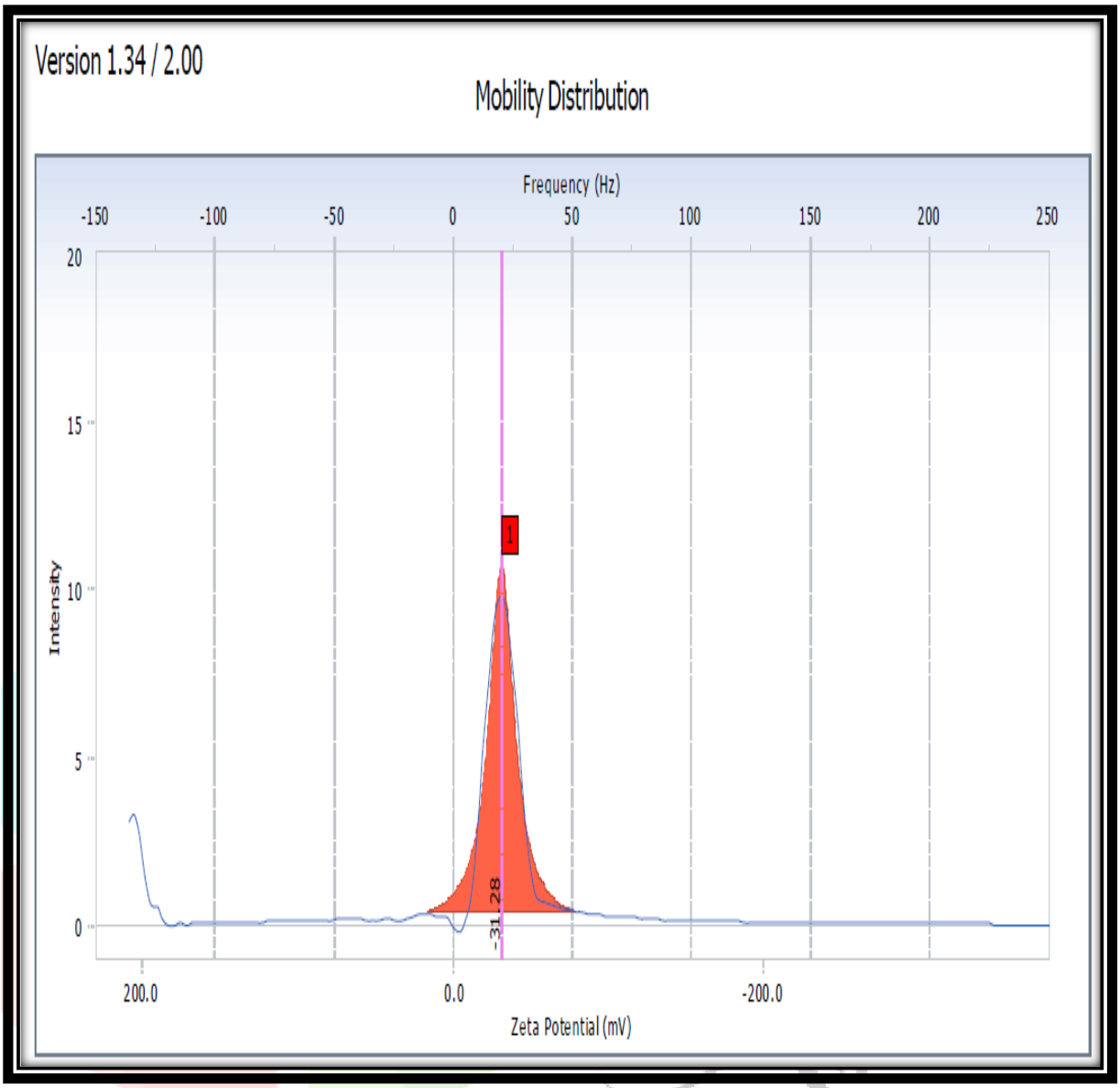

Figure 8: Zeta potential of nanoemulsion Results

Zetapotential:-31.28mV **Peak frequency:** 20.39Hz

Conductivit:0.0584mS/cm **Intensity** : 10.37

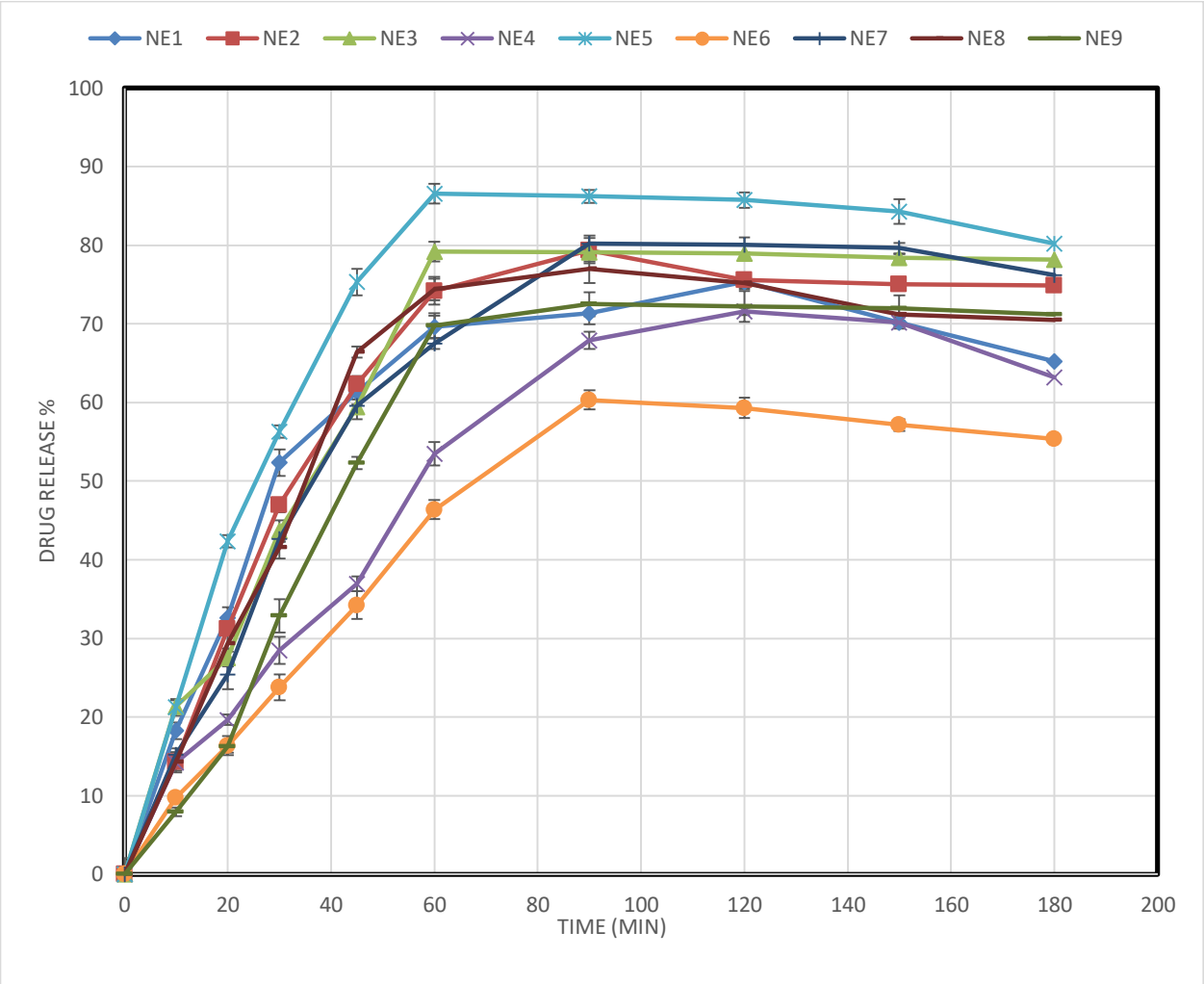

Figure 9: Drug Release study of nanoemulsion