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RESEARCH ARTICLE

Formulation and Evaluation of Chitosan Nanoparticles Containing Glibenclamide

Patel CJ[1*], Patel DV[2]

[1]*Research Scholar, Shri J. J. T. University, Jhunjhunu, Rajasthan, India.*

[2]*B. K. Mody Govt. Pharmacy College, Rajkot, Gujarat, India*.

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ABSTRACT

The objective of the present study was to develop Glibenclamide loaded nanoparticles using Chitosan as release control polymer. Different concentrations of Chitosan and Sodium Tripolyphosphate (TPP) were tried. A 3^2 full factorial design was used for optimization. The prepared nanoparticles were evaluated for particle size, zeta potential, % yield, and association efficiency, CPR after 1 hr and CPR after 20 hr. results obtained were subjected to regression analysis to obtain mathematical model. Using the developed model a checkpoint cum optimized formulation was developed on bases of desired characteristics and evaluated. *In-vivo* anti-hyperglycemic activity of the checkpoint batch was studied. The results obtained were in correlation with the predicted results. The formulation containing 0.75% w/v Chitosan and 0.4% w/v TPP was selected as optimized formulation.

KEYWORDS

Glibenclamide, Chitosan, Nanoparticle, Factorial Design, Optimization

INTRODUCTION

Interest has grown in the design of drug-containing formulations which deliver drugs to specific 'targets' in the body as well as providing drug over longer periods of time at controlled rates. A nanoparticle is a submicroscopic solid particle with a size ranging 10 nm to 1 µm. They can be prepared from emulsion, micelles, interfacial polymerization, preformed polymers, and coacervation. Nanoparticles occupy a unique position in drug delivery technology due to their attractive properties. In particular, nanoparticles have several advantages in pharmaceutical applications. In addition, they offer drug targeting possibilities and a sustained release action.[1]

***Address for Correspondence:**
Chirag J. Patel
Research Scholar,
Shri J. J. T. University, Jhunjhunu,
Rajasthan, India.
E-Mail Id: chirag999patel@yahoo.co.in

Introduction to Drug Glibenclamide

Glibenclamide is a potent sulfonylurea and has established potential benefits such as lower dose, rapid onset, lower insulin levels and less-pronounced glucagon tropic effects, insulin-sensitizing and insulin-mimetic affects. However it is a poorly soluble drug (b8 µg/ml in pH 7.4 phosphate buffers) with relatively high permeability through CaCo-2 cell monolayer's which warrants it to be classified under BCS Class II classification.

Glibenclamide is effectively absorbed from the gastrointestinal tract, but the presence of food and certain dietary supplements interfere with its dissolution and in turn its absorption. Glibenclamide may be more effective if given 30 min prior to meal. The application of nanotechnology drug delivery for improving the dissolution characteristics of Glibenclamide is still in the early hours.[2]

Glibenclamide is a second-generation sulphonylurea that is an orally bioavailable hypoglycemic agent used in the management of type 2 diabetes. It is administered in low doses (5 mg), is quickly cleared from the body, and its active metabolites have a considerable hypoglycemic effect.[3] Different research has reported that Glibenclamide has a low bioavailability, which is attributed to its poor dissolution properties.[4-6]

Different methods have been reported to determine Glibenclamide levels in various biological fluids, such as plasma and serum,[7-9] in pharmaceutical formulation analyses[10-12] or in simultaneous determination of anti-diabetic drugs.[13]

Introduction to Polymer Chitosan

Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite common during precipitation and the Chitosan may look 'cottonlike'. Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of Chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies.

Chitosan has been processed into several pharmaceutical forms including gels, films, beads, microspheres, tablets, and coatings for liposomes. Chitosan has favorable biological properties such as nontoxicity, biocompatibility and biodegradability. Polysaccharides has the advantage of being widely approved as food ingredient, which suggests its acceptability as a new excipient for oral administration.[14]

MATERIALS AND METHOD

Materials

Glibenclamide was gift sample from Zydus Pharm. Ltd., India. Chitosan (Protasan UP G 113, M. Wt. 150-200 kDa, DOA 75-90%) was procured from Novamatrix, Norway. Sodium Tripolyphosphate was procured from S. D. Fine Chem. Ltd., Mumbai, India. All other materials and solvents used in the study were of LR Grade.

Drug-Excipient Compatibility Study

For the compatibility study of drug-polymer and stability of drug during formulation process Fourier transform infra-red (FT-IR) spectroscopy analysis was piloted. Potassium bromide (KBr) pellet method was used to record FT-IR spectrums of moisture free samples. FT-IR spectrum of pure Glibenclamide, physical mixture of Chitosan and Glibenclamide with excipients and nanoparticles were analyzed after stability study.

Preparation of Glibenclamide Nanoparticles

Chitosan nanoparticles were prepared according to the procedure testified by Calvo et al. (1997) based on the ionic gelation of Chitosan with Sodium Tripolyphosphate (TPP) anions. Quantity of drug in all formulations were kept constant i.e. 100 mg. Chitosan was dissolved in acetic aqueous solution (1 mg/ml).

Calculated quantity of drug was added to the Chitosan solution and sonicated for uniform distribution. 0.5 %w/v of poloxamer was added to the Chitosan solution maintained at 25°C as a suspending agent, to prevent particle aggregation while stirring 4 ml TPP aqueous solution with various concentrations was added into 15 ml Chitosan/drug solution, respectively. Three kinds of phenomena were observed: solution, aggregates and opalescent suspension.

The zone of opalescent suspension was further examined as nanoparticles. Nanoparticles were collected by centrifugation at 8,000 rpm for 30 min. Then supernatants were discarded and the concentrated nanoparticles solution was freeze dried. Above procedure was followed for the selection of concentration range of drug, polymer and TPP.

Optimization of Formulation Using 3^2 Full Factorial Designs

It is desirable to develop an acceptable pharmaceutical formulation in shortest possible time using minimum number of man-hours and raw materials. Traditionally pharmaceutical formulations after developed by changing one variable at a time approach.

The method is time consuming in nature and requires a lot of imaginative efforts. Moreover, it may be difficult to develop an ideal formulation using this classical technique since the joint effects of independent variables are not considered. It is therefore very essential to understand the complexity of pharmaceutical formulations by using established statistical tools such as factorial design. In addition to the art of formulation, the technique of factorial design is an effective method of indicating the relative significance of a number of variables and their interactions.

The number of experiments required for these studies is dependent on the number of independent variables selected. The response (Y_i) is measured for each trial.

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

Model is fitted by carrying out multiple regression analysis and F-statistics to identify statistically significant terms.

The full Equation, an Equation containing only statistically significant terms, is then used for drawing counter plots to visualize the impact of changing variables at a glance. The optimum point may be identified from the plot and replicate trials may be run to verify the prediction of optimum response. A 3^2 randomized full factorial design was utilized in the present study.

In this design two factors were evaluated, each at three levels, and experimental trials were carried out at all nine possible combinations. The design layout and coded value of independent factor is shown in Table 1. The factors were selected based on preliminary study Effect of polymer:

TPP concentration ratio has been statistically evaluated for the study on various characterization parameters like In-vitro release study, Zeta potential, Particle size, Percentage drug loading and Percentage process yield. The formulations of the factorial batches (F1 to F9) are shown in Table 1.

Table 1: Full Factorial Design Layout

Batch Code	Coded value		Un-Coded value*	
	X_1 (mg/ml)	X_2 (mg/ml)	X_1 (mg/ml)	X_2 (mg/ml)
F1	-1	-1	0.5	0.2
F2	-1	0	0.5	0.6
F3	-1	1	0.5	1
F4	0	-1	1.0	0.2
F5	0	0	1.0	0.6
F6	0	1	1.0	1
F7	1	-1	1.5	0.2
F8	1	0	1.5	0.6
F9	1	1	1.5	1

X_1 code for concentration of polymer and X_2 code for concentration of Sod. TPP

*****Concentration of Glibenclamide – 100 mg constant

Evaluation of Nanoparticles

Particle Size Analysis and Zeta Potential

Particle size distribution of prepared nanoparticle formulations was studied by Laser Diffraction Particle Size Analyzer (SHIMADZU & METROHM). The data obtained after the observation were analyzed accordingly. The zeta potential of the samples was measured by a Zetatrac (METROHM).

Percentage Process Yield

The percentage yield of different formulations was determined by weighing the nanoparticles after freeze drying. The percentage process yield was calculated as follows:

Percentage process yield = $(W_1/W_2) \times 100$… (2)

Where, W_1 – Total weight of nanoparticles

W_2 – Total initial weight of solids

Percentage Association Efficiency

The nanoparticle association efficiency of Glibenclamide was determined upon separation of nanoparticle from the aqueous preparation medium containing the non-associated drug by centrifugation (16,000×g, 30min, 15 ◦C). Concentrations of Glibenclamide in the supernatant (C_2) were determined by UV-visible spectrophotometry at 230 nm after suitable dilution. The entrapment efficiency was calculated according to the following equation:

$$\text{Percentage association efficiency} = (C_1 - C_2) / C_3 \times 100 \ldots\ldots\ldots (3)$$

Where, C_1 – Total amount of drug taken for the formulation

C_2 – Concentration of drug in supernatant layer

In-vitro Release Studies

At the start of the study *in-vitro* release studies were carried out by dialysis bag method. An amount of nanoparticle suspension equivalent to 60 mg pure Glibenclamide was filled in dialysis bag (10 ml) (Hi media). In the acid stage, dialysis bag was placed in a round bottomed cylindrical vessel of USP dissolution test apparatus II; containing 675 ml of 0.1 N HCl. Stirring speed was 100 rpm and the temperature was maintained at 37 $\pm$ 0.5^0C as per given in *BP 2009*. Aliquots were withdrawn at predetermined time intervals and immediately replaced with the fresh medium equilibrated at 37^0C. After 2 h, 225 ml of 0.2 M tribasic sodium phosphate was added to change the pH of test medium to 7.4. The sink condition was maintained throughout the experiment. The withdrawn samples were diluted and analyzed for drug content using U.V. spectrophotometer at 230 nm keeping 0.1 N HCl and phosphate buffer pH 7.4 as blank depending on the time interval for sample taken. All the determinations were made in triplicate.

Kinetic Modeling

In order to understand the kinetic and mechanism of drug release, the result of *in-vitro* drug release study of nanoparticles were fitted with various kinetic equation like zero order (equation 4) as cumulative percentage release vs. time, higuchi's model (equation 2) as cumulative % drug release vs. square root of time. r^2 and k values were calculated for the linear curve obtained by regression analysis of the above plots.

$$C = k_0 t \ldots\ldots\ldots\ldots\ldots (4)$$

Where k_0 is the zero order rate constant expressed in units of concentration/time and t is time in h.

$$Q = k_H t^{1/2} \ldots\ldots\ldots\ldots\ldots (5)$$

Where, k_H is higuchi's square root of time kinetic drug release constant.

To understand the release mechanism *in-vitro* data was analyzed by peppas model (equation 3) as log cumulative drug release vs. log time and the exponent n was calculated through the slope of the straight line.

$$M_t / M_\infty = bt^n \ldots\ldots\ldots\ldots\ldots (6)$$

Where M_t is amount of drug release at time t, M_∞ is the overall amount of the drug, b is constant, and n is the release exponent indicative of the drug release mechanism. If the exponent n = 0.5 or near, then the drug release mechanism is Fickian diffusion, and if n have value near 1.0 then it is non-Fickian diffusion.

Stability Study

Effect of Different Temperature on Chitosan Nanoparticle Size Distribution

The prepared Chitosan nanoparticles of selected batches (FN1, FN5 & FN9) were subjected to storage at two different temperature conditions i.e. 4°C and 25°C for 12 months. The stability of Chitosan nanoparticles was determined by studying the particle size distribution.

Accelerated Stability Study

Samples from each batch were withdrawn after the definite time intervals and the residual amount of drug in the vesicles was determined. All the batches of freeze dried Glibenclamide nanoparticles were tested for stability. The preparations were divided into 3 sets and were stored according to ICH guidelines for long

term at 25°C ± 2°C/60% RH ± 5% RH, 12 months and for accelerated stability study at 40°C ± 2°C/75% RH ± 5% RH, 180 days. Drug content of all the formulations was determined by U.V. spectrophotometer at 230 nm.

In-Vivo Anti-Hyperglycemic Activity

The *in vivo* release behavior of the formulation was studied by measuring anti-hyperglycemic activity in normal healthy male albino rats (weighing 250 to 300 g each) using the glucose-measuring instrument. Rats were caged under controlled temperature and 12 h light/dark cycle. They were fed with standard laboratory chow and water. The experiments were designed and conducted in accordance with the guidelines of institutional animals' ethics committee. For the induction of diabetes, rats were kept on fasting for 24 h prior to alloxan injection. On the day of administration, Alloxan Tetrahydrate was freshly dissolved in 0.01 M (pH 4.5) citrate buffer and subcutaneous injection was given at the dosage of 250 mg/kg.

Blood glucose concentration was checked by the glucose oxidase and Glucometer (Roche) after 1 week of Alloxan injection. The animals with glucose concentration exceeding 250 mg/dl were considered diabetic. To evaluate the anti-hyperglycemic activity of drug formulation, the male Wistar Albino rats were divided into three groups, each group consisting of six animals.

One group served as control, second group served as diabetic control while in third group, again it was divided in two groups of three animals, one group received Glibenclamide solution and another group received nanoparticles containing Glibenclamide orally (5 mg/kg of Glibenclamide) once daily during experiment. Glibenclamide nanoparticles were administered orally to third group by stomach intubation. Blood samples were collected at particular time intervals upto 24 h and the blood glucose level was measured as described.

RESULTS AND DISCUSSION

Drug-Excipient Interaction Study

The results of the FT-IR spectroscopy analysis conducted for the analysis of drug-polymer interaction shows no chemical interaction between Glibenclamide and Chitosan.

Table 2: Tabulated study results of various evaluation parameters

Sr. No.	Formulation code	Particle size (nm)	Zeta potential (mV)	Process yield (%)	Association Efficiency (%)
1	F1	204.53 ± 37.71	38.54 ± 0.166	45.74 ± 1.019	37.95 ± 0.081
2	F2	246.16 ± 33.23	37.25 ± 0.817	51.38 ± 0.445	40.65 ± 0.264
3	F3	323.41 ± 43.09	35.28 ± 0.320	54.36 ± 0.737	41.94 ± 0.237
4	F4	217.63 ± 27.15	41.63 ±0.643	56.43 ± 0.935	38.73 ± 0.493
5	F5	296.24 ± 46.12	37.69 ± 0.323	67.89 ± 0.980	41.74 ± 0.423
6	F6	318.87 ± 34.81	35.87 ± 0.275	75.45 ± 1.568	44.38 ± 0.513
7	F7	231.10 ± 30.04	43.78 ± 0.837	38.82 ± 0.703	34.71 ± 0.673
8	F8	296.32 ± 22.31	41.84 ± 0.158	43.65 ± 0.980	37.47 ± 0.586
9	F9	421.04 ± 36.84	39.94 ± 0.876	47.27 ± 0.861	39.74 ± 0.572

Evaluation of Nanoparticle

Particle Size Analysis and Surface Morphology

Particle size analysis results for different formulation were collected and tabulated in Table 2. The maximum particle size of nanoparticles was observed in F9 (421.04 ± 36.84 nm) as compared to other formulations and the least size was seen in F1 (204.53 ± 37.71). The particle size tends to increase with increasing the concentration of sodium TPP. Results of Particle size distribution where analyzed statistically (using Statistica 8.0). It was observed that there was increase in particle size with increase in concentration of Chitosan and sodium TPP. But when the data obtained after the factorial analysis were treated further to derive polynomial equation 7, it was found that important variable in deciding the particle size was the concentration of sodium TPP and not the concentration of Chitosan, surface plot and contour plot was shown in Figure 1.

$$Y = 283.92 + 29.06X_1 + 68.34\ X_2 \quad \text{.....(7)}$$

The increase in particle size observed with increase in Chitosan concentration from F1 to F4 may be the effect of change in drug to polymer concentration. This could be supported by the finding of Rajendran et al. (2010) who have used same polymer for preparation of nanoparticle, reported that the increase in drug concentration could increase the mean particle size. In the equation 7 positive values of arithmetic mean X_1 and X_2 indicates that the particle size increases as concentration of sodium TPP and Chitosan increases. Study of the surface morphology from the above figure suggests that the prepared Chitosan nanoparticles have the drug absorbed on the surface of it and also that the presence of pores in the nanoparticles were indicative that the diffusion of drug from the Chitosan nanoparticle would be easy once it gets dissolved in dissolution medium. Dissolution of drug was an important step in case of Glibenclamide as it is poorly soluble in water. As drug was incorporated successfully in Chitosan nanoparticles due to the increase in overall surface area for dissolution now the drug could dissolve at faster rate and could diffuse afterwards; out of the polymer matrix which will give controlled and steady drug release over a period of time.

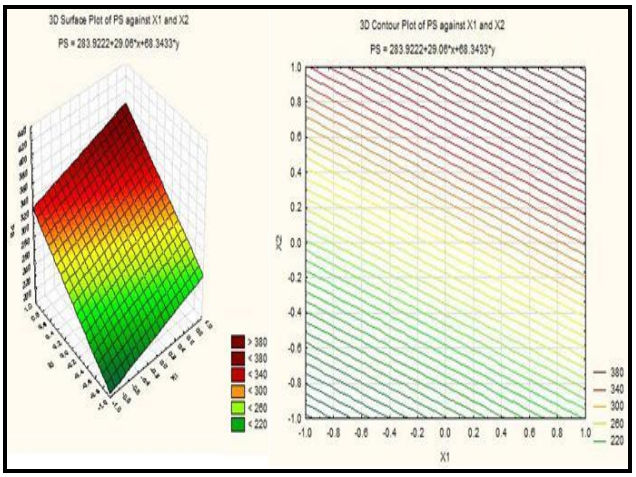

Figure 1: 3D surface plot and 3D contour plot of particle size vs X_1 and X_2

Zeta Potential

The zeta potential values ranged from 35.28 ± 0.320 mV to 43.78 ± 0.837 mV as shown in Table 2. Zeta potential showed higher positive values which indicated the stability of the nanoparticles during the process of formulation. It was observed from the results that the value of zeta potential increases with the increases in the concentration of polymer but at the same time it was also evident that there was a definite effect of concentration of sodium TPP on the zeta potential value. The significance of zeta potential is that its value can be related to the stability of colloidal dispersions. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate as outlined. The zeta potential results when treated for factorial analysis interesting results were found confirming the above discussion. After the factorial analysis as shown when equation 8 was

obtained, it confirmed the conclusion that the concentration of Chitosan has the positive effect as the value of X_1 is positive. The data obtained was also reported as surface plot and contour plot in Figure 2.

$$Y = 39.09 + 2.41X_1 - 2.14X_2 \quad \cdots\cdots\cdots\cdots(8)$$

The increase in the zeta potential with the increase in Chitosan concentration could be combined with the decrease in ratio of drug to polymer as drug concentration kept constant in this factorial design. As seen in the equation 8 the term X_2 which is the concentration of sodium TPP got the negative sign which indicates that as the concentration of it increases there be a decrease in the zeta potential value. Surface plot and contour plot are given in Figure 2.

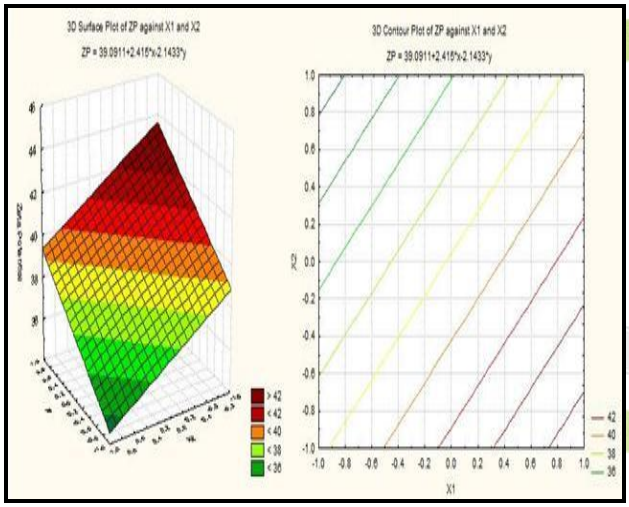

Figure 2: 3D surface plot and 3D contour plot of zeta potential vs X_1 and X_2

Percentage Process Yield

The percentage process yield for different prepared formulation was evaluated and results obtained are tabulated in Table 2. The results obtained were showing that the percentage process yield for most of the formulation was above 40%. Among all formulations F6 had shown highest percentage process yield with the value 75.45 ± 1.568 %w/w with the polymer to sodium TPP ratio of 3.75:1. Process yield is an important parameter regarding the formulation aspect for the large scale production. The polynomial equation 9 was derived after statistical analysis. Surface plot and contour plot are shown in Figure 3. The equation thus derived showed positive values for arithmetic mean, 53.44 %w/w and for the concentration of sodium TPP which indicated that it had positive effect on the process yield but at the same time effect of Chitosan concentration showed negative effect and outlined the positive effect of sodium TPP.

$$Y = 53.44 - 3.62X_1 + 6.01X_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots(9)$$

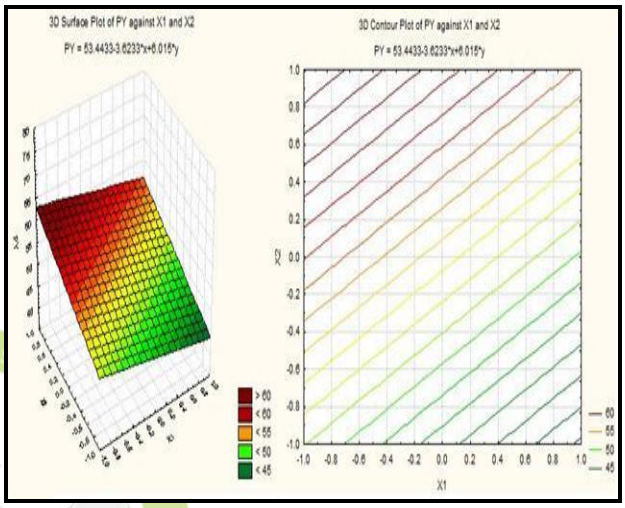

Figure 3: 3D surface plot and 3D contour plot of process yield vs X_1 and X_2

Percentage Association Efficiency

The evaluation of nanoparticles for percentage association efficiency was done and the results obtained are tabulated in Table 2. Association efficiency of the drug with the polymer was ranging from 34.71 ± 0.673 % to 44.38 ± 0.513 %. The association efficiency was seemed to be affected by the drug to polymer concentration which is evident if the results were compared with respect to drug to polymer ratio which is decreasing with the increase in concentration of Chitosan polymer; as concentration of drug was kept constant. The results were treated to derive the equation 10 from the factorial design. The results suggest that drug to polymer ratio was not the sole factor affecting the association efficiency but also the concentration of sodium TPP could have the effect on percentage association efficiency. The equation derived supported the discussion that the concentration of sodium TPP also affects the association efficiency.

$$Y = 39.70 - 1.43X_1 + 2.445*X_2 \ldots\ldots\ldots\ldots (10)$$

39.7%w/w is the arithmetic mean of all the nine formulation. The negative value of the coefficient of X_1 indicates that as concentration of Chitosan increases association efficiency decreases. The positive coefficient of the variable X_2 indicates that as the concentration of sodium TPP increases association efficiency also increase. For F7, which was having least association efficiency, the reason could be the ratio of X_1 to X_2, highest among all formulations. This can be seen in Figure 4 of surface and contour plots, respectively.

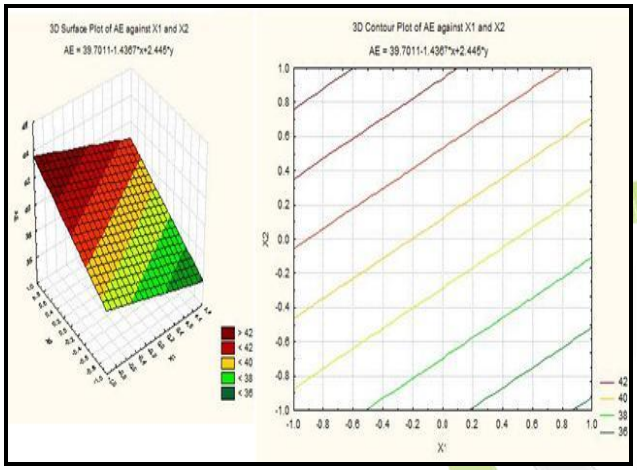

Figure 4: 3D surface plot and 3D contour plot of association efficiency vs X_1 and X_2

In-Vitro Release Studies

In the research field, mainly when the work to be carried out also includes the *in-vivo* release behavior of drug from the formulation, *in-vitro* release profile is a principal tool that predicts in advance how the drug will behave *in-vivo*. Drug release studies are in process quality control tool for any ongoing formulation process, anywhere. It is also useful to waive the *in-vivo* study, provided the bio-equivalency data has been established. It also helps in predicting the reproducibility of the rate and duration of drug release. The percentage of drug release at each time interval was calculated and plotted against time. The different formulations have shown significant difference in the drug release behavior. The formulation of nanoparticles allows one to control overall release of the drug as well as for poorly soluble drug surface area for dissolution can be increased. This increase in surface area allows drug dissolution at comparatively faster rate and as being a polymeric nanoparticle it also gives controlled and steady release by various mechanisms. The release profile of prepared nanoparticles has been reported in the Figure 5.

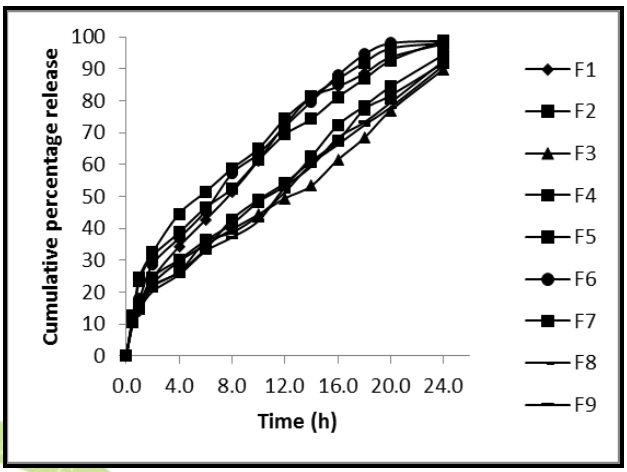

Figure 5: *In-vitro* dissolution profile of formulations F1 to F9

When studied the release of drug from nanoparticle formulations, it was observed that in all formulation (F1 to F9) there was a rapid release of drug in 1 h time interval followed by the release of drug in controlled manner. The reason behind this phenomenon could be the entrapped drug on the surface of the Chitosan nanoparticle which when comes in contact with dissolution medium released from the surface and goes into the dissolution medium.

The dissolution study results of all the formulations, at time interval of 1 h, subjected to statistical analysis to derive equation 11. The value 22.32 was the arithmetic mean CPR for all the nine formulations. There is significant effect of polynomial term i.e. square of concentration polymer on the release profile of the drug from nanoparticles but the value is low. This may be due the combined effect of drug to polymer ratio and polymer to sodium TPP ratio. Increase in drug release initially which could be combined with high association efficiency of drug with nanoparticle as observed.

$$Y = 22.32 - 1.55X_2 - 7.31X1^2 \ldots\ldots..(11)$$

To derive another equation 12, the dissolution study results of all the formulations, at time interval of 20 h were subjected to factorial analysis. Here the value 95.64 is indicative of the arithmetic mean CPR of all the formulations after 20 h of release study.

$$Y = 95.64 - 4.40X_2 + 3.12X_1X_2 - 12.13X_1^2 \ldots (12)$$

Equation 12 was similar to equation 11 and leads to the same derivation regarding the effect of square of polymer concentration on the drug release from the formulated nanoparticle formulations. 3D contour plot and surface plot were given in Figure 6 for the data collected at 1h and in Figure 7 for the data collected at 20 h. It would be easy to understand the effect of variables on the dependent variable value by going through the given figures.

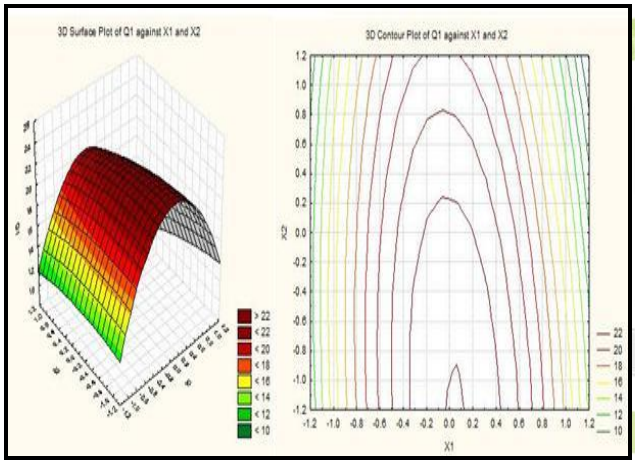

Figure 6: 3D surface plot and 3D contour plot of dissolution Q1 vs X_1 and X_2

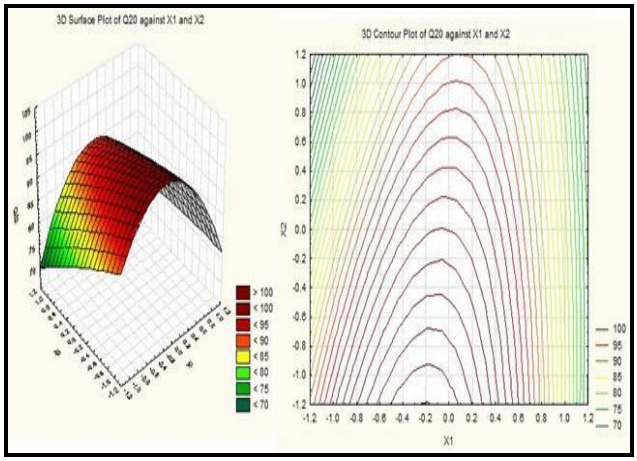

Figure 7: 3D surface plot and 3D contour plot of dissolution Q20 vs X_1 and X_2

Kinetic Modeling

The kinetic modeling is an important evaluation parameter with regard to the drug release mechanism from the formulation. It is also a tool by which we can further analyze the *in-vitro* dissolution release data of prepared and evaluated formulation. The kinetic data for formulations F1 to F9 are given in Table 3. From the obtained kinetic data it was clear that the drug release from the nanoparticles follows zero-order kinetic as the regression co-efficient values are indicative of the same. But the regression co-efficient obtained by the Higuchi model suggests that the drug release from the nanoparticles was not only by diffusion but other mechanism may be there. It is indicative from the n-values obtained by korsmeyer-peppas model that the prepared nanoparticle formulations have followed super case II mechanism with regard to the drug release from the nanoparticles.

Table 3: Kinetic modeling of F1 to F9

Form^n code	Zero order	Higuchi's	Peppas
	r^2 value	r^2 value	n value
F1	0.96483	0.99776	0.47428
F2	0.98763	0.98531	0.5159
F3	0.98204	0.98872	0.4999
F4	0.97028	0.9956	0.45813
F5	0.97557	0.99666	0.4873
F6	0.9789	0.99462	1.1879
F7	0.99095	0.98629	0.5495
F8	0.98989	0.98351	0.5722
F9	0.99194	0.98216	0.56692

Evaluation of Optimized Formulation

After evaluating the results of different evaluation parameters for factorial analysis they have been studied for the predicted values in between the given extremes of independent variables. The first criteria for selecting the optimized formulation were to select formulation which gives approximately 20% release within 1h and approximately 95%

release at 20h. The second criteria for selecting the optimized formulation were values of zeta potential, process yield and association efficiency. After statistically analyzing the different levels between minimum and maximum concentration values of X_1 and X_2, a batch with coded values of X_1 = -0.5 and X_2 = -0.5 (Un-coded values X_1 = 0.75 and X_2 = 0.4) was selected as check point cum optimized batch based on the values of first and second selection criteria. The composition and predicted values of this batch are given in Table 4.

Table 4: Composition and predicted response of check point cum optimized batch

Parameter	Predicted Values
Particle size (nm)	235.22
Zeta potential (mV)	38.955
Process yield (%)	52.245
Association efficiency (%)	39.1925
CPR after 1 hr (Q_1)	21.2675
CPR after 20 hr (Q_2)	95.5875

When both the formulations evaluated for the *in-vitro* release they have shown the drug release in same manner as that of the other formulations i.e. release was steady and controlled over a period of 20 h, Figure 8.

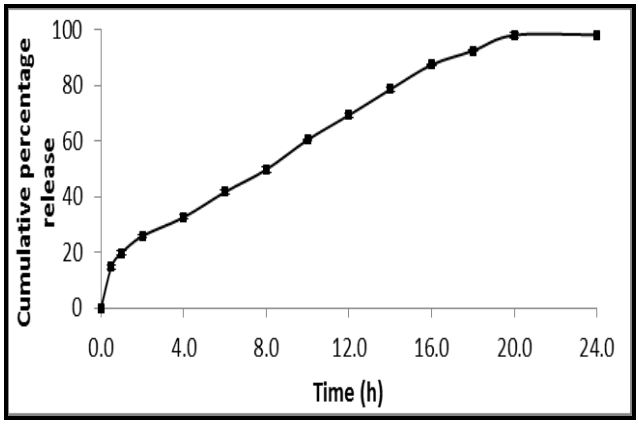

Figure 8: *In-vitro* release profile of checkpoint cum optimized formulation

Stability Study

Effect of Different Temperature on Chitosan Nanoparticle

The results obtained of study undertaken to evaluate the effect of different temperatures on Chitosan nanoparticles reflected that at 4^0C and 25°C the size of nanoparticle formulations remained similar to those of the freshly prepared samples.

The shape of the nanoparticle during this period was not studied as there was no change in the particle size which in case may have some bearing on the particle diameter.

The size of nanoparticle plays an important physiological role in its in-vivo interactions with biomolecules.

Accelerated Stability Study

The results of accelerated stability study shows no significant change in Particle size, zeta potential, CPR after 1 hr and CPR after 20 hr, indicates the formulation is stable.

In-Vivo Anti-Hyperglycemic Activity

In the case of Glibenclamide nanoparticles, the reduction in blood glucose levels was sustained for 24 hours after oral administration. It is suggested that a 25% reduction in blood glucose levels is a significant hypoglycemic effect.

The sustained hypoglycemic effect observed over a longer period of time in the case of nanoparticles, is due to the slow release and absorption of Glibenclamide over longer periods of time. Nanoparticle loaded with Glibenclamide showed decrease in SGL upto 48% within 4h and showed t_{min} at 8h.

Glibenclamide solution showed t_{min} at 4h but again at 8h when readings were taken, it showed the increase in glucose level.

Decrease in SGL for normal and diabetic control was also seen which may be due to the fasting condition of the rats taken for the examination.

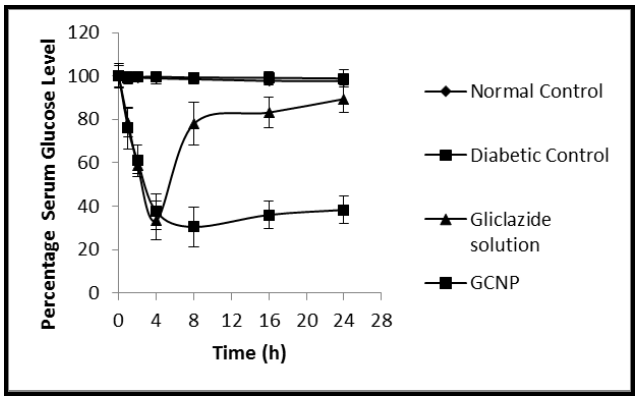

Figure 9: *In-vivo* anti-hyperglycemic activity of checkpoint cum optimized formulation and Glibenclamide solution compared to normal and diabetic control

CONCLUSION

From the present study it was found that 0.75% w/v of Chitosan & 0.4% w/v TPP desired results in terms of Particle size, Zeta potential, Process yield, Association efficiency, CPR after 1hr & CPR after 20 Hrs. Further the design formulation was stable and hence it can for tide a better option for controlled & targeted delivery of Glibenclamide.

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