Formulation and development of nanotechnology-based oral preparations of antidiabetic drugs using biodegradable polymer

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Abstract

Introduction: Diabetes mellitus is a chronic metabolic disorder characterized by elevated blood glucose levels and impaired insulin secretion. Inadequate insulin release from the pancreas or insulin's ineffective management of glucose leads to this disease.

Material and Methods: Tolbutamide was identified and described based on the characteristics indicated in preformulation investigations, and the sample was generously provided by Quantum Drugs and Chemicals Ltd. in Tamil Nadu, India. Preformulation studies were used to assess the TBM, and the results of these investigations contribute to the development of a stable dosage form.

Results: An effective oral formulation of TBM based on nanotechnology and biodegradable polymer was created. Clinical tests of the formulations, as well as the establishment of an in vitro-in vivo correlation for TBM-PC nanoparticles, the use of other biodegradable polymers to enhance drug entrapment, and so on, are all within the realm of possibility for future research.

Conclusion: The use of alternative biodegradable polymers that might boost the drug entrapment, and the establishment of an in vitro-in vivo correlation for TBM-PC nanoparticles. Health care can be made more efficient and economical through the use of modern technology, which is essential to meeting the high expectations of the masses for enhanced quality of life.

Keywords: Nanotechnology, oral preparations, antidiabetic drugs, biodegradable polymer

INTRODUCTION

Diabetes mellitus is a lifelong full-fledged persistent metabolic condition which occurs due to lack of insulin secretion, characterised by high glucose condition (hyperglycemia). This condition is an outcome of deficient secretion of insulin from pancreas or glucose is not properly managed by the insulin. This disease is canonically assorted by the Diabetes Association of America (ADA) [1, 2]. Thiscondition is characterised due to lack of beta cells of islets of langerhans in the pancreas thereby leading to little production or no insulin production. *Diabetes Mellitus 2* or Adult–Onset Diabetes. This condition is characterised by insulin resistance by body cells. The schematics physiology. *Gestational diabetes*: this condition is characterised during pregnancy, generally gets corrected automatically after the birth of child [3, 4].

The approach of drug delivery is confined with dosage form and route of administration which involves different devices which safely access a pharmacological entity in body to attain the predictable therapeutic effect. Constraints with the other delivery devices endorse the advent of nanoformulations for the drug delivery by utilizing biopolymer (polysaccharides) carrier. Nanotechnology which entangles the designing of formulations with 1–1000nm, intensely increases the therapeutic efficacy of loaded compounds [4, 6]**.**

Technology of nanoscaling delivers the platform where different substances and devicesare drafted, integrated and characterized. The critical functional component of this technology should be sized on the scale of nanometer. Scientifically, nanotechnology is employed to describe materials, devices and systems along their construction and constituent exploiting new and symbolically better physical, chemical and biological features as well as the phenomena and processes enabled by the ability to control properties at nanoscale [7-10].

Recently polymeric nanoparticulate systems have been examined effectively and competently for the delivery of drugs. The use of polymeric nanoparticles, especially those comprised of natural polymers, have procured concerned as promising candidates for the evolvement of drug carriers due to their excellent biocompatibility, and esteemed biodegradability with low toxicity and versatile biological applications [11, 12].

Nano-formulations composed of polymer contains

submicron –scaled colloidal particles where of concerned biologically active agent can be incorporated or encapsulated internally in the matrix of polymer or adsorbed on the surface of polymer. Biodegradable nanoparticles are the most promising and exciting formulations of nano- system with sub-micron (less than 1 µm) scaled messengers and are fabricated to acquire long term or sustained system to enhance stability, bioavailability and to target drug at specific sites of the systemic circulation. These implicate a broad array of research areas and industrial operations from basic technology to applied technology on the nanoscale. Nanoparticulate system becomes the necessity of the hour for medication industry as substitution delivery system for the therapy of highly prevailing and long lasting condition like type II diabetes mellitus [13, 14].

The problems related to conventional oral drug delivery system involves the sudden increase or decrease in drug plasma levels, which result in side effects of therapy or complete failure of the therapy. Such system requires frequent administration of drug to maintain the therapy and it leads to the patient non-compliance. Such problems becomea major issue when one has to deal with the management of a disease which is a curse to human being. From the past few years, nanotechnology is widely used for delivery of various drugs to the body for efficient absorption and enhanced bioavailability owing to high surface to volume ratio, reducing toxicity and sustained release. This new technology is now being utilized in designing of new drug delivery systems. The submitted proposal is also basedon similar lines [15, 16].

The main objectives of the present proposal are to develop nanotechnology based oral formulation of some anti-diabetic drug, to increase the solubility by reducing the size of drug leading to enhanced bioavailability of BCS class II drug and to eliminate the frequent administration of the conventional dosage form, which is proposed to be done by making a nanotechnology based formulation where these nanoparticles shall be prepared by using a biodegradable polymer.

MATERIAL AND METHODS:

The gift sample of drug Tolbutamide (TBM) was obtained from Quantum Drugs and Chemicals Ltd., Tamil Nadu, India and was further it was identified and characterized on the parameters mentioned in preformulation studies. The TBM was evaluated based on certain preformulation studies and the findings of these studies helps in formulating a stable dosage form at various stages of formulation development.

Characterization and Identification of TBM:

Organoleptic characteristics:

TBM was characterized on the basis of organoleptic characteristics like the color, smelland savor.

Melting point:

The melting point of TBM was determined by using melting point apparatus (Micro Scientific Works®) with capillary method. Prior to performing the study, the TBM was dried for about 24 hours by placing in a vacuum desiccator at room temperature.

FT-IR spectral analysis:

The TBM (1 mg) was triturated with KBr (100 mg) in a mortar. A transparent and smooth pellet was made by placing a little amount of mixed sample into a pellet maker with a compression force of 10kg/cm^2. The pellet was placed in the sample holder and scanning was done over a range of 4000 - 400 cm^{-1} by using Bruker, Alpha, ECO- Fourier Transform Infra-Red spectrophotometer.

a. Differential Scanning Calorimetry:

The DSC measurement of TBM was carried out on a Differential Scanning Calorimeter (Universal instruments V4.1D TA). The calibration of instrument was done by using indium as standard. The accurately weighed TBM (2mg) was placed and sealed in aluminium sample lid and heated over a range of 25ºC to 250ºC with an increment of 10ºC/min temperature under nitrogen atmosphere at a flow rate of 20ml/min [17].

b. X-Ray Powder Diffraction:

To ascertain the crystalline/amorphous nature of TBM, X-Ray powder diffraction (XRPD) studies were carried out. The XRPD study was performed by using X'Pert Pro, spinner PW3060 with Nickel-Filtered, Cu-Kα radiation, voltage: 45kV and current: 40mA. The study was conducted with continuous scan speed of 4º/min over a 2θ range of 0º - 40º.

c. Particle size analysis:

The particle size of TBM was obtained using a Malvern particle size analyzer. The driedpowder TBM sample was suspended in Milli-Q water by agitating the particle with the help of sonicator before final measurement. The particle size was determined by laser diffraction technique [18].

***d. pH solubility studies of drug*:**

The equilibrium solubility of TBM was determined by Shake Flask Method, in which excess of TBM in the solvent for a prolonged period was dissolved by stirring until equilibrium to obtain the saturated solution of TBM. The pH solubility study of TBM was conducted in the below mentioned media:

Drug Excipient Interaction Study:

i. Physical drug excipient compatibility studies:

The samples of TBM, polymer and mixture of TBM and polymer were placed in glass vials (sealed and open) at 60°C and 40°C/75% RH which represent the accelerated conditions. The ratios in which the mixtures of TBM and PC were made have beenbased upon the likely amount of drug and polymers in the final preparation

[18].

ii. Chemical drug excipient compatibility studies: The mixture of TBM and polymer in ratio 1:2 was prepared. The prepared samples were sealed and placed for study at temperature and humidity condition of 40ºC and 75% RH respectively for a period of one month. Thereafter, the samples were analyzed for any possible interaction between drug and polymer by using FTIR and the relevant peaks of drug and polymer at fixed wave numbers were studied [19].

Analytical method development for TBM:
For the quantitative analysis of TBM, quantitatively an analytical methodology wasneeded to be developed by using spectrophotometric technique.

Formulation development:
From the literature survey and the compatibility studies of the excipients, the most favorable excipients were selected. From literature survey and its following characteristics, Poly (ε-caprolactone) was selected as release modifying polymer for formulation of TBM loaded nanoparticles [20].

Selection of preparation technique:
There are various techniques for the preparation of nanoparticles i.e. solvent extraction technique, interfacial deposition method, nanoprecipitation, quasi-emulsion solvent evaporation technique, solvent displacement technique etc. The nanoparticles formulation can be prepared by numerous techniques depicted above. Furthermore, there should be no adverse effect on the therapeutic efficacy of drug, how well the method can be reproduced, the stability of the prepared formulation should be high and no side product which have toxic effect should be produced during preparation. Due to these reasons and based uponliterature survey, quasi-emulsion solvent evaporation technique was selected as themethod of the choice for the preparation of nanoparticles formulation [21].

Fabrication of TBM loaded Nanoparticles:
TBM and release modifying biodegradable polymer (Poly (ε-caprolactone)-PC) were dissolved in common solvent (ethyl acetate) in varying ratio to prepare internal phase. The so prepared internal phase was added drop wise in the external phase containing liquid paraffin and Span 80 as emulsifier, with stirring. The collected nanoparticles were freeze dried for 24 hrs at a temperature of -55ºC and vacuum condition of 0.5 kPa by using Lyoprotectant (glucose and lactose) to obtain the stable nanoparticles as fine powder which can be investigated further. A schematic representation of the preparation technique of TBM loaded nanoparticles is shown in Figure [22].

Optimization of TBM loaded Nanoparticles:
However, for the formulation research, the optimization of critical formulation and process parameters are very important. Earlier, the formulation development was based on the experience of the developer alone due to which a novel formulation takes lot of time to develop [23].

Factorial Designs:
The response equation made by factorial design estimate main effects and interactions but this design at two levels has an inherent assumption of linearity. These designs are used for optimization of various factors at varying levels based upon some prefixed responses. In the present study, the factors which are to be optimized are stirring speed-SS, stirring time-ST and sonication time-SCT. All the factors were studied at two level and the responses for the quality attributes like particle size-PS,drug entrapment efficiency-DEE and cumulative drug release-CDR were recorded [24].

Optimization of various parameters:

Optimization of critical formulation attribute (TBM-PC ratio):
Based on the literature survey and profile of polymer, PC was selected as release modifying biodegradable polymer. However, ratio of TBM and PC is also an important factor in the formulation development. Therefore, a series of formulations by keepingthe amount of drug constant and increasing the amount of PC as indicated in Table 5.5 were prepared. All such prepared formulations were analyzed to study the effect on critical quality attributes such as particle size (nm), zeta potential (mV), drug entrapment efficiency (%) and percent cumulative drug release [25].

a. Optimization of critical process parameters:
The optimization of critical process parameters was done by using 2^3 full factorial design along with evaluation of the main and interaction effects of CPP. The four factorswhich have no or insignificant effect on the quality attributes were kept constant. The remaining three factors were optimized using 2^3 full factorial design at two levels i.e. low and high level as given in Table. The uncontrollable conditions beside the point that can affect the experiments, randomization technique were used to avoid such effect on the results. The response was recorded for the attributes particle size (nm), drug entrapment efficiency (%), cumulative percent drug release.

b. Validation and optimization of 2^3 full factorial design:
The levels of factors were constraint so as enhance the DEE (drug entrapment efficiency) and minimize the PS (particle size) and to sustain the release of drug over prolonged period of time. The polynomial equations were generated as follows:

$$\textbf{Response} = \beta_o + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 + \beta_{123}X_1X_2X_3$$

***In vitro* drug release studies:**
Dialysis bag method was used to study the *in vitro* drug release from TBM-PC nanoparticles. The formulated TBM-PC nanoparticles samples were accurately weighed and were packed in a dialysis bags (12,000 D cut off) and placed in a vessel containing phosphate buffer pH 7.4 with constant stirring at 37±1°C. At predetermined intervals, the aliquot of 1 ml was taken and sink conditions were maintained by replenishing with an equal amount of fresh phosphate buffer. Drug is released from nanoparticles to the outer compartment and sampled for analysis. The samples were analyzed at 226 nm using UV-VIS spectrophotometer [28].

Drug release kinetics study from TBM-PC nanoparticles:
The data obtained from the drug release study was further studied for release kinetics of drug. The obtained values of CDR along with time were tested by using various models as depicted under: Zero order model which form a relation between CDR and time First order model which form a relation between log CDR and time Higuchi model which form a relation between CDR and Sq. rt. of time Korsmeyer-Peppas model which form a relation between log CDR and log time [29].

Stability Study:
The stability studies were performed to assess the stability of the prepared formulations throughout its shelf life. To assess it, well-designed protocol is mandatory. The stability depends on various surrounding factors like temperature and humidity conditions during storage and pharmaceutical factors like type of formulation, the natureof therapeutic agent etc.

***In-vivo* studies:**

Animals:
In-vivo studies to access the anti-diabetic activity were carried out by using female wistar albino rats weighing between 250-300gm. The wistar albino rats were kept free for the standard animal diet and water *ad libitum*. The protocols of the *in vivo* studies were approved by Institutional Animal Ethical Committee and experiments were carried out according to CPCSEA guidelines [30].

Oral acute toxicity study:
A 30-day oral acute toxicity study under good laboratory practices was conducted. Wistar albino rats were administered TBM-PC nanoparticles according to OECD test guidelines 425. The animals were kept at fast for 12hrs before administration of dose of TBM-PC nanoparticles. The animals were selected randomly and grouped as follows (n=6):

Group I: Control group- treated with normal saline.
Group II: TBM-PC nanoparticles treated test group (250 mg/kg body weight)

Streptozotocin-induced anti-diabetic study:
Diabetes was induced in Wistar albino rats by injecting streptozotocin at a dose of 60 mg/kg body weight dissolved in pH 4.5 citrate buffer via intraperitoneal (I/P) injection. 5% w/v glucose solution was provided to the animals to prevent the drug induced hypoglycemia. The animals having blood glucose levels more than 350 mg/dl during fasting stage were selected for anti-diabetic study. The blood glucose levels were monitored with calibrated glucose meter Accu-Chek®.

Statistical analysis:
The data obtained after the *in vivo* study was analyzed by using t-test to check the significant difference between two groups at p value of <0.0001 and ANOVA to check significant difference between multiple groups at p value of <0.0001 using GraphPad Prism 8.3.0.

RESULTS AND DISCUSSION

Preformulation studies

Characterization and Identification of TBM:

a. Organoleptic characteristics:
The TBM is a white to off-white crystalline powder, practically odorless and bitter in taste. Physical appearance of the TBM sample under investigation was found to be sameas that of the official reports.

b. Melting point:
It was found that the melting point of TBM was in a range of 128ºC±2ºC, which is in conformity of official reports and certificate of analysis supplied by the manufacturer. It confirms that the TBM is pure as it exhibit sharp melting point.

c. FT-IR Spectral analysis:
The FT-IR spectrum of TBM as recorded in Figure 6.1 was found in accordance withthe FT-IR of standard TBM **(107)**. Figure 6.2 shows graphs of standard TBM. Band assignments for the observed spectrum are summarized.

The FTIR spectral analysis and respective band assignments to major peaks indicatesthe presence of functional groups at their respective wave numbers.

d. Differential Scanning Calorimetery (DSC study):
The DSC thermogram of TBM is shown in Figure 6.3, which exhibits a melting endotherm peak at 128.66ºC with enthalpy of 75.9 J/g, from which it can be concluded that the procured TBM is pure and authentic.

e. X-Ray Powder Diffraction Studies:
The XRPD analysis indicates the solid form structure and provides information about physical form of powder. The diffraction spectrum of drug as shown in Figure 6.4shows several different peaks indicating the state of drug is highly crystalline.

f. ***Particle size analysis:***

The distribution of PS-(particle size) of TBM was compared with the percent volume and average diameter of D_{50}. The PS-(particle size) distribution of TBM is indicated in Table 6.2 and depicted in Figure.

g. ***pH solubility studies of drug*:**

The bioavailability of drug is dependent upon the solubility of drug. The solubility is a crucial factor for the drug which belongs to class II as the drug release rate andtherefore, the pharmacological effect of the prepared formulation depends on it. The solubility of TBM in different media was calculated and is tabulated in table.

h. ***Partition Coefficient:***

Partition coefficient is the key factor for determination of its hydrophilic or lipophilic nature. More the compound is lipophilic in nature; the better crossing of biological membranes will take place. Whereas, the hydrophilic compound stays in the aqueous compartment and which influence the release of drug from formulation **(125)**. The partition coefficient (log P) of TBM is found to be 2.31±0.18 indicating a relatively lipophilic molecule.

i. ***Hygroscopicity Study of drug:***

Results obtained at the end of four weeks after keeping the drug at different RHconditions showed negligible % weight gain. There was only 0.01% weight gain at 30% RH, 0.01 % at 50% RH, and 0.02 % at 75% RH from the initial weight. It wasconcluded that the TBM was not hygroscopic and can be used in formulation at any humidity condition.

Drug Excipient Interaction study:

Physical Compatibility Study: The ratio for preparing mixture of drug and the polymerwas selected based on the probable concentration of the excipients in the formulation. The physical changes were observed for drug and excipients compatibility study. The data obtained is shown in Table 6.6, indicating TBM and the PC are compatible as per pharmacopoeial standards. However, at 60ºC the wet mass was obtained which is due to the reason that PC melts in the range of 58-63ºC.

Preparation of calibration curve of TBM in PBS of pH 7.4:

The prima facie requirement of any calibration technique is linearity. The presence of linearity ensures that the analytical method shall produce the accurate results which are proportional and correlated with the varying concentrations of the drug in samples. More accurate technique shall be developed keeping in view the linearity of the techniques after experimenting over a wide range of drug concentrations.

Formulation Development:

Optimization of TBM loaded Nanoparticles:

Optimization of critical formulation attribute (TBM-PC ratio):

The TBM loaded PC nanoparticles were formulated by Quasi-emulsion solvent evaporation (QESE) technique with varying drug polymer ratio. The prepared nanoparticles were analyzed for PS-particle size (nm), ZP-zeta potential (mV), DEE- drug encapsulation/entrapment efficiency (%) and CDR-cumulative drug release (%). The results are indicated in Table.

The PS of various batches vary from 213.5 ± 3.71 nm to 967.1 ± 4.12 nm and the smallest PS found to be 213.5 ± 3.71 nm from the TN4 batch. The increasing amount of polymer from batches TN5 to TN8 leads to agglomeration of particles and subsequently the PS increases. Whereas, the batches having lower amount of polymer than drug i.e. TN1 to TN3 exhibits lower entrapment efficiency due to less amount of polymer to form polymeric matrix. The DEE varies from 12.56±5.71% to 71.27±3.17% i.e. lowest for TN1 batch and highest for TN4 batch. The increase in DEE leads to decrease in the loss of drug increase in the production yield [8-19]. Further, the increased DEE also leads to achievement of the therapeutic effect of the drug even in small doses.

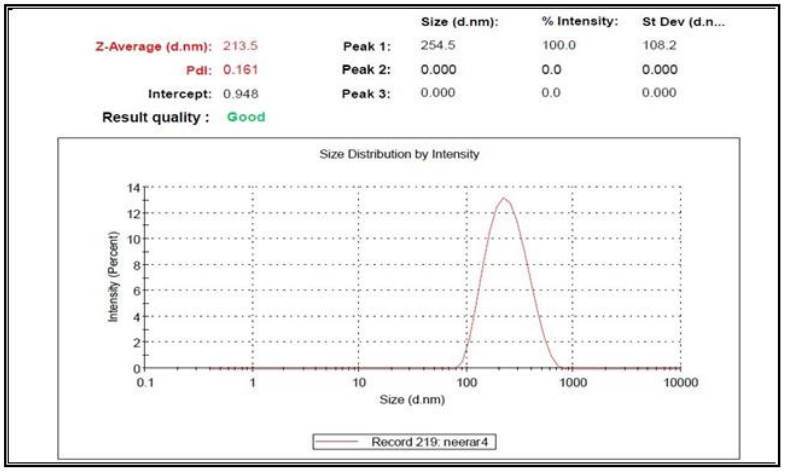

Figure 1: PS report of TBM-PC nanoparticles

Among the all batches having varying ratio of drug and polymer, batch TN4 has the least PS of 206.1±3.71 nm, highest DEE 71.27±3.17%, stable having negative ZP of - 17.5±1.75 mV and drug release up to 12 hours is 87.73 ± 0.29 % indicating prolonged release of drug. Keeping in view of the above quality attributes, the ratio of TBM and PC was selected as 1:2 for further optimization of critical process attributes.

Composition of batches and results for CDR:

The batches of TBM-PC nanoparticles as given by the 2^3 full factorial design were characterized for the third quality attribute viz. CDR (%) and the results obtained along with the composition of all the eight batches are shown in figure. The consolidated layout of composition of TBM-PC nanoparticles indicating the levels of independent variable viz. critical process variables i.e. SS-stirring speed (rpm), ST- stirring time (min) and SCT-sonication time (min) and the results in average values of dependent responses viz. critical quality attributes i.e. PS (nm), DEE (%) and CDR (%) are portrayed.

Experimental Design:

Y_1- PS-Particle Size:

Calculation of main and interaction effects:

The effects of the main independent factors and their interactions on PS were calculated and tabulated in Table.

Table 1: Main effects of independent factors on PS

Factors Level	**A: SS-stirring speed (RPM)**	**B: ST-stirring time (Min)**	**C: SCT-sonication time (Min)**
High (+1) Settings	267.28	280.75	293.81
Low (-1) Settings	364.87	351.40	338.34
Effect	**-97.60**	**-70.65**	**-44.53**

Table 2: Interaction effects of independent factors on PS

Factors Level	**A×B**	**A×C**	**B×C**	**A×B×C**
High (+1) Settings	317.49	313.49	313.37	311.32
Low (-1) Settings	314.66	318.66	318.79	320.83
Effect	**2.83**	**-5.16**	**-5.42**	**-9.51**

Analysis of Variance (ANOVA) for DEE:

The F-value of the applied model is 74.40 which suggest that the selected model is significant and the possibility that this value could be due to noise of experiment is only 0.06%. The P-values of 0.0006 which is below 0.0500 suggest that the studied model terms are significant. In this case, the model terms are A, B and C. The complete ANOVA table.

a. Y_3- CDR-Cumulative Drug Release:

Calculation of main and interaction effects:

The effects of the main independent factors and their interactions on CDR were calculated and tabulated in Table.

Table 3: Main effects of independent factors on CDR

Factors Level	**A: SS-stirring speed (RPM)**	**B: ST-stirring time (Min)**	**C: SCT-sonication time (Min)**
High (+1) Settings	83.91	81.82	81.60
Low (-1) Settings	72.95	75.05	75.26
Effect	10.96	6.77	6.34

Table 4: Interaction effects of independent factors on CDR

Factors Level	**A×B**	**A×C**	**B×C**	**A×B×C**
High (+1) Settings	78.54	79.05	78.37	78.55
Low (-1) Settings	78.33	77.82	78.49	78.32
Effect	**0.21**	**1.23**	**-0.12**	**0.23**

Mathematical Modeling:
The polynomial equations as given below have various coefficients related to intercept, main and interaction effects. The sign and extent of the coefficient predict the influence of each term on the overall response.

Summary statistics for the model drug:
Analysis of variance (ANOVA) of the responses as shown in Table 6.23 indicated that the developed response surface models for PS, DEE and CDR were significant and adequate, without significant lack of fit.

Table 5: Significance of design

Response	**F-value (Model)**	**P-value**
Y_1 (PS)	142.38	0.0002
Y_2 (DEE)	74.40	0.0006
Y_3 (CDR)	169.19	0.0001

Table details the model summary statistics for the selected significant models. The high level of correlation between the responses obtained experimentally and thosepredicted by the design was confirmed by the high values of R^2. The predicted and adjusted R^2 values were found in good agreement, which results in reliable models.

Response Surface Analysis:
Figure portray the 3D response surface plots (RSP), while Figure 6.33, 6.38 and 6.43 are the corresponding 2D contour plots for PS, DEE and CDR of TBM-PC nanoparticles.

PS-Particle Size (nm):
The equation generated revealed that all the factors exerted a significant influence on PS of TBM-PC nanoparticles. The effect of the main factors on the PS of the optimization of TBM-PC nanoparticles was further explained using the 3D RSP and contour plot which reveal that PS varies linearly with increasing amount of each factor.

DEE-Drug Entrapment Efficiency:
The mathematical model generated (Eq. 6.2) indicated that the entire factor's i.e. SS-stirring speed, ST-stirring time and SCT- sonication time were found to exert a significant influence on DEE on TBM-PC nanoparticles. A positive influence of all three factors is clearly demonstrated by the 3D RSP and the respective 2D contour plot. However, the effectof SS-stirring speed was much prominent followed by ST-stirring time and SCT- sonication time. Some interactions also exists as shown by the plots, the interaction of SS-stirring speed-ST-stirring time, SS-stirring speed-SCT-sonication time and ST- stirring time-SCT-sonication time has negative effect on DEE due to leaching of drug at higher energy levels and it is also evident from the observations.

At the constant level of ST-stirring time and SCT-sonication time and at a SS-stirring speed of 500 rpm, the DEE is 71.01% and at 1500 rpm, the DEE increases up to 83.79% i.e. a 1.18 fold increase in DEE. The same effect was observed for ST-stirring time at constant level of SS-stirring speed and SCT-sonication time i.e. the DEE increases from 74.39% to 80.41% on increasing the ST-stirring time from 30 to 120 min leads to 1.08 folds increase in DEE of TBM-PC nanoparticles. This may be due to the reason that these factors leads to decrease in PS that eventually enhance the surface area andsubsequently higher DEE. The effect of SS-stirring speed and ST-stirring time on DEE of TBM-PC nanoparticles is depicted in Figure 6.39 and 6.40 respectively.

CDR %:
The mathematical model generated (Eq. 6.3) indicated that all the three factors have a significant effect on CDR. A positive effect of all the factors and interactions and a negative effect of only one interaction of ST-stirring time and SCT-sonication time is clearly demonstrated by the 3D RSP and the respective contour plot, which suggest that the CDR increases with the increase of amount of any factor.

Optimization provided by 2^3 full factorial design:

To check the prediction made by the design during optimization procedure, a new batch of TBM-PC nanoparticles with the variables values given by the design was prepared. The design give prediction that SS-stirring speed on 1480 rpm, ST-stirring time of 110 min and SCT-sonication time of 5 min would provide the desired responses as shown in Table 10. The batch with highest desirability was selected and manufactured by using the procedure mentioned earlier and evaluated further for various characteristics.

Table 6: Optimized batch of TBM-PC nanoparticles

Code	SS	ST	SCT	PS	DEE	CDR	Desirability
OB1	1480	110	5	219.51	86.39	89.50	0.940

The Figure shows the cube graphs and 2-D surface graphs for theoptimized batch along with desirability and predicted values of the dependent responses.

Evaluation of Optimized Batch:

Differential Scanning Calorimetry study:
The optimized batch (OB1) of TBM-PC nanoparticles was evaluated on the basis of differential scanning calorimetry (DSC) study along with polymer PC, drug TBM,physical mixture of TBM and PC. The results of DSC are shown in Figure 6.49. From the thermogram it is evident that the polymer PC exhibits an endothermic peak at 60^{o}C and drug TBM exhibits a sharp endothermic peak at 128^{o}C which also supports its crystalline nature. Further, no interaction in the peaks of physical mixture of TBM and PC is shown and it supports the presence of physical compatibility. However, theintensity of the peaks during analysis of TBM-PC nanoparticles is lessened due to the dilution effect caused by the polymer i.e. formation of polymeric matrix and it also suggests that the polymer is evenly distributed around the surface of TBM.

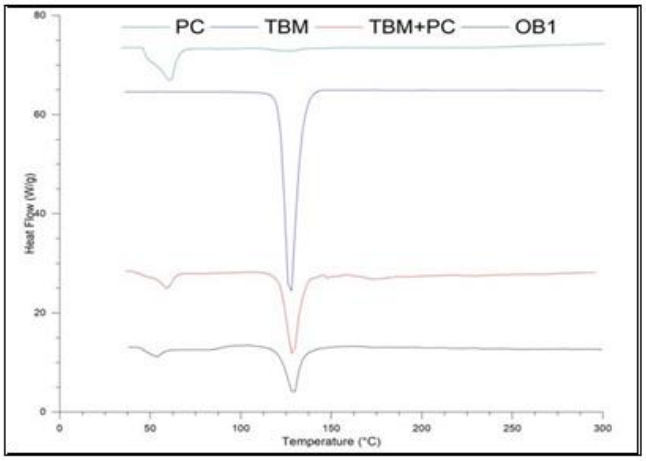

Figure 2: DSC result of PC, TBM, TBM + PC and TBM-PC nanoparticles(OB1)

X-Ray Powder Diffraction:
The results obtained from Powder X-Ray Diffraction study is shown in Figure 6.50. From the XRPD patterns it is suggested that the polymer PC is in the semi-crystalline state, whereas, the drug TBM is crystalline in nature and shown numerous peaks with high intensity. The physical mixture of TBM and PC shows the peaks of both drug and polymer which indicates the physical compatibility between drug and polymer. The XRPD of TBM-PC nanoparticles were carried out to identify the nature of the formulation. In the XRPD pattern, there are no characteristic peaks of drug which support the dilution effect of polymer and it was revealed that the TBM-PC nanoparticles formulation is amorphous in nature. These changes of crystalline to amorphous nature confirm the entrapment of TBM in PC polymeric matrix.

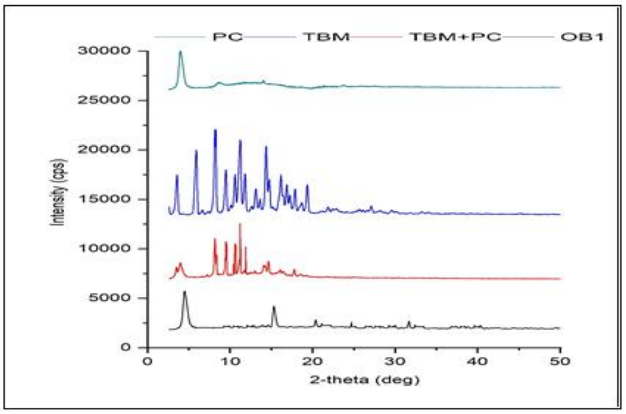

Figure 3: XRPD patterns of PC, TBM, physical mixture of TBM and PC andTBM-PC nanoparticles (OB1)

FT-IR spectral analysis:

FTIR spectral analysis was conducted for the optimized batch of TBM-PC nanoparticles, the characteristic peaks of TBM were present with slightly reduced intensity i.e. 1721.43 cm^{-1} for C=O stretch, 1548.27 cm^{-1} and 1459.93 cm^{-1} for aromatic and C=C stretch, 1333.04 cm^{-1} for sulfonamide S=O stretch and 727.66 cm^{-1} for N-H wagging. The characteristics peaks of polymer PC are also present with reduced intensity indicating the presence of polymeric matrix with minor shift in peaks. This study confirms that the TBM and PC did not interact significantly with each other. The FTIR spectra of OB1 batch of TBM-PC nanoparticles is depicted in Figure.

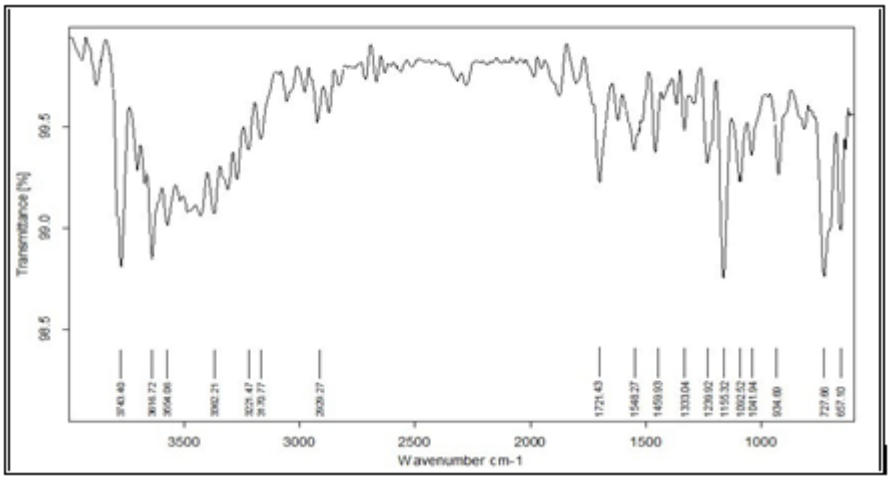

Figure 4: FTIR spectral analysis of TBM-PC nanoparticles (OB1)

Surface and shape analysis:

The OB1 batch of TBM-PC nanoparticles was studied for the surface and shape characteristics by using field emission scanning electron microscopy and transmission electron microscopy. The micrograph of SEM and TEM reveals that the prepared nanoparticles were spherical in shape having small pores on the surface which may develop due to the reason that solvent evaporates from the surface and the formation of pores takes place. In some micrographs some drug seems to be present on the polymeric surface which may contribute to the burst effect of the drug and onset of therapy. The SEM and TEM micrographs are portrayed in Figure.

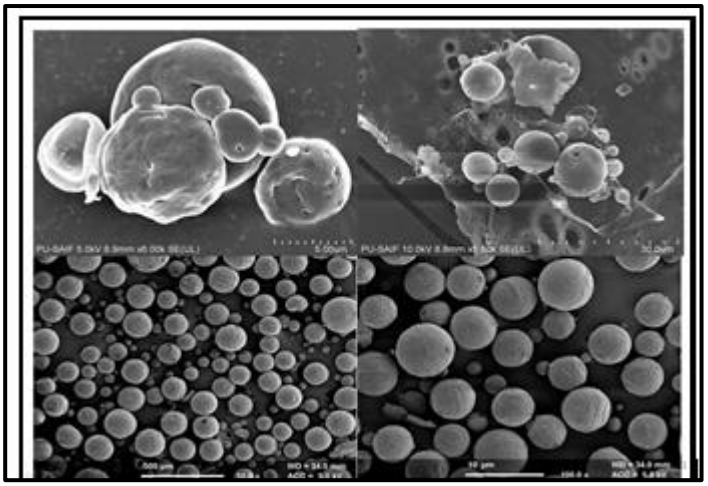

Figure 5: FE-SEM micrographs of TBM-PC nanoparticles (OB1)

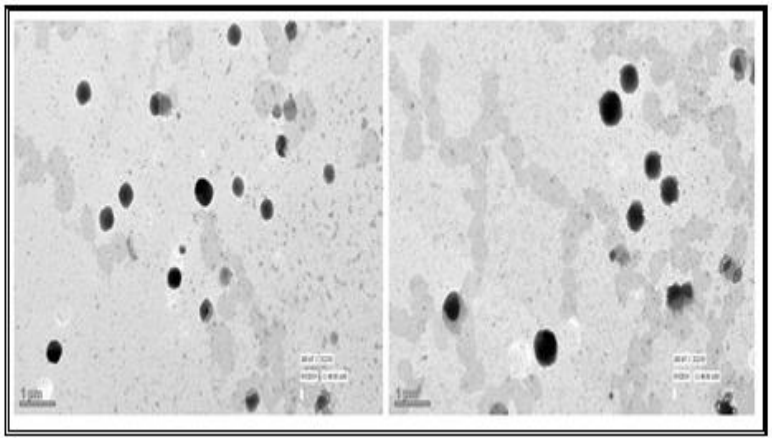

Figure 6: TEM micrographs of TBM-PC nanoparticles (OB1)

Particle Size and Zeta Potential studies:

The average PS of OB1 batch of TBM-PC nanoparticles was found to be 206.1 nm with PDI of 0.157 which is in close relation with the PS as given by the design of experiment. The PS report is shown in Figure 6.54. ZP of the nanoparticles indicates the stability of the formulation. Either negative or positive charge of high magnitude shows the presence of repulsive force and leads to physical stability and it also suggests thatthe stabilizer used in the system prevents the agglomeration. The ZP of OB1 batch was -17.5mV which indicate the physical stability of prepared TBM-PC nanoparticles.

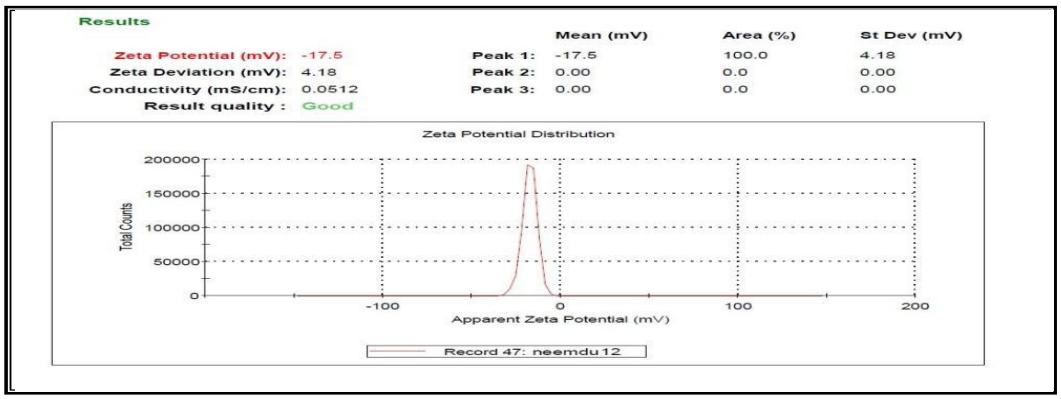

Figure 7: ZP of TBM-PC nanoparticles (OB1)

Drug Entrapment Efficiency:
The DEE of the optimized batch of TBM-PC nanoparticles was found to be 85.35±1.26which is in similar as that of the predicted value given by the 2^3 full factorial design i.e. 86.39 with a residue of 1.04.

***In-vitro* drug release studies:**
The in vitro drug release profile of OB1 batch of TBM-PC nanoparticles vis-à-vis TBM is shown in Figure. The release of TBM is found to be almost 100% in 2 hours whereas the release of the drug from nanoparticles was found to be 38.89±0.73% during the same time period which indicates the prolonged release of the drug for 12 hours. At the 12th hour, the release of drug from nanoparticles was found to be 64.17±1.72%. Further, the study was continued up to 24 hours to find out the release patterns of drug, and at 24th hours the CDR was found to be 90.21±1.12%.

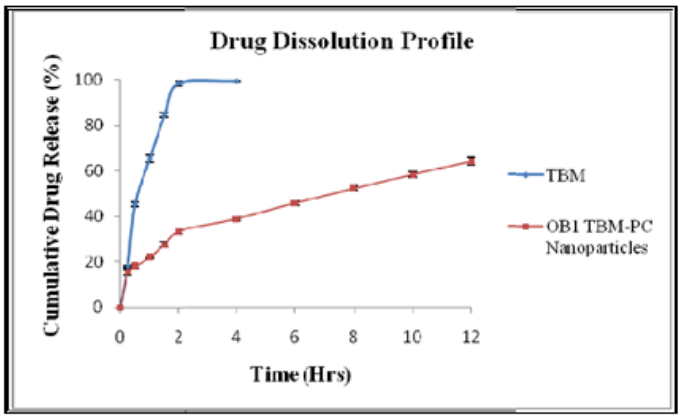

Figure 8: Drug dissolution profile of TBM-PC nanoparticles of OB1 batch andTBM

Response after optimization procedure:
Table illustrates the results of design predicted values and experimentally observed values for the quality attributes for the optimized formulation. From the results, it was evident that both predicted and observed values for PS, DEE and CDR are in close proximity with very low residuals.

Drug release kinetics from TBM-PC nanoparticles:
The data of CDR of the optimized batch of TBM-PC nanoparticles was further studied for release kinetics study. For the said purpose the data was fitted in to various models viz. zero order (CDR and time), first order (log CDR and time), Higuchi kinetics (CDR and Sq. rt. of time) and Korsmeyer-Peppas (log CDR and log time). Table enlists the values of regression coefficient obtained from various kinetics models.

Stability studies:
The stability depends on various surrounding factors like temperature and humidity conditions during storage and pharmaceutical factors like type of formulation, the nature of therapeutic agent etc. The prepared nanoparticles (OB1) were studied for stability for three months at 5±3ºC and 25 ºC and the results are summarized in Table.

Table 7: Stability studies – OB1 of TBM-PC nanoparticles

Storage Period	PS (nm)	ZP (mV)	Residual Drug Content (%)
Initial	206.1±3.71	-17.5±1.75	100
At 5± 3 ºC			
1 month	210.1 ± 4.64	-18.3± 1.02	99.74 ± 1.23
3 months	218.1 ± 5.13	-19.05± 0.97	99.12 ± 3.14
At 25 ºC			
1 month	231.7 ± 5.24	-18.7 ± 1.12	99.12 ± 2.17
3 months	245.2 ± 3.36	-19.47 ± 1.21	98.28 ± 3.63

During the study, there was no change in the visual appearance of the nanoparticles. The prepared nanoparticles were easily dispersed under the storage conditions. On chemical evaluation, it was observed

that the % residual drug content of the optimized batch atthe end of 3 months was found to be 99.12 ± 3.14% at 5±3ºC and 98.28±3.63% at 25ºC. There was no significant change in the mean PS of TBM-PC nanoparticles. Hence, the results related to PS (nm), ZP (mV) and Residual Drug Content (%) indicated the stability of the prepared nanoparticles upon storage over a period of three months. From the results, it is also evident that the temperature of 5±3 ºC for storage of prepared nanoparticles is optimum.

***In-vivo* studies:**

The approval of the project from IAEC of the Institute was obtained vide no. IAEC/68- 80 dated 28-03-2017 prior to conduction of *in vivo* study.

Oral acute toxicity study:

The wistar albino rats were exposed to a dose of 250 mg/kg body weight of TBM-PC nanoparticles for oral acute toxicity studies. The results of various parameters studied are summarized in Table 6.29. Further, no mortality was observed during the study period which suggests the given dose to be safe even after the nanosizing of the drug in TBM-PC nanoparticles.

Streptozotocin-induced anti-diabetic study:

The samples of blood were withdrawn at predetermined time intervals and assayed for the blood glucose concentration in mg/dl. The results obtained are shown in Table 6.30 and in Figure 6.61. From the obtained results, the group treated with the TBM shows a decrease in blood glucose concentration within half an hour and peak decrease was shown at 2 hrs thereafter the blood glucose concentration again started to raise showing the effect of conventional drug delivery. This finding is also in consonance with the dataobtained by in vitro drug release study.

Further, the TBM-PC nanoparticles shows a decrease in blood glucose level at half an hour of study which may be due to the initial sudden release of drug for onset of action from the nanoparticles and the glucose level were kept on decreasing up to 6 hrs and are in permissible limits up to long terms of 24 hrs. This shows that the release of drug from TBM-PC nanoparticles is prolonged for long duration therapy.

CONCLUSION

Diabetes mellitus is a lifelong full-fledged persistent metabolic condition which occurs due to lack of insulin secretion, characterised by high glucose condition (hyperglycemia). This condition is an outcome of deficient secretion of insulin from pancreas or glucose is not properly managed by the insulin. The later condition is termed as type II diabetes. Thus in a nutshell, the nanotechnology based oral formulation of TBM by using biodegradable polymer was successfully developed. Further, the establishment of *in vitro-in vivo* correlation for TBM-PC nanoparticles, use of different biodegradable polymers which might increase the drug entrapment, clinical studies ofthe formulations can be the future scope of the work for further investigations. The high expectation of masses for improved quality of life can be achieved by making healthcare more efficient and affordable by using advancing technology.

Funding
None

Conflict of Interest
None

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