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Qbd-Based Formulation Optimization and Characterization Of Polymeric Nanoparticles of Cinacalcet Hydrochloride with Improved Biopharmaceutical Attributes

Geliştirilmiş Biyofarmasötik Özelliklere Sahip Sinakalset Hidroklorürün Polimerik Nanopartiküllerinin Qbd Tabanlı Formülasyon Optimizasyonu ve Karakterizasyonu

Short running title: Freezed-dried Polymeric Nanoparticles of CIH
Kısa süreli başlık: CIH'nin Dondurulmuş-Kurutulmuş Polimerik Nanopartikülleri

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ÖZ:
Amaç: Mevcut çalışma, PLGA ve poloksamer-188 kullanılarak nanopresipitasyon ve ultrasonikasyon yöntemleriyle üretilen sinakalset hidroklorürün dondurularak kurutulmuş polimerik nanopartiküllerinin hazırlanmasını, QbD'nin optimizasyonunu sağlaması ve oral biyoyararlanımının iyileştirilmesiydi.
Gereç ve Yöntemler: Formülasyonlar için ilk tarama ve optimizasyon, Taguchi ve Box-Behnken tasarımları kullanılarak yapılmıştır. FT-IR ve DSC, seçilen ilaç ve polimerler arasında hiçbir etkileşim ve uyumsuzluk ortaya koymadı. Nanopartiküller,% ilaç salımı, partikül boyutu analizi, zeta potansiyeli, PDI, SEM, TEM, P-XRD, TGA, DTA, in vitro ve in-vivo ilaç salımı için karakterize edildi.
Sonuçlar: In vitro çalışma, ilacın optimize edilmiş partiden difüzyon mekanizması ile sürekli salındığını göstermiştir. Optimize edilmiş polimerik nanopartikül formülasyonu, deneysel modelin doğrulanması kullanılarak sayısal ve grafiksel yöntemlerle tanındı. Optimize edilmiş parti, ICH stabilite kılavuzlarına göre altı ay boyunca stabildi ve partikül boyutu, tuzak etkinliği ve in vitro ilaç salımı açısından önemli bir değişim bildirilmedi. AUC ve Cmax verilerinin farmakokinetik parametreleri, saf ilaç süspansiyonuna kıyasla optimize edilmiş

partinin 3 ve 2.9 katı arttı.
Sonuç: Bu nedenle hazırlanan polimerik nanopartikül formülasyonu, hiperparatiroidizmin ön koşulu olan hastalarda uzun vadeli normokalsemiyi yönetmek için bir model ilacın iyileştirilmiş terapötik etkinliği ve biyoyararlanım potansiyeli için alternatif bir dağıtım sistemi.

Anahtar Kelimeler: PLGA, Polimerik nanopartiküller, P-XRD, Optimizasyon, Biyoyararlanım potansiyeli.

ABSTRACT:
Objective: The present work was to prepare, QbD enabled optimization, and improve the oral bioavailability of freeze-dried polymeric nanoparticles of cinacalcet hydrochloride manufactured by nanoprecipitation and ultrasonication methods using PLGA, and poloxamer-188.
Materials and Methods: The initial screening and optimization have been carried out for the formulations by employing Taguchi and Box-Behnken designs. The FT-IR and DSC revealed no interactions and incompatibility among the selected drug and polymers. The nanoparticles were characterized for % drug release, particle size analysis, zeta potential, PDI, SEM, TEM, P-XRD, TGA, DTA, *in-vitro*, and *in-vivo* drug release.
Results: *In-vitro* study showed sustained release of drug from the optimized batch by diffusion mechanism. The optimized polymeric nanoparticle formulation was recognized by numerical and graphical methods using validation of the experimental model. The optimized batch was stable as per ICH stability guidelines for six months with no considerable alternation has been notified in particle size, entrapment efficiency, and *in-vitro* drug release. The pharmacokinetic parameters of AUC and C_{max} data increased 3 and 2.9 folds of the optimized batch compared to the suspension of pure drug.
Conclusion: Hence, the prepared polymeric nanoparticles formulation an alternative delivery system for enhanced therapeutic efficacy and bioavailability potential of a model drug to manage long-term normocalcemia in patients with the preliminary condition of hyperparathyroidism.
Keywords: PLGA, Polymeric nanoparticles, P-XRD, Optimization, Bioavailability potential.

INTRODUCTION

Nano-drug delivery systems in medicine have evolved as a dependable and reliable technological boon as site-specific drug targeting in nano-drug development in the previous two decades[1,2]. Polymeric based nanoparticles are the colloidal nature systems measuring around 10-100nm. The experimental findings achieved are mostly in the range of 100-500nm. The polymeric based nanoparticulate systems have been considered an area of extensive research in novel drug delivery system because of its all-encompassing biocompatibility and ease of altering properties[3].

For the study of nanoparticles, worldwide numerous scientists have discovered abundant approaches such as nano-precipitation, solvent evaporation, salting out, emulsification-diffusion, and the supercritical fluid technology (SCT)[4]. The polymeric nanoparticulate systems offer high applicability as an active delivery system. These polymeric nanoparticulate versions consistently deliver the drug to the active site with enhanced therapeutic response and minimize possible side effects[5]. These biopolymers have the core advantages of reduced toxicity and maximum therapeutic index[6,7].

The polymeric micelles have and additional characteristics for being safer and trustworthy for drug targeting, biocompatibility, and stability during effective drug release[8-10]. The recent innovation of the drug cinacalcet hydrochloride (CIH) includes the investigations anticipated to enhance oral bioavailability because of precipitation-ultrasonication techniques[11-15]. These findings can explain to achieve enhanced stability, effective dissolution rate, and less toxicity[16].

Nowadays, a heavy focus of attention has evolved in applying such materials for drug delivery implementation. These polymeric micelles are usually composed of biodegradable and biocompatible hydrophobic polymer blocks such as PCL, PLA, PEG,etc.[17-18]. Among all, the PLGA is a well defined and most effective co-polymer that is applicable in a wide range of FDA approved therapeutic devices that undergo hydrolytic degradation at its ester linkage position when assisted with water[19-23].

A modern-day first line, well-known calcimimetic drug Cinacalcet Hydrochloride is indicated for the safer management of tertiary hyperparathyroidism in people with chronic renal disorder, dialysis, and also for hypercalcemia in people with parathyroid carcinoma[24,25.] The oral form of CIH is considered the frontline medication in the generation of agents, i.e., the calcimimetics, with an innovative mechanism of action with absolute bioavailability of 20-25% and the log P value of 6.8[26,27].

The current utilization of QbD in optimizing the suitable experimental design space is gaining immense significance in pharmaceuticals development. QbD-based statistical design of RSM is frequently employed to justify the specificity relied on the precision of a range of linear, quadratic, or multiple factors in the model. It permits a systematic understanding of a process or product-related variables, emphasizing the optimum optimization of reproducible and robust product development[28,29]. The Box-Behnken Design (BBD) is an accepted configuration of RSM, which is valuable in depicting the precise interactions within the parameters selected for optimization[30-32].

As per the research literature concern, due to fewer number of research findings for freeze-dried polymeric nanoparticles of CIH, is prepared by the nanoprecipitation with the ultrasonication method except for few commercially marketed tablets of the strength of 30, 60 and 90 mg). An alternative drug delivery system has developed for enhanced therapeutic efficacy and bioavailability potential of cinacalcet hydrochloride.

MATERIALS AND METHODS

Materials

Cinacalcet HCl was obtained as a gift sample from Cadila Healthcare Pvt. Ltd. (Mumbai, India). PLGA (Poly- (lactic-co-glycolic acid) was acquire as a gift sample by Dr.Reddy's lab, Hyderabad, India. Poloxamer 188, received from Hi-Media (Mumbai, India), (USA). Mannitol was received from Himedia Chemicals Pvt. Ltd. (Mumbai, India). Further, the necessary reagents, chemicals, and solvents were found out as analytical grade [AR], with utmost quality, were applied in the research work. The most authentic ARRIVE guidelines recommended that all the animal studies or experimentation to be conducted in alliance with (Scientific Procedures) Act, 1986, in the UK and insist on allied guidelines, EU Directive 2010/63/EU for animal experimentations was permitted by the Animal Care Committee, RIPS, Berhampur, Institutional Animals Ethics (926/PO/Re/5/06/CPCSEA, Approval No. 87).

Methods

Target Product Profile (TPP)

TPP was defined for target polymeric nanoparticle drug delivery system (PNDDS) formulation of cinacalcet hydrochloride to augment the drug's oral bioavailability. The essential rudiments of QTPP such as types, strength, and the route of administration of dosage forms or formulations, pharmacokinetic variables or determining factors, stabilities packaging attributes, the release of a drug, and also pharmacokinetic profiles of the drug[17,28].

Critical Quality Attributes (CQAs)

Among the intact target product profile (TPPs), several crucial and potential quality attributes (QAs) are designated as CQAs based on their criticality of influence upon patients' benefit. For the marked nanoparticle formulation, the time intended for stirring (crucial for faster dissolution of the drug in the digestive fluid), zeta potential (TD), and Mean particle size(nm) (essential for drug liberate from the formulation or dosage forms) [17,27].

Screening of formulation excipients

Intrinsic solubility analysis

Drug substance intrinsic solubility was estimated in various solvents such as water, acetonitrile, phosphate buffer pH of 6.8 and 7.4, 0.1N HCl, methanol, ethanol, DMSO, acetone, PEG200, and PEG 400,n-octanol. The drug's adequate capacity was included in each of the solvents and set aside on a mechanical shaker (Rivotek, Rivieria Glass Pvt. Ltd., Mumbai, India) along with water bath regulated at 37 ± 0.5 °C for 72 h. The Vials were subsequently observed from distinct intervals for absolute solubilization of the drug substance, and afterward, the drug was further included if essential. All of the notable excipients were permitted to move into the centrifuged tubes (Spinwin MC02, TarsonsPvt Ltd.) Kolkata, India) for isolation of the undissolved or immiscible drug, the distinct quantity of the solubilized drug was detected and analyzed by UV-Vis spectroscopy (UV-Vis Spectrophotometer, Labindia Ltd., Mumbai, India) at wavelength maximum 279 nm (i.e., λ max of the drug) from the supernatant fraction[20].

Development of analytical method by UFLC

A simple, rapid RP-UFLC method was used for the quantification of CIH. Drug separation

was performed on 250×4.6 mm ID, ODS C18 column. The mixture of 50:50 (v/v) acetonitrile: phosphate buffer i.e., TBHS solution of 25ml as mobile phase and filtered through a 0.45µm Millipore filter at a flow rate of 0.5 ml/min. Chromatographic detection was performed at a wavelength of 223 nm, and an analytical column was maintained at constant temperature (25 ± 1 °C)[33].

Identification of QTPPs and CQAs in product development

The QTPPs and CQAs are the major QbD elements to achieve product development and objectives for CIH nanoparticles, and the QTPP elements were set up based on drug sustained-release, zeta potential, polydispersibility index, and particle size. The QTPPs and CQAS parameters as depicted in Table1.

Preliminary screening of influential factors using Taguchi design

The initial screening was undertaken by utilizing a 7-factors 2-levels Taguchi design to identify the key influential factor(s) affecting the CQAs. For the Taguchi design matrix, a series of 8 formulations were suggested and prepared.

Preparation of polymeric nanoparticle formulation

The development of polymeric nanoparticle was attempted by adopting nanoprecipitation, followed by the ultrasonication method. The required quantity of PLGA was dissolved in the organic phase (acetone) at 50°C and added to the acetone solution of drug CIH. The organic phase was included drop-wise into the additive (stabilizer) solution of poloxamer-188 (Aqueous phase) with the glass syringe outfitted with a needle (gauge size 26) at 3ml/min and a stirring speed of 5000-15000 rpm at 25°C (Sample homogenizer T18 DIGITAL IKA RV Germany). The ultrasound state parameter was set to 3secs with an interval of 2secs at 40W for 5minutes. The residual amount of acetone was evaporated at 40°C beneath condensed pressure, using a Rotary evaporator (IKA RV 10 digital, Germany) for 2mins. The obtained nano-suspension was centrifuged (RC 4815F, Eltek India) at 9000 rpm for 30 min. Finally, lyophilized for 36h at-54°C.

Systematic formulation optimization studies

A 3-factor and 3-level containing BBD response surface design with mixture components were adopted for optimizing the polymeric nanoparticle composition. Design expert ver. 12.1.1 software (Stat-Ease, Minneapolis, MN, USA) was used for generating the experimental trials, where the ratio of Drug: PLGA (mg) (X1), poloxamer 188 concentration %w/v (X2), and stirring Speed (X3) was utilized as the independent variables or factors and with 3-levels (-1, 0,and1)was built to estimate the significant effect of these assorted variables or responses namely, cumulative % drug release QT24% (Y1), particle size in nm (Y2), zeta potential in mV(Y3) and polydispersity index (Y4). A sum of 17 trial formulations was organized together with consecutive of five cumulative replicates of the center point trial and further CQAs formulation, for evaluation.

Lyophilization of optimized PNs of CIH

Literature reviews show that samples achieved after lyophilization were typical characteristics with porous structure enormous redispersibility and extended-term steadiness[34]. The optimized PNs of CIH of Run No. 16,12, 15 were lyophilized by (ALPHA 1- 2 LO Plus CHRIST (lyophilizer) to produce the powdered freeze-dried form at a vacuum pressure of 0.01 KPa for 48h at -50°C to obtain lyophilized PNs of CIH, i.e., run No. 16. The selected optimized formulation was further freeze-dried to the powder form by applying

suitable cryoprotectant,i.e., mannitol (2%), and then subjected to micrometric characterization.

Characterization of freeze-dried PNs

FT-IR

The FT-IR spectroscopy was performed effectively for estimating the possible physical interactions of drug cinacalcet hydrochloride.FT-IR spectra of selected cinacalcet hydrochloride and physical mixture (PM) with PLGA and poloxamer188 were recorded on IR using KBr with a resolution of 4 cm-1. The drug-excipients compatibility studies were carried out by computing the transmittance range from 4000 to 400cm-1. Matching among peaks was performed to distinguish any potential interactions among the other additives of cinacalcet hydrochloride[35].

Differential scanning calorimetry

DSC studies were done in order to assess the interaction among the drug and the polymer. All the required samples (10 mg) were subjected to heat in aluminum pans through effluent gas containing dry nitrogen. The DSC thermograms of cinacalcet hydrochloride, PLGA, poloxamer 188, along with their respective physical mixtures of CIH and PLGA, CIH, and poloxamer 188 was determined[36].

Entrapment efficiency

The percent EE of CIH in the formulated or prepared PNs was anticipated directly by collecting the CIH content in the polymeric nanoparticles. Samples of 10 ml of the PNs of CIH were allowed to centrifuged at 9000 rpm for 30min at -4 °C using a cooling centrifuge. The un-encapsulated free drug can be removed using centrifugation dialysis[37]. The supernatant free drug was calculated and validated, employing the UV-Spectrophotometric method at wavelength 279nm. The drug entrapment efficiency (DEE)or(DEE%)of nanoparticles were determined and calculated as indicated below Eq.(1).

$$\%\ \text{Entrapment Efficiency} = \frac{\text{Total amount of drug} - \text{Free Drug}}{\text{Amount of total drug content}} \times 100 \ldots\ldots (\text{Eq. 1})$$

Particle size and zeta potential measurement

Particle size, polydispersity index, and zeta potential were found out effectively by PCS by using Zeta sizer Nano-ZS Make-Malvern instrument. Zeta potential implies that its value can be associated with the steadiness of colloidal dispersions. A high zeta potential will present the immovability, or steadiness, intended for molecules and particles that are little enough[38].

In-vitro diffusion studies

In this in-vitro drug release study, the dialysis bag diffusion technique was implemented for pure drug CIH. 5 ml of the formulations were placed in the dialysis bag hermetically sealed and dropped into 150ml of 0.1NHCl under sink conditions for the first 2h. Then it transferred into phosphate buffer solution pH 6.8 for 24 h. The whole system was kept at 37ºC with incessant magnetic stirring at 200 rpm. Sample (2ml) were removed with the utmost care from the compartment of the receptor at predestined time intervals and substituted by fresh 0.1 N HCl and phosphate buffer pH 6.8. Then 1ml of this sample was taken, and 1ml of ethyl

acetate was added. Then vortexed in a cyclomixer, and 0.5ml of this solution's supernatant layer was made in a test tube. Kept for drying and later on, the mobile phase was added to this test tube and analyzed under RP-UFLC[38-41]. Using a non-Fickian diffusion mechanism, kinetic studies are analyzed, allied with a concentration gradient, diffusion mechanics, and the extent of swelling[42, 43].

Solid-state characterization

Powder X-RD

Powder X-ray diffractometer (Rigaku, Japan, Smart Lab 9 kW) was implemented for diffraction studies. Powder-XRD studies were performed on the samples by exposure to nickel filtered CuKa radiation (40 kV, 30 mA) and allowed for the scan. Samples required for P-XRD related investigation were pure drug and optimized lyophilized PNs of CIH. The results were then recorded as peak height (intensity) versus time(h).

Scanning electron microscopy

SEM studies the texture or exact appearance of nanoparticles. (SEM, Jeol, Japan, JSM-6390LV) with a high resolution at 30 kV. The formulation bearing the sample of has first adhered to the carbon-coated metallic stub. SEM is useful for a detailed study surface morphology. High-energy electron helps to scan across the surface of a specimen, having an Au and Pt coating, which assists in improving contrast and the signal-to-noise ratio[44]. The pure drug and optimized lyophilized PNs of CIH were studied and appropriately examined for determining surface morphology.

Transmission emission microscopy

The exterior appearance or outline of the PNs was determined by TEM (100s, JEOL Ltd, Japan), the PNs of CIH, which was lyophilized and diluted with 2mL of distilled water and consistently mixed by ultra-sonication for 3 minutes. The samples were arranged by insertion a drop of PNs of CIH upon a coated copper grid and air-dried[45].

TGA

TGA studies were implemented to justify the moisture content associated with weight loss in isothermal or non-isothermal stability studies. TGA provides a vital tool to characterize and quantify the moisture content in pharmaceutical material[46].In the pre-formulation studies, TGA is the appropriate technique for differentiation of polymorphs from hydrates or identification of monohydrates from among other hydrates, which may not be possible by DSC alone[34,47].

DTA

It is well understood that thermal analytical techniques are highly requisite to study the polymorphisms, predict drug stability, solvation, degradation, drug compatibility with excipients, and impurity studies. Moreover, as compared to all, DTA is a well-established thermal method intended for an improvement to the melting point determination[48].

In-vivo pharmacokinetic study

A single dosage bioavailability technique was intended in animals under an unfed state. The oral bioavailability of the optimized CIH formulation and an aqueous suspension of the pure drug was estimated in rabbits[49]. A healthy breed of male rabbits was taken for investigation. Then 1ml of blood was drawn from the ear vein of the animal as a blank sample. Then 6.3 mg of pure drug dissolved in 12.6ml of distilled water was given to the animal orally and 2.6 ml of formulation to another animal. The blood sample from the ear vein of both rabbits was drawn periodically of 2 h interval at a range of time points (0, 2, 4, 6, 12, 18, and 24 h). Then the blood samples were subjected to a centrifuge for 20min., at 5000rpm, after 20min. The supernatant layer of serum was carefully collected by the aid of micropipette. All the samples were analyzed using the analytical UFLC technique. A collection of pharmacokinetic criteria like half-life (t1/2), maximum plasma concentration (Cmax), elimination rate constant (K),

maximum time to attain peak plasma concentration (Tmax), area under the curve (AUC) were calculated. The Animal Care Committee permitted the pharmacokinetic study, RIPS, Berhampur, Institutional Animals Ethics (926/PO/Re/5/06/CPCSEA, Approval No. 87). All the animal experimentation complied with the ARRIVE guidelines and performed in association with the UK Animals (Scientific Procedures) Act, 1986 and connected guidelines, EU Directive 2010/63/EU for animal experiments.

Accelerated stability study

The accelerated stability study was performed as per the ICH guidelines of optimized nanoparticles filled with hard gelatin capsules dosages form was subjected to accelerated stability at temperature 40ºC and relative humidity of 75% for six months using a stability chamber (TH-200G, Thermolab, and Thane, India) and packed in a glass bottle with a cotton plug. The samples were removed from stability at 0, 1, 2, 3, and 6 months time periods, and evaluated for particle and zeta potential analysis, and drug release.

RESULTS

Excipients selection on the basis of solubility studies

Cinacalcet hydrochloride (CIH) revealed a mean saturation solubility in selected solvents of 3660µg/ml, 3256µg/ml, and 2471µg/ml in acetone, DMSO, and ethanol. The comparable mean saturation solubility profile of CIH in various solvents is depicted in Figure 1. Among different solvents, acetone showed the highest solubility, quantitative solubility for other co-solvents, and hence acetone was selected. Least solubility was observed in methanol 0.000345µg/ml.

Taguchi Screening Design for Identifying Critical factors

The preliminary screening (Taguchi OA design) was applied to filter out the most influential factors with several trials for each element; two levels opted low and high, (1 and 2) Table 2. shows the respective coded and actual values for the formulations on the basis of the CQAs. The influence of multiple factors, like A-PLGA concentration, B-Poloxamer 188 concentration. C-Stirring speed, D-Stirring time, E-Ultra- sonication time, F-Temperature, and G-Stirring type were studied. Table S1 signifies the summarized ANOVA values obtained from screening design. The importance of individual factors on the responses were determined from their P-values. The model factors A, B, and C, are significant since their obtained P-value is less than standard α value (0.05), compared with other factors with P values more than 0.1000 shows insignificant model terms. Thus, the factors A-Drug: PLGA concentration B-Poloxamer-188 concentration and C-Stirring speed were finally selected as influential factors for further optimization.

Experimental design, optimization, and analysis

By keeping the other factors constant at a low level, the concentrations of PLGA, poloxamer 188, and stirring speed were changed. On the basis of preliminary data from Pareto- chart analysis, three levels were selected (-1, 0, and 1) for each of the factors. Table 3. represents a total of 17 runs on applying a three-factor at three-level 33 Box-Behnken Design. The characterization studies of each formulation were done to investigate the effect of different factors like A-Drug: PLGA concentration., B-Poloxamer188 concentration, and C-Stirring speed on individual CQAs.

Response surface analysis of 2D and 3D plot

Effect of the factor on CQA QT24%

Figure 2a. It portrays the 2D (contour) and 3D plot of the CQA QT24%.On thorough understanding, it is anticipated that at a low level (-1) of Drug: PLGA concentration and high level (1) level of poloxamer 188 concentration, the red region is prevalent, more than 75% of drug release in 24 hr. The run No.16 has a maximum percentage of drug release, 76.945 %. In contrast, run No. 14 has a minimum QT24% value, 29.411 % due to the high level (1) of Drug: PLGA concentration. Increased polymer concentration increases the level particle size distribution and aggregation, which retards the release behavior. The result suggests an optimum drug concentration: polymer ratio is required for better dissolution of the drug. It can also be inferred that the concentration of stabilizer (poloxamer-188) has a prominence impact on improved drug dissolution.

Effect of the factor on CQA PS

Figure 2b. Portrays the 2D (contour) and 3D plot of the CQA PS. This shows the size distribution range from 147.898 nm for run 16 to 450.211nm for run 8. It was also observed that at a low level (-1) of factors A-Drug: PLGA concentration and high level (1) of B-Poloxamer-188 concentration shows the prevalence of blue region, the low value of particle size is achieved[50]. Thus estimating that lower level of A-Drug: PLGA concentration helps achieve lesser particle size, and it significantly increases concerning a higher level, shown by the dark yellow region. An increase in the stirring rate also influences the particle size, i.e., it reduces its size[51].

Effect of the factor on CQA ZP

Figure 2c It portrays the 2D (contour) and 3D plot of the CQA PDI. Both A-Drug: PLGA concentration and B-Poloxamer 188 concentration seem to influence the CQA ZP. It ranges from -6.321mV for run 12 to 22.7 mV for run 16 at a low level 0.5 of A-Drug: PLGA concentration and B-Poloxamer 188 concentration at more than level 1 show higher value, which predicts to have a substantial impact on the above CQA[52].

Effect of the factor on CQA PDI

Figure2d. It portrays the contour plot and the 3Dplot of the CQA PDI. Both A-DRUG: PLGA concentration and B-Poloxamer188 concentration seem to equally influence the PDI. It ranges from 0.12 to run 14 to 0.65 for run 8. The results showed that PDI's value remains below 0.2 only when both A-Drug: PLGA concentration and B-Poloxamer 188 concentration have a value above the level 0.5 Uniform-size distribution is a vital requirement for getting drug absorbed at GI membrane. The stabilizer system is responsible for maintaining uniform-size distribution.

Analysis of variance (ANOVA) of BBD Design

Table S2 depicts the summarized ANOVA values of various factors. The resultant model F-value of QT24%, PS, ZP, and PDI is measured as 21.76, 11.80, 5.06, and 5.72. The observed P values for various CQAs are ≤ 0.05 ($\alpha = 0.05$), which justify the significant quadratic model. The lack-of-fit values for QT24%, PS, ZP, and PDI were calculated as 0.565, 0.157, 0.001, and 0.455. For QT24%, the model terms such as A, B, and C^2 are substantial. For PS, the model terms B is substantial, and A, AB, A^2 are substantial in ZP. In PDI, the model terms such as B, AB, and B^2 are significant.

DISCUSSION

Summary of BBD quadratic model

Table S3 renders a concise outline for the Box-Behnken quadratic model applied to optimize the PNs of CIH. In CQA QT24%, the predicted Regression coefficient (R^2) of 0.7627 is in acceptable agreement with the adjusted Regression coefficient (R^2) of 0.9211. The precision ratio of 17.021 estimates a good signal-to-noise ratio. In PS's case, the Predicted Regression coefficient(R^2) of 0.2844 is not as close to the Adjusted R^2 of 0.8586 due to may indicate a significant block effect, with the precision ratio of 12.012 indicating an adequate signal. For ZP, the predicted R2 of -1.0737 implies that the overall mean may be a better predictor with the adjusted R2 of 0.6956. The precision ratio of 8.268 indicates an adequate signal. For PDI, the predicted Regression coefficient(R^2) of 0.0433 is not as close to the Adjusted R^2 of 0.7264, with a precision ratio of 7.696, indicating an adequate signal.

Analysis for Identification of Overlay Plot and Design Space

In the case of optimization, the preferable target was allotted for various responses QT24%, PS, ZP, and PDI as per the target identified in identifying various QTPPs and CQAs. Based on the required QTTP, limits for different CQAs were set, then processed for optimization. The run 16 was the optimized PNs of CIH, which was achieved by BBD comprising of 30 mg of CIH: 30mg of PLGA, of poloxamer-188 (1.5 %w/v) concentration, and stirring speed 10000rpm. The summarized responses of the experimental and predicted values for the optimized formulation obtained after the optimization process are shown in Table S4. The evaluation of the proposed optimized formulations showed QT24% of 76.945%, PS of 147.898nm, ZP of 22.7 mV, and PDI of 0.398. The optimized PNs of CIH exhibited to

achieve the QTTP in an optimum composition.

Characterization of PNs

FT-IR

Cinacalcet hydrochloride-polymer interactions were assessed for CIH and physical mixture (PM) with PLGA and poloxamer 188. The observations were recorded on IR using KBr with a resolution of 4 cm-1 over the region 4000-400 cm-1 FT-IR analysis for pure cinacalcet hydrochloride exhibited absorption spectral bands as shown in Figure 3. at 1517 cm-1 designated to methyl (-CH3),1338 cm-1 designated to (-CH2), 2909 cm-1 designated to amide(-NH), 796 cm-1 designated to the trifluoromethyl(-CF3), and absorption bands at 805cm-1 assigned to designated to benzene (-C6H6). The corresponding peaks obtained for the physical mixture from the spectral analysis showed no alterations. The outcome showed the compatibility between CIH and other excipients.

DSC

DSC curve of cinacalcet hydrochloride exhibit an endothermic peak at a temperature of 181.9ºC, the onset temperature of 178.3 ºC, and the end set the temperature of 184.9ºC, matching its melting point. The DSC thermograms of CIH and physical mixtures of CIH with excipients were observed. The thermogram of CIH showed an intense endothermic sharp peak at 181.90ºC (Tfus), with onset at 178.33ºC and latent heat of fusion (ΔHfus) was observed to be -28.26mJ, predicted crystalline drug nature whereas that of physical mixtures also depicted the same as shown in Figure 4. Studies indicated no change in peak characteristics for pure drug and formulation; thus, no interactions between drug and excipients were inferred in the present research.

Micromeritic studies

Table 4. enlists micrometric properties of lyophilized polymeric nanoparticles, where the angle of repose is 27.96±1.5 degrees, and % moisture content 2.8±0.4, respectively. Based on these micrometric properties, run 16 was selected to be the best formulation with better flow properties.

Entrapment efficiency

Among all the trials run 16, i.e., Drug: PLGA concentration 30:30mg, poloxamer-188 1.5%w/v at stirring speed of 10000 rpm, found to have higher entrapment efficiency (69.56%).

Particle size and zeta potential determination

The zeta-sizer instrument analyzed the particle size of all formulations. The optimized size range for the PNs of CIH was 147.8nm, i.e., for run 16 Figure5a. The developed cinacalcet hydrochloride loaded PLGA- NPs exhibited spherical and uniformity in the particle size distribution of <200 nm. The increase in PLGA concentration had a potentiating behavior on the particle size, which produced a hazy appearance, i.e., due to the increased aggregation level. The zeta potential results for the respective formulation was 22.7 mV for Run No. 16. Figure 5b**.**

P-XRD

The XRD patterns of optimized PNs of CIH and pure drug CIH are depicted in Figure6a and Figure 6b. Pure-drug CIH showed sharp peaks at the diffraction angles such as 11.9°, 15.3°, 16.9° 19.3°, 22.4°, 23.6°, and 25.2°, indicating a typical crystalline pattern. Optimized PNs of

CIH showed a reduction, i.e., the minimal peak intensity at those angles, indicating amorphous form and confinement of the drug at the molecular level in the freeze-dried form.

SEM and TEM

Figure7a and Figure7b illustrated the scanning electron microscopic pictures of pure-drug CIH and optimized PNs of CIH. The SEM of pure-drug CIH appears to be a rough surface with crystalline structures. However, the TEM studies of the optimized PNs of CIH predict the amorphous structure with spherical smooth-surfaced particles Figure8.

TGA

The TGA curve of pure drug CIH exhibits that at an initial temperature of 23ºC the weight loss is found to be 0.045mg, with (% weight loss 2.76), which observed a straight line up to temperature 280ºC with 1.42mg with %weight loss 93.42. The TGA curve of optimized PNs of CIH exhibited at 24^0C temperature the weight loss found to be 0.24mg (%weight loss 8.22)., followed by a sharp decrease of curve observed at 180ºC with weight loss1.70 mg (57.58%) as depicted in Figure 9a and 9b, and its decline up to 480ºC but later on, it showed a straight line up to 800ºC. This curve indicates that the optimized formulation seems to be significant and thermo-stable concerning % weight loss compared to CIH's pure drug.

DTA

The curve of CIH exhibited that, at 181^{o}C, a significant decrease in intensity of peak, which signifies an endothermic reaction with respect to the melting point. Similarly, in the case of optimized polymeric nanoparticles at 226^{o}C sharp change in peak curvature has been noticed due to the change in melting point (348.6mJ). Here the onset of peak appearance observed during 21^{o}C to 67.31ºC (endset 67.31 with 13.5J). The details of DTA thermograms of optimized PNs formulations and its pure drug of CIH are depicted in Figure 10a.and10b.

In-vitro diffusion studies

The behavior pattern of drug release for the optimized PNs of CIH and pure drug of CIH is illustrated in Figure 11. The optimized PNs of CIH showed a better dissolution profile with nearly two times the cumulative %drug release than the pure drug in 6hr. Hence, an optimum combination of Drug: PLGA, poloxamer-188, provides a better dissolution profile than the pure drug. After applying different kinetic models, the pure-drug CIH R2 values calculated as 0.921 for zero-order, 0.934 for first-order and 0.940 Higuchi model, and optimized PNs of CIH formulation, the R2 found to be 0.835, 0.868, and 0.944 respectively. The regression coefficient (R2) obtained for different kinetic models suggested the highest fitness toward the Higuchi model for pure-drug CIH and optimized PNs of CIH. The value of release exponent (n) for the pure-drug CH and optimized PNs of CIH formulation was 0.689 and 0.478. Hence, the drug release from pure drug follows Fickian diffusion kinetics, whereas for optimized PNs of CIH follows non-Fickian diffusion kinetics.

In-vivo pharmacokinetic study

Figure 12. exemplifies the plot between the mean plasma concentration of CIH vs. the time for optimized PNs of CIH to that of pure drug. Table 4. shows the determined values for individual pharmacokinetic criterion. The optimized formulations (Tmax) were determined as 6h compared with 4h for pure-drug CIH, which indicated sustained release time of the drug. Cmax of optimized PNs of CIH was 1.945mcg/ml, whereas, in the case of the pure drug of CIH, it is 0.671 µg/ml. AUC of optimized PNs of CIH 31.558 (µg/h)/mL) was found to be

more than three-fold increase as compared with AUC of the pure drug 10.457(µg/h)/mL). The rationale in the boosting up in bioavailability is its improvement in the dissolution and absorption profile of drugs through the gastrointestinal membrane. In-vivo studies proved a significant elevation in the drug CIH absorption and permeation profile concerning optimized PNs of CIH, which is evident from the distinctly superior pharmacokinetic parameter in contrast to pure drug.

Accelerated stability outcomes

P-values of the ANOVA design during accelerated stability study shown in Table S5. The P-value is more than 0.05, value for all the CQAs, indicating no significant change. Hence, the optimized freeze-dried PNs of CIH were found to satisfy the stability criteria as minimal substantial alterations in CQAs throughout the stability period.

CONCLUSION

The current research instigates a systematized elaboration of PNs of a novel therapeutic for hyperparathyroidism; cinacalcet hydrochloride using a quality-by-design approach to improve drug bioavailability and sustained drug release. In the process of QbD, first QTTPPs and CQAs were identified with proper justification. Taguchi screening resulted in primary screening, followed by orderly optimization by using Box-Behnken design. The regression equation and response surface were analyzed. ANOVA model assisted in the identification of the specific appreciable model term. Optimization of freeze-dried PNs of cinacalcet hydrochloride was undertaken by coding the high and low-value range for various CQAs. The design space identification is confirmed from the overlay plot. The optimized single dose of freeze-dried PNs of drug obtained using BBD consisted of 30 mg of CIH, 30 mg of PLGA, poloxamer-188 1.5%w/v. The optimized freeze-dried polymeric nanoparticle formulation showed an optimum particle size of 147.89nm, zeta potential at 22.7 mV, entrapment efficiency of 69.56%, in-vitro drug release more than 75% after24h. In-vivo studies showed 3folds enhancement in oral bioavailability with increased C_{max} for optimized formulation compared with an aqueous suspension of pure drug. Accelerated stability study of the optimized PNs of CIH validates the insubstantial changes in the CQAs during a stored period of 6months, which was distinct by p-values for all CQAs. The present research concluded that an optimum combination of 30 mg of CIH: 30 mg of PLGA, 1.5 w/v% of poloxamer 188 for the PLGA- based polymeric nanoparticles of the drug, effectively achieved the desired objective sustained drug release and enhancement in bioavailability.

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AUTHORS' STATEMENT

Conflicts of interest: No conflict of interest was declared by the authors. The authors alone are responsible for the content and writing of this article.

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Declaration of Competing Interest

The authors declare that they have no known competing for financial interests or personal relationships that could have appeared to influence the work reported in this paper.

ETHICAL ISSUES

All the animal studies performed in the present work were carried out before the approval of the study protocol. The pharmacokinetic study was permitted by the Animal Care Committee, Roland Institute of Pharmaceutical Sciences, Berhampur, Institutional Animals Ethics (926/PO/Re/5/06/CPCSEA, Approval No. 87). All the animal experimentation complied with the ARRIVE guidelines and performed in association with the UK Animals (Scientific Procedures) Act, 1986 and connected guidelines, EU Directive 2010/63/EU for animal experiments.

LIST OF ABBREVIATIONS

PDI: Polydispersibility index
UFLC: Ultra-fast liquid chromatography
PNs: Polymeric nanoparticles
CIH: Cinacalcet hydrochloride
EE: Entrapment efficiency
TGA: Thermogravimetric analysis
DTA: Differential thermal analysis
SEM: Scanning electron microscopy
CQAs: Critical quality attributes
TPPs: Target product profile
(P-XRD): Powder X-ray diffractometry
BBD: Box-Benkhen design
C_{max}: Maximum concentration
t_{max}: Maximum time
PCL: poly-p-caprolactone
PLA: poly (lactic acid)
PCS: Photon Correlation Spectroscopy

REFERENCES

1. Peer D, Karp JM, Hong S, Faro Khzad OC, Margalit R, Langer R. Nanocarriers as an emerging platform for cancer therapy. NatNanotechnol. 2007; 2:751–760.
2. Davis ME,Chen Z, Shin DM. Nanoparticle therapeutics: an emerging treatment modality for cancer Nat Rev DrugDiscov.2008;7:771–782.
3. Quintanar-Guerrero D, AllemannFessi EH, Doelker E. Preparation techniques and mechanisms of formation of biodegradable nanoparticles from preformed polymers. Drug DevInd Pharm.1998;24:1113–28.
4. BhanojiRao ME, Swain S, Patra CN, Mund SP. Formulation design, optimization and characterization of eprosartanmesylate nanoparticles. Nanosci and Nanotech-Asia. 2018;8:2130–143.
5. Kreuter J. Nanoparticles-a historical perspective.Int J Pharm.2007;331:1–10.
6. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Rel.2001; 70:1–20.
7. Shenoy DB, Amiji MM. Poly (ethylene oxide)-modified poly-(epsilon-caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer. Int J Pharm.2005;293:261–270.
8. Glen A. The impact of nanotechnology in drug delivery: global developments, Market Analysis Future Prospects, 2005,Availablefrom:URL:http://www.nanomarkets.com.(Cited 31.03.09).
9. Safra T, Muggia F, Jeffers S, Tsao-Wei DD, Groshen S, Lyass O, Henderson R, Berry G, Gabizon A. Pegylated liposomal doxorubicin (doxil): Reduced clinical cardio toxicity in patients reaching or exceeding cumulative doses of 500mg/m^2. Ann Oncol.2000;8:1029– 1033.
10. Schroeder U, Sommerfeld P, Ulrich S, Sabel BA. Nanoparticle technology for delivery of drugs across the blood–brain barrier. J Pharm Sci.1998;87:1305–1307.
11. Raghuvanshi RS,Katare YK, Lalwani K,Ali MM,Singh O,Panda AK.Improved immune response from biodegradable polymer particles entrapping tetanus toxoid by use of different immunization protocol and adjuvants. Int J Pharm.2002;245:109–121.
12. Kreutera J, Petrov VE, Kharkevich DA, Alyautdin RN. Influence of the type of surfactant on the analgesic effects induced by the peptide dalargin after its delivery across the blood– brain barrier using surfactant-coated nano-particles. J Control Rel.1997;49:81-87.
13. Allemann E, Gurny R, Christophe JL. Biodegradable nanoparticles- from sustained release formulations to improved site specific drug delivery. J Control Rel.1996;39:339.
14. Moffitt M,KhougazK,EisenbergA,Micellization of ionic block copolymers. AccChem Res.1996; 29:95–102.
15. Sushant SK, Yogesh M, ChoudhariNazma NI, and Vishnukant M. Polymeric micelles: authoritative aspects for drug delivery. Designed Mono and Poly.2012;15:465-521.
16. Burt HM,Zhang X,Toleikis P,Embree L,Hunter WL.Development of copolymers of poly (dl-lactide) and methoxypolyethylene glycol as micellar carriers of paclitaxel. Colloids and Surf B: Biointerfac.1999; 16:161–171.
17. Xiaoqing XU, Guoguang C, Yaning LI, Jingjing W, Jun Y, Lili R. Enhanced dissolution and oral bioavailbility of cinacalcet hydrochloride nanocrystals with no food effect. Nanotechnol.2019;30:55-102.
18. Yoo HS,Park TG.Biodegradable polymeric micelles composed of doxorubicin conjugated PLGA-PEG block copolymer. J ControlRele.2001;70:63–70.

19. Jain RK, Stylianopoulos T. Delivering nanomedicine to solid tumors. Nat Rev ClinOncol. 2010;7:653–654.
20. Lammers T, Kiessling F, Hennink WE, Storm G. Drug targeting to tumors: principles, pitfalls and (pre-) clinical progress. J Control Rel. 2012;161:175–187.
21. Bae YH, Yin H. Stability issues of polymeric micelles. J Control Rel.2008;1312–4.
22. Read ES,Armes S.P.Recent advances in shellcross-linked micelles.ChemCommun. 2007;29: 3021–3035.
23. Deng C, Jiang Y, Cheng R, Meng F, Zhong Z. Biodegradable polymeric micelles for targeted and controlled anticancer drug delivery: promises, progress and prospects. NanoTod.2012;7:467–480.
24. Abdollahi S, Lotfipour F. PLGA-and PLA –Based Polymeric Nanoparticles for Antimicrobial Drug Delivery. Biomed Inter.2012;3:1–11.
25. Burlington MA, Jones, Bartlett. Nurses Drug Hand book, (13rd ed.)Jones &BartletLearning.2014.
26. YousafF,CharytanC.Review of cinacalcet hydrochloride in the management of secondary hyperparathyroidism. Ren Fail.2014;36:131–8.
27. Padhi D, Harris R. Clinical pharmacokinetic and pharmacodynamic profile ofcinacalcet hydrochloride. ClinPharmacokinet.2009;48:303–11.
28. Swain S, Parhi R, Jena BR, Babu SM. Quality by design: concept to applications. Curr Drug Discov Technol. 2019;16:240-250.
29. Podczeck F. Pharmaceutical Experimental Design, Lewis GA, Mathieu D, and R. Phan-Tan-Lu.eds. Marcel Dekker, Inc., New York, 1999;498, Int J of Pharm. 182.1,1999, 127-128.
30. Box GEP, Wilson KB.On the experimental attainment of optimum conditions.J. Royal. Stat. Soc. Ser. B.1951;1:1–45.
31. Swain S, Jena BR, MadugulaD, Beg S. Application of Quality by Design Paradigms for Development of Solid Dosage Forms. In: S.Beg,M.d.SaquibHasnain,eds.Pharmaceutical Quality by Design; Principles and Applications.(1st ed.)Elsevieracademicpress,2019;109-130.
32. Box GEP, Behnken DW. Some new three level designs for the study of quantitative variables.Technometrics.1960; 2:455–75.
33. PanigrahiKC,Patra CN,Rao MEB.Quality by Design Enabled Development of OralSelf- Nanoemulsifying Drug Delivery System of a Novel CalcimimeticCinacalcetHCl Using a Porous Carrier: In Vitro and in Vivo Characterisation, AAPS Pharm Sci Tech.2019;20:216.
34. Neupane YR, Sabir MD, Ahma N, Ali M, Kohli K. Lipid drug conjugate nanoparticle as a novel lipid nanocarrier for the oral delivery of decitabine: ex vivo gut permeation studies. Nanotechnology.2013; 24:415102.
35. Hu C, Rhodes DG**.** Proniosomes: a novel drug Carrier preparation. Int J Pharm.1999;185:23–35.
36. GregorC Mc, Bines E. The use of high-speed differential scanning calorimetry (Hyper- DSC) in the study of pharmaceutical polymorphs. Int J Pharm. 2007;350:48-52.
37. DidemAS, MuharremS,JohannaGW, Frank S, Thomas S. Nano structured Biomaterials and applications. J of Nanomater.2016;13.
38. Dynamic Light Scattering Particle Size and Zeta Potential Analyzer.https://www.iitk.ac.in/dordoldn/dynamic-light-scattering-particle-size-and-zeta-potential- analyzer (Accessed November 3,2015).
39. Cruz CN, Tyner KM, Velazquez L, Hyams KC, Jacobs A, Shaw AB, Jiang W,

Lionberger R, Hinderling P, Kong Y, Brown PC, Ghosh T, Strasinger C, Suarez-Sharp S,Henry D, et al. "CDER risk assessment exercise to evaluate potential risks from the use of nonmaterial's in drug products, AAPS PharmSciTech.2013;15:623–628.
40.Langer R. New methods of drug delivery.Science.1990;
249:1527–1533.
41. Artifin DY, Lee LY, Wang CH. Mathematical modeling and simulation of drug release from microspheres: implication to drug delivery systems. Adv. Drug Deliv Rev.2006;58:1274–1325.
42. Siepmann J, Siepmann F. Mathematical modeling of drug delivery. Int J Pharm2008;364:328–343.
43. Lin SB, Hwang KS, Tsay SY, Cooper SL. Segmental orientation studies of polyether polyurethane block copolymers with different hard segment lengths and distributions. Colloid Polym Sci.1985;263:128–140.
44. Carter M, Jennifer S. In Guide to Research Techniques in Neuroscience (2nd ed).Marcel Dekker Inc; USA;2015.
45. Peter Christoper GV, VijayaRaghavan C, Siddharth K, Siva Selva KM, Hari Prasad R. Formulation and optimization of coated PLGA– zidovudine nanoparticles using factorial design and *in-vitro in-vivo* evaluations to determine brain targeting efficiency.Saudi Pharm J 2014;22:133–140.
46. Haines, P.J. Principles of Thermal Analysis and Calorimetry, RSC paperbacks, Royal soc of chem.2002:1-9.
47. Nakashima D, TakamaH, Ogasawara Y, Kawakami T, NishitobaT, Hoshi S, Uchida E, Tanaka H. Effect of Cinacalcet hydrochloride, a new calcimimetic agent, on the pharmacokinetics of dextromethorphan: in vitro and clinical studies. J Clin Pharmacol.2007;47:1311-9.
48. Differential thermal Analysis. https://www.sciencedirect.com/topics/medicine-and- dentistry/differential-thermal-analysis (Accessed December 1,2018).
49. Janga KY, Jukanti R, Velpula A, Sunkavalli S, Bandari S, Kandadi P, Veerareddy PR. Bioavailability enhancement of zaleplon via proliposomes: role of surface charge. Eur J PharmBiopharm.2012;80:347–57.
50. Legrand P, Lesieur S, Bochot A, Gref R, Raatjes W, Barratt G. Influence of polymer behaviour in organic solution on the production of polylactide nanoparticles by nanoprecipitation. Int J Pharm.2007; 344:33–43.
51. Chorny M, Fishbein I, Danenberg HD, Golomb G. Lipophilic drug loaded nanospheres prepared by nanoprecipitation: effect of formulation variables on size, drug recovery and release kinetics. J Contr Rel.2002;83:389–400.
52. RostamizadehAHK,SalariD,Hamidi M.Preparation of biodegradable nanoparticles of tri- block PLA–PEG–PLA copolymer and determination of factors controlling the particle size using artificial neural network. J Microencapsul.2011;28:406–416.

Table captions

Table 1. Quality target product profile (QTPP) and critical quality attributes (CQAs) for developing PNs of cinacalcet hydrochloride

Table2. Taguchi design matrix for screening of factors along with the experimental results of various CQAs and along with factors and their respective low and high levels are coded

Table 3. Composition of various PNs of CIH as per BBD along with the obtained CQAs responses and their coded levels

Table 4. Carr's index, angle of repose, moisture content and in vivo pharmacokinetic parameters values of pure drug and optimized formulation batch

Figure captions

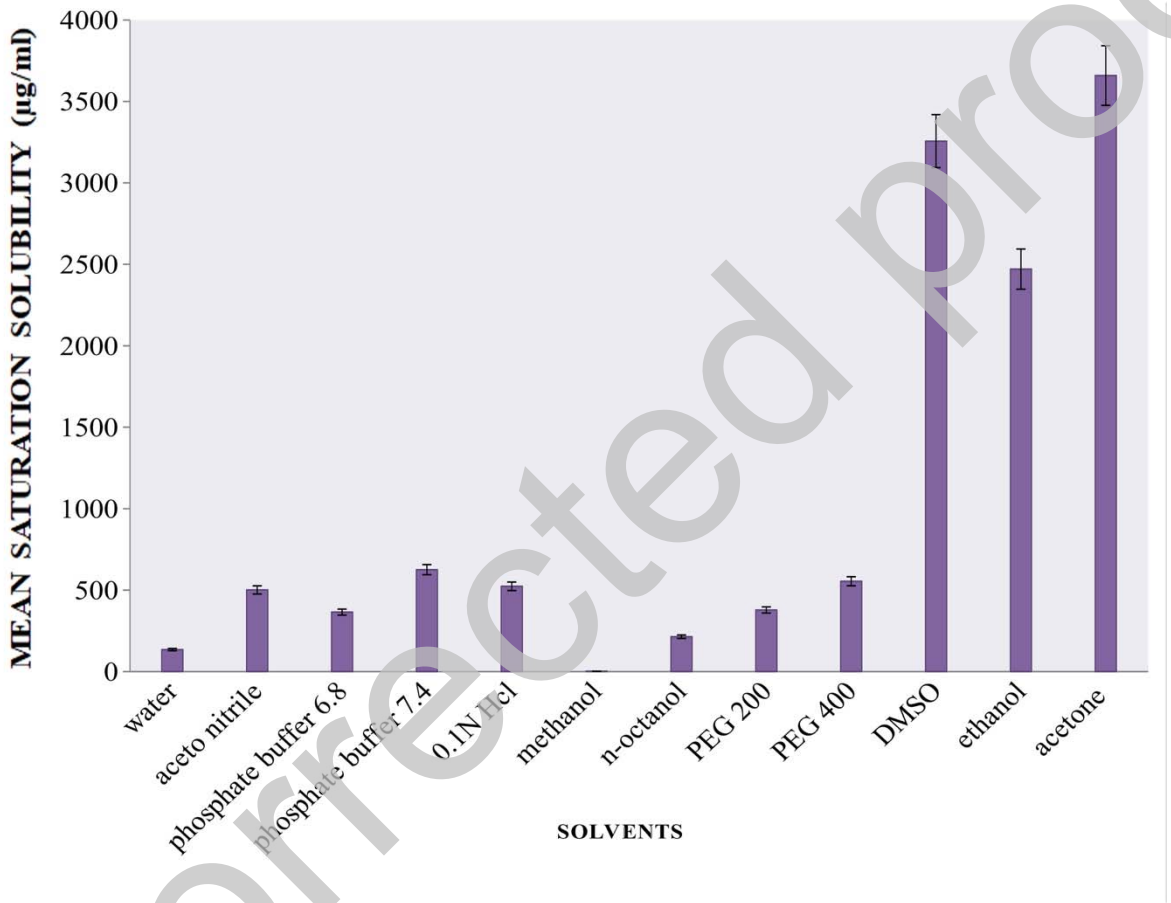


Figure1. Mean saturation solubility data curve of cinacalcet hydrochloride in selected solvents.

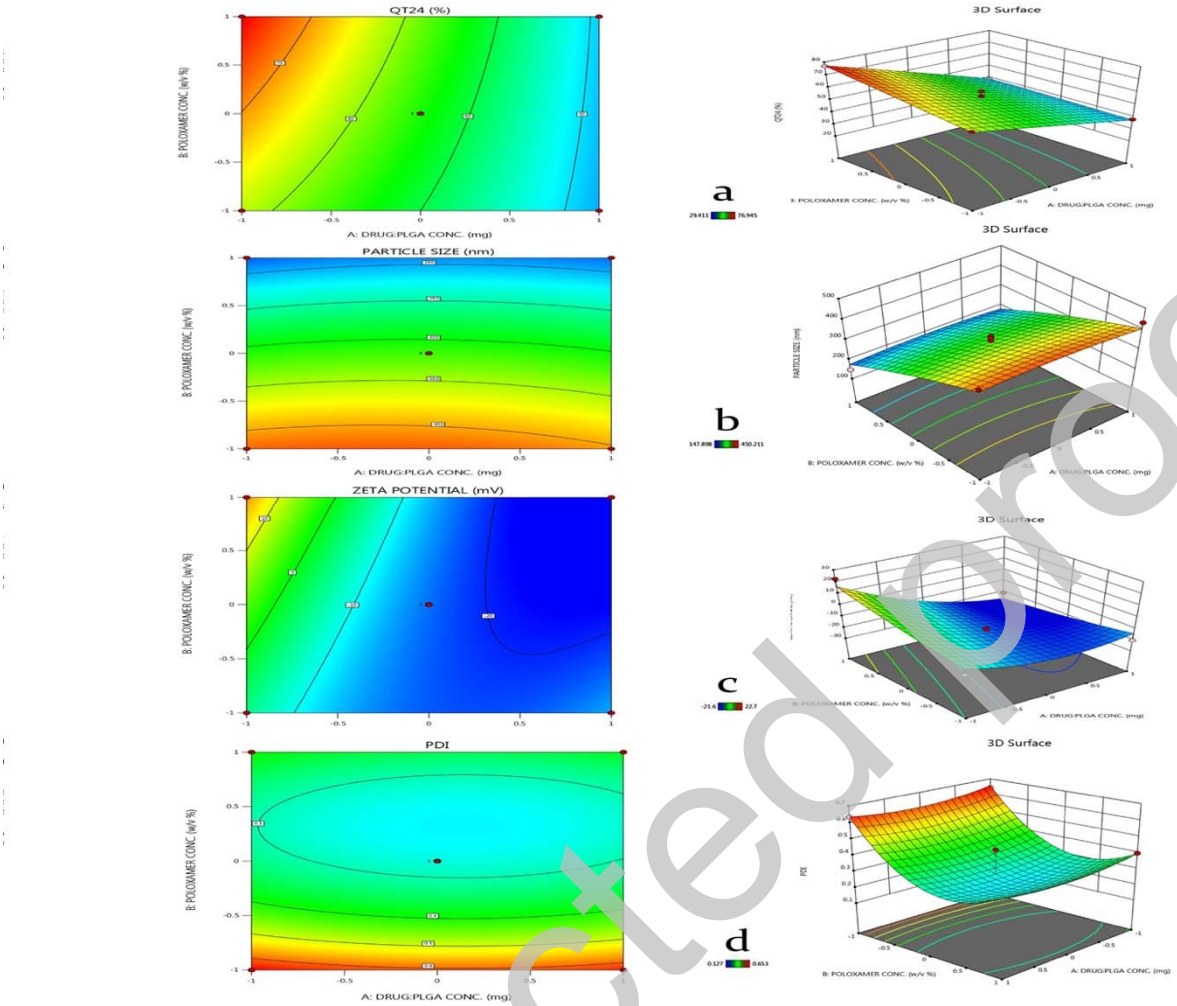


Figure 2. Contour plots (2D) and response surface plots (3D) of selected independent factors on selected dependant factors QT24% (a); particle size (b); zeta potential (c) and polydispersibilityindex(d).

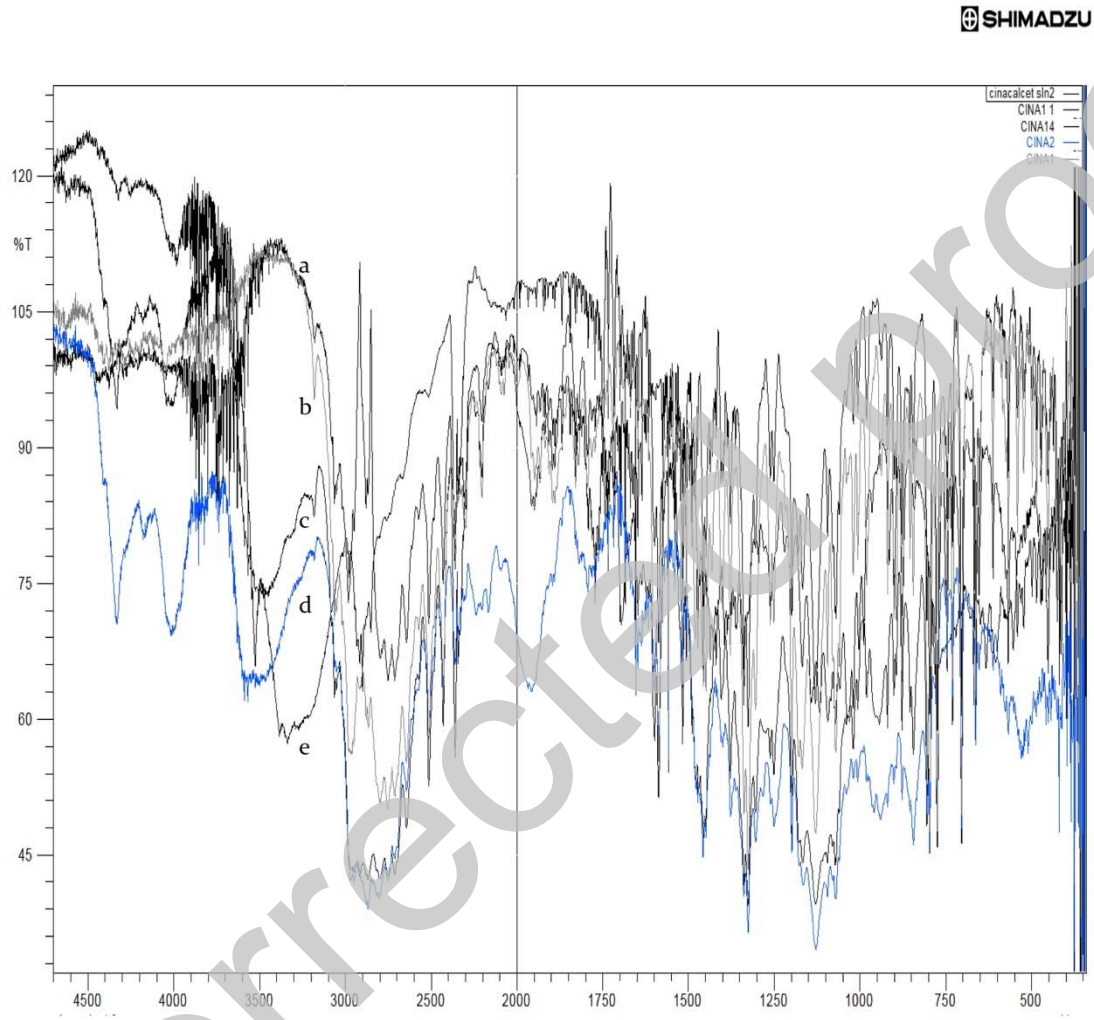


Figure 3. FT-IR spectrum of pure drug (a); physical mixture of drug with PLGA (b); physical mixture of drug with polaxomer-188 (c); polaxomer-188 (d) and PLGA polymer (e)

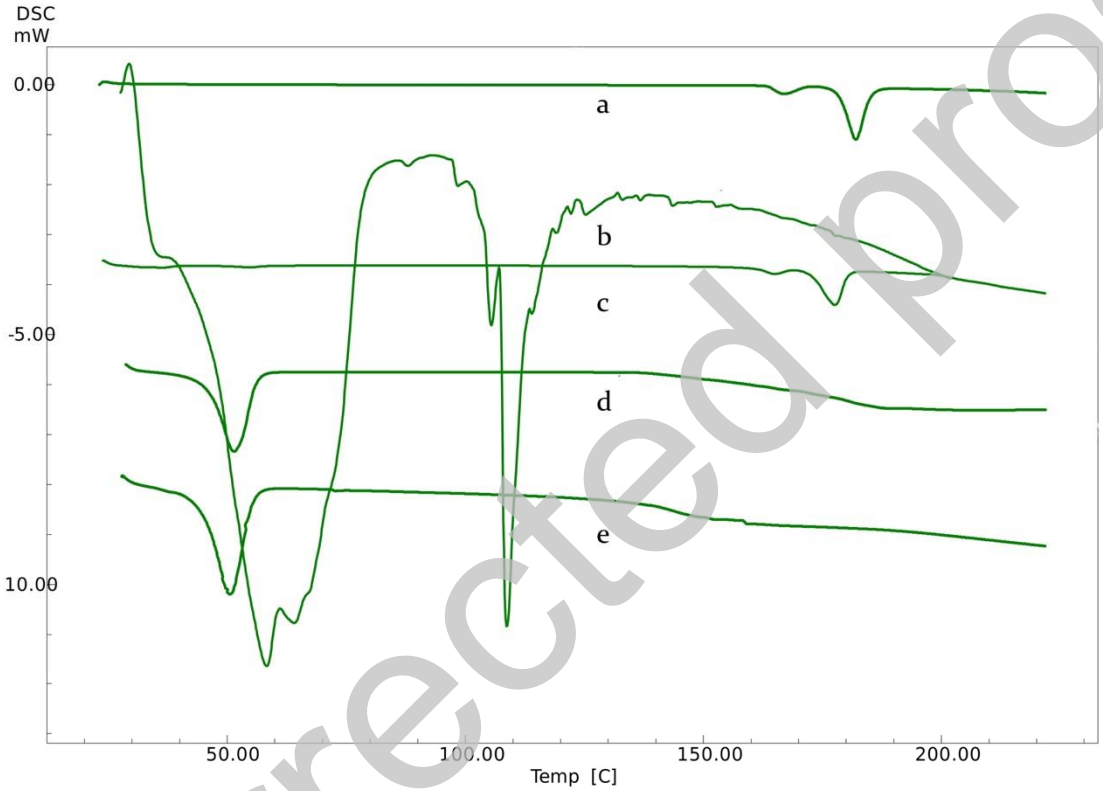


Figure 4. DSC thermogram of pure drug (a); PLGA polymer (b); drug with PLGA polymer (c); drug with polaxomer-188 (d) and polaxomer-188 (e).

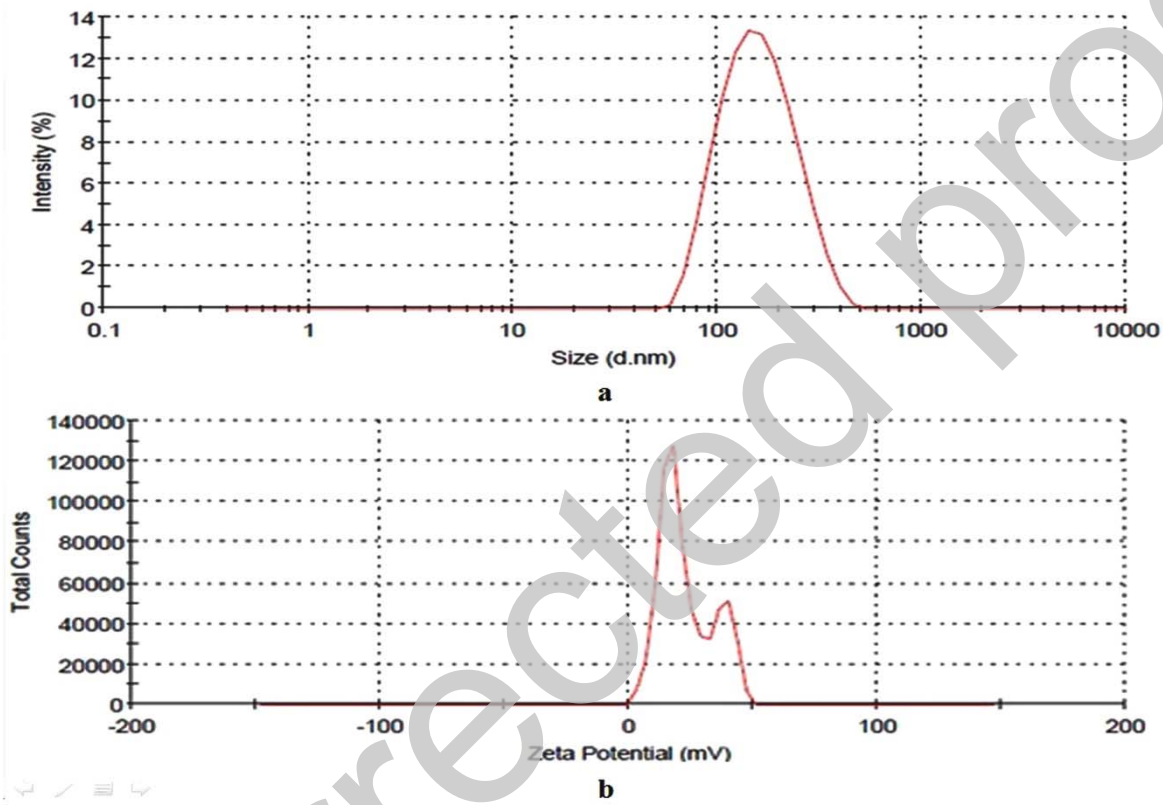


Figure 5. Particle size analysis and zeta potential curves of optimized formulation batch.

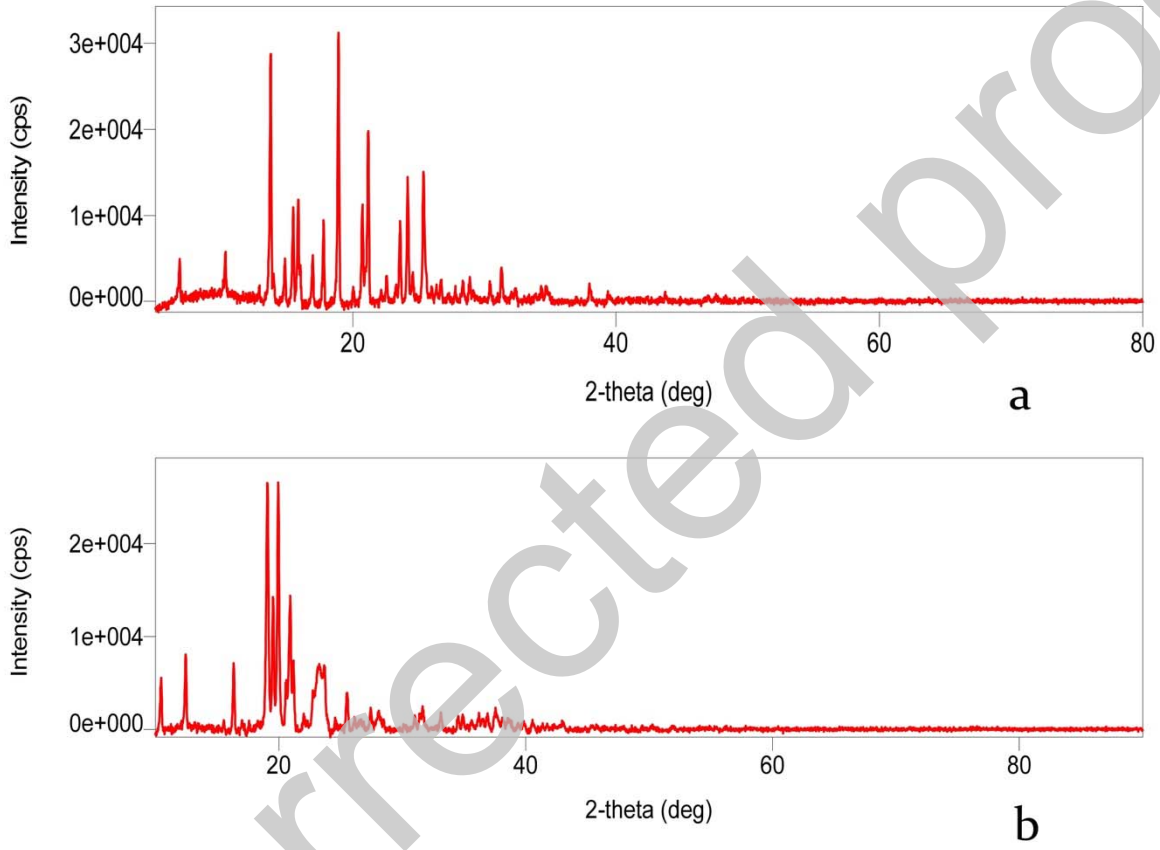


Figure 6.X-RD curves of pure drug (a) and optimized formulation batch (b).

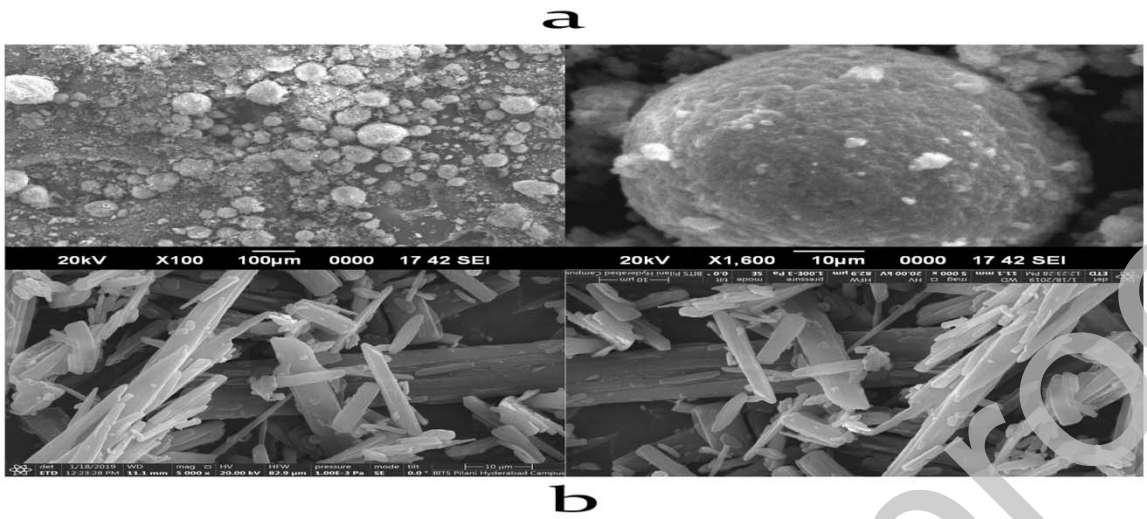


Figure 7. SEM images of optimized formulation batch (a) and pure drug (b).

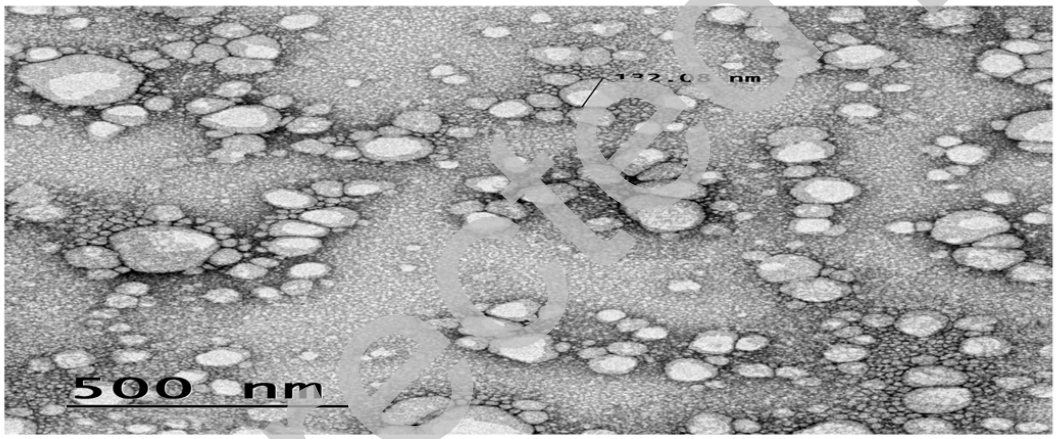
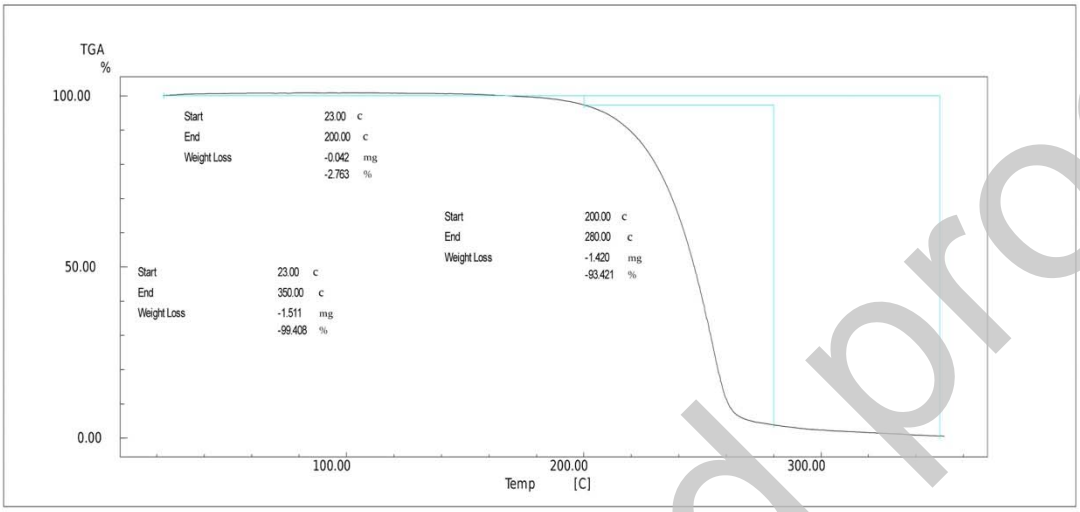
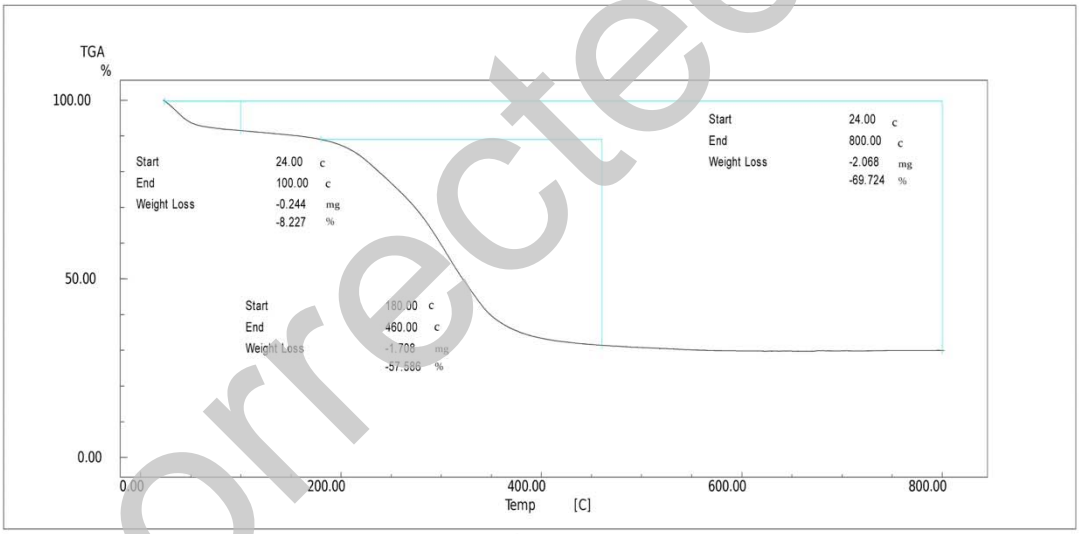


Figure 8.TEM image of optimized formulation batch.

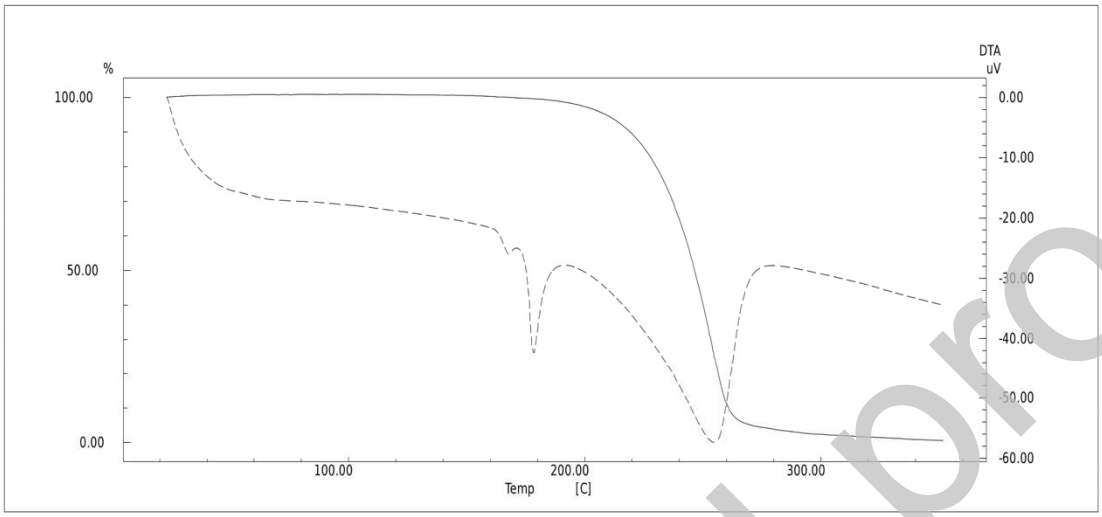


a

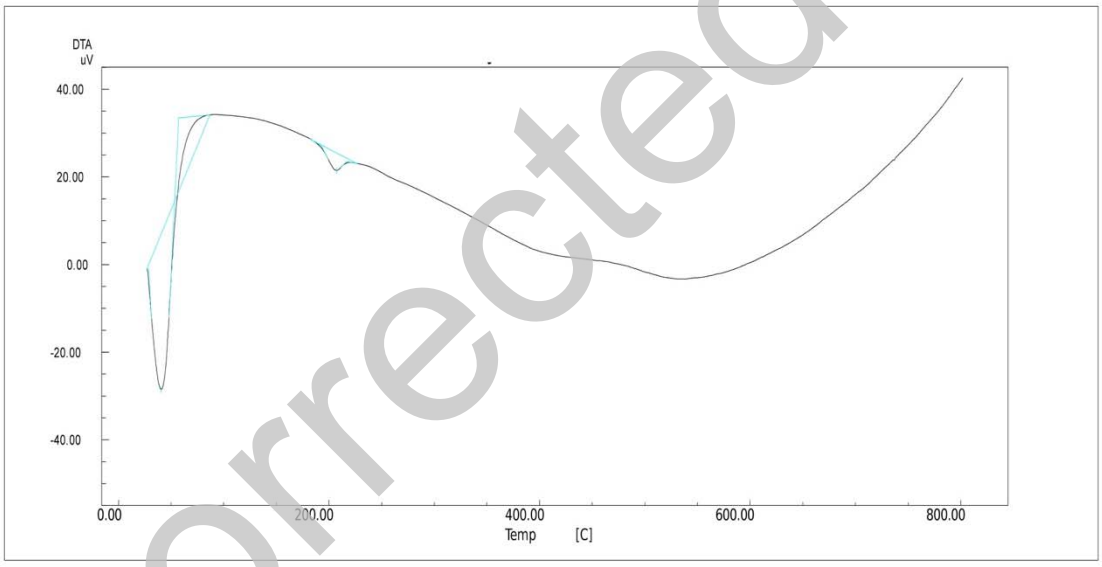


b

Figure 9.TGA plot of pure drug (a) and optimized formulation batch (b).



a



b

Figure 10. DTA plot of pure drug (a) and optimized formulation batch (b).

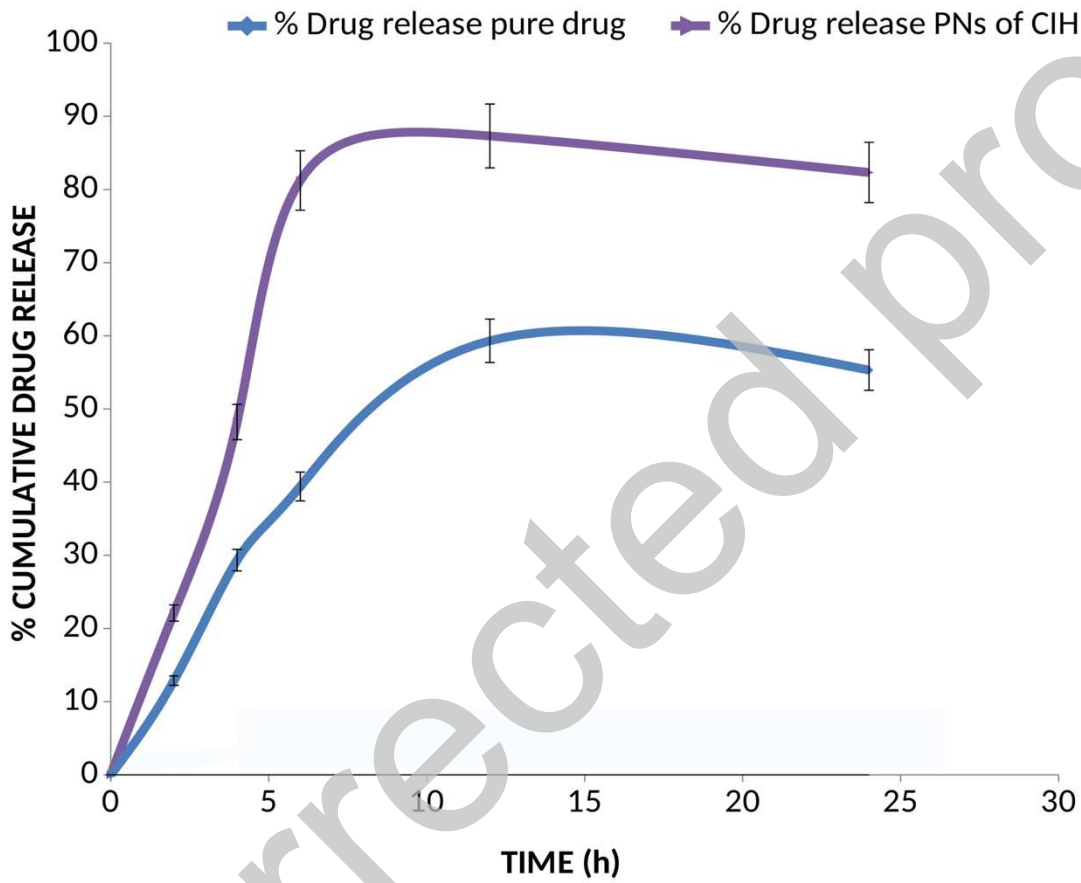


Figure 11.*In-vitro* drug release curve of pure drug vs optimized formulation batch.

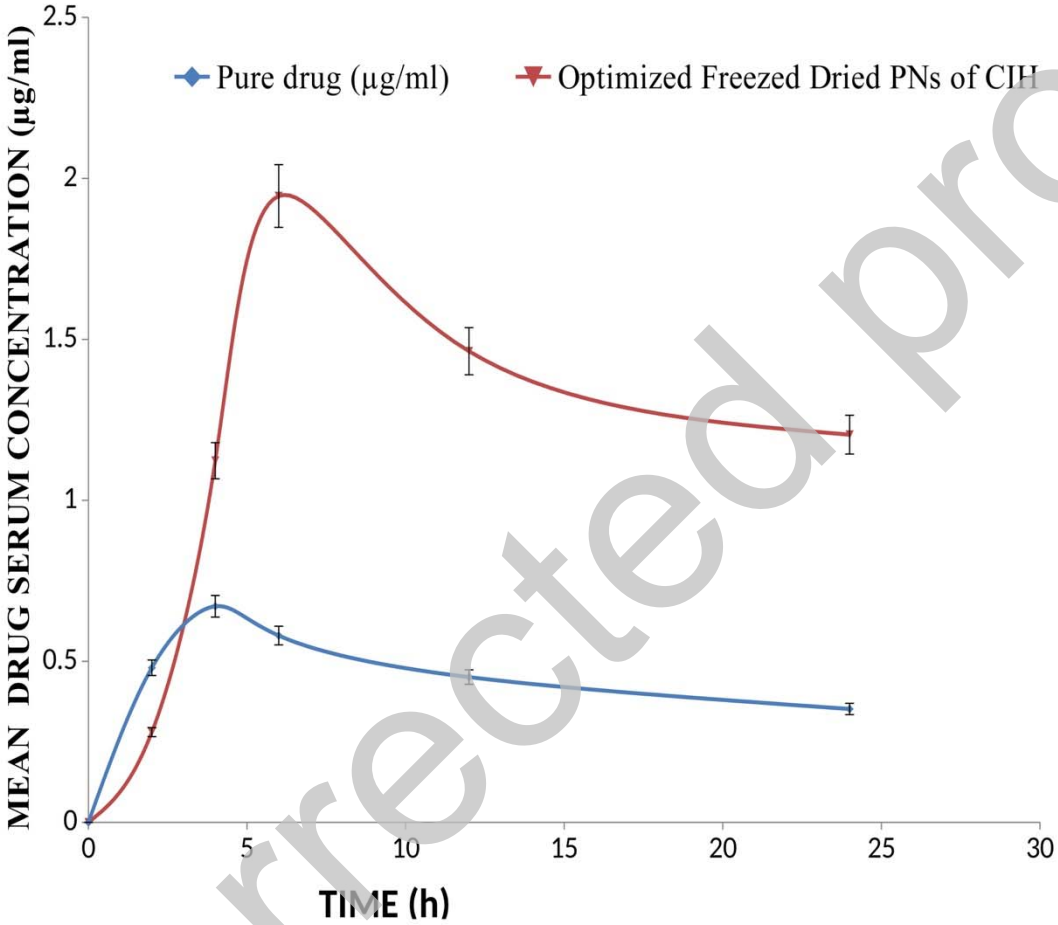


Figure 12. Serum concentration (µg/ml) v/s time (h) curve of optimized formulation batch vs pure drug suspension.

Supplementary data table legends

TableS1:Summary of ANOVA for factor screening and its significance as per Taguchi design
Table S2: Summary of ANOVA for different factors and its significance with respect to quadratic model
TableS3:Summary of design of experiment with various parameters fitting to quadratic model
TableS4:Constraints for the process of optimization of PNs of CIH using design of experiment
Table S5: Drug release, particle size, zeta potential and PDI of optimized PNs formulation at accelerated stability conditions

Table 1. Quality target product profile (QTPP) and critical quality attributes (CQAs) for developing PNs of cinacalcet hydrochloride

QTTPs	**Target**	**CQAs**	**Pre-determined target**	**Justification**
Dosage type	Extended release dosage forms	Cumulative drug release at 24hrs (QT24 %)	75-85%	Sustained release of drug is the objective of the study and is important for better absorption.
Dosage form	Polymeric nanoparticles	Zeta potential	≥±20mV	Highly critical factor as per the stability perspective of the Nano suspensions.
Drug release and absorption	C_{max} and AUC higher comparedto pure drug	Mean particle size (nm)	100nm-200nm	Particle size in these ranges is highly critical and important for better absorption of drug.
Dispersity	High dispersity	PDI (Polydispersity index)	0-0.4	Uniformity in the particle distribution by their size is essential for therapeutic activity hence highly critical.

Table 2. Taguchi design matrix for screening of factors along with the experimental results of various CQAs and along with factors and their respective low and high levels are coded

Runs	A	B	C	D	E	F	G	QT24%	Particle size (nm)	ZP (mV)	PDI
1	2	1	2	1	2	1	2	47.983	252.4	-27.8	0.299
2	2	2	1	1	2	2	1	67.342	181.0	22.32	0.175
3	1	2	2	2	2	1	1	32.341	276.3	-22.3	0.356
4	2	2	1	2	1	1	2	72.903	191.5	18.231	0.127
5	1	1	1	2	2	2	2	47.785	266.7	-22.3	0.368
6	2	1	2	2	1	2	1	70.234	224.1	-17.312	0.221
7	1	1	1	1	1	1	1	59.234	266.7	-23.6	0.368
8	1	2	2	1	1	2	2	63.456	286.7	-29.1	0.653

Factors	Codes	Low level (-1)	High level (+1)
PLGA conc. (mg)	A	20	60
Poloxamer 188 conc. (gm%)	B	0.5%	1.5%
Stirring speed (rpm)	C	5000	10000
Stirring time (h)	D	1	2
Ultra-sonication time(mims)	E	5	10
Temperature ^{0} C	F	25	40
Stirring type	G	Magnetic	Mechanical

Table 3. Composition of various PNs of CIH as per BBD along with the obtained CQAs responses and their coded levelsQT24% cumulative % drug release at 24h; PS, Particle size; ZP, Zeta potential; PDI, polydispersity index

Runs	Factor 1	Factor 2	Factor 3	Response Y1	Response Y2	Response Y3	Response Y4

	A:X1 PLGA: Drug ratio (mg)	B:X2 Polaxomer 188 conc. (%w/v)	C:X3 Stirring speed (rpm)	QT24%	Particle size (nm)	Zeta potential (mV)	PDI
1	-1	0	-1	60.345	390.311	13.23	0.453
2	1	0	-1	35.692	348.781	-21.21	0.432
3	0	0	0	48.824	345.453	-18.28	0.428
4	1	1	0	38.567	168.312	-21.6	0.389
5	-1	-1	0	62.542	432.367	-10.324	0.642
6	0	1	1	60.321	232.345	-18.674	0.299
7	0	1	-1	45.432	236.544	-19.421	0.349
8	0	-1	-1	42.871	450.211	-11.984	0.653
9	0	0	0	53.567	334.021	-15.27	0.257
10	0	0	0	54.987	323.237	-15.311	0.234
11	0	0	0	55.342	293.245	-16.322	0.231
12	-1	0	1	67.311	290.312	-6.321	0.171
13	1	-1	0	37.985	432.211	-20.3	0.634
14	1	0	1	29.411	286.768	-21.24	0.127
15	0	0	0	59.093	290.578	-18.431	0.231
16	-1	1	0	76.945	147.898	22.7	0.398
17	0	-1	1	44.252	403.231	-12.234	0.543

Independent Variables	**Levels**		
	Low level (-1)	**Middle level (0)**	**High level (+1)**
X1: DRUG:PLGA ratio (mg)	1:1(30 mg)	1: 1.5 (45 mg)	1:2(60mg)
X2: Polaxomer 188 conc. %	0.5%	1%	1.5%
X3:Stirring speed (rpm)	5000	10000	15000

Table 4. Carr's index, angle of repose, moisture content and in vivo pharmacokinetic parameters values of pure drug and optimized formulation batch

Formulations	**Carr's index**	**Angle of repose (Θ)**	**Moisture content (%)**	**C_{max} (µg/ml)**	**t_{max} (h)**	**K_e**	**AUC_0^∞ (µg/h)/mL**	**$t_{1/2}$**
Pure drug (CIH)	16.66±1.08	35.5±1.98	3.2±0.31	0.671	4	190.773	10.457	0.0036
Optimized formulation	12.20±0.98	27.96±1.5	2.8±0.4	1.945	6	192.737	31.558	0.0035

SUPPLEMENTARY DATA TABLES

Table S1: Summary of ANOVA for factor screening and its significance as per Taguchi design

QT24% cumulative % drug release at 24hr; PS, Particle size; ZP, Zeta potential; PDI, polydispersity index;

*Significant values, i.e. less than α value (0.05)

Source	P values of obtained from screened responses			
	QT24%	PS	ZP	PDI
PLGA	0.0288*	0.0446*	0.0206*	> 0.1000
Poloxomer-188	> 0.1000	0.0128*	0.0117*	0.0184*
Stirring speed	0.0197*	> 0.1000	0.0135*	> 0.1000
Stirring time	0.0524	0.0421*	0.0119*	> 0.1000
Ultra sonication time	0.0124*	> 0.1000	> 0.1000	> 0.1000
Temperature	0.0444*	> 0.1000	> 0.1000	> 0.1000
Stirring type	> 0.1000	> 0.1000	> 0.1000	> 0.1000

Table S2: Summary of ANOVA for different factors and its significance with respect to quadratic model

QT24% cumulative % drug release at 24hr; PS, Particle size; ZP, Zeta potential; PDI, polydispersity index;

*Significant levels, i.e. less than α value (0.05)

Source	QT24%		PS		ZP		PDI	
	F value	p-value	F value	p- value	F value	p- value	F value	p-value
Model	21.76	0.0003*	11.80	0.0018*	5.06	0.0220*	5.72	0.0157*
A-Drug:PLGA CONC.	158.24	< 0.0001*	0.0689	0.8005	29.10	0.0010*	0.1114	0.7483

B-Poloxomer conc.	11.35	0.0119*	97.34	< 0.0001*	0.8630	0.3838	17.82	0.0039*
C-Stirring speed	2.89	0.1330	5.08	0.0588	0.9868	0.3536	9.25	0.0188*
AB	3.84	0.0909	0.0946	0.7673	6.38	0.0394*	0.0000	0.9956
AC	3.53	0.1025	0.3228	0.5877	2.06	0.1939	0.0175	0.8984
BC	3.67	0.0970	0.4094	0.5426	0.0054	0.9435	0.1193	0.7399
A^2	0.0124	0.9146	0.5268	0.4915	5.87	0.0460*	0.7705	0.4092
B^2	0.0089	0.9276	0.3985	0.5479	0.1601	0.7010	22.87	0.0020*
C^2	12.11	0.0103*	2.09	0.1914	0.0030	0.9582	0.1729	0.6900
Lack of fit	0.7762	0.5652	3.01	0.1572	43.49	0.0016	1.07	0.4555

Table S3: Summary of design of experiment with various parameters fitting to quadratic model

Responses	QT24%	PS	ZP	PDI
R^2	0.9655	0.9382	0.8668	0.8803
Adjusted R^2	0.9211	0.8586	0.6956	0.7264
Predicted R^2	0.7627	0.2844	-1.0737	0.0433
Adeq. Precision	17.0206	12.0122	8.2677	7.6964
Std. Dev.	3.53	33.43	6.79	0.0868

QT24% cumulative % drug release at 24hr; PS Particle size; ZP, Zeta potential; PDI, polydispersibility index; R^2 Correlation coefficient; Std. Dev; Standard deviation

Table S4: Constraints for the process of optimization of PNs of CIH using design of experiment

Response	Predicted Mean	Predicted Median	Observed/ Experimental values	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
QT24%	77.352	77.3529	**76.945**	3.526	3.05442	70.1303	84.5754	55.2393	99.4665
Particle size	176.54	176.541	**147.898**	33.431	28.9524	108.08	245.003	-33.0701	386.153

Zeta potential	16.385	16.3852	**22.7**	6.792	5.88234	2.47572	30.2948	-26.2021	58.9726
PDI	0.396	0.396625	**0.398**	0.086	0.075209	0.218784	0.574466	-0.147878	0.941128

Table S5: Drug release, particle size, zeta potential and PDI of optimized PNs formulation at accelerated stability conditions

Time (Months)	QT24% (Cumulative drug release)	Particle size (nm)	Zeta potential (mV)	PDI
0	76.945	147.898	22.7	0.398
1	73.394	168.493	18.342	0.232
2	71.452	182.312	-13.41	0.421
3	68.341	168.301	-12.311	0.311
6	66.311	190.451	-22.212	0.390
p-value $\alpha \leq (0.05)$ significant difference exists	0.072	0.263	0.217	0.481