Enhancement of Dissolution Rate and Bioavailability of Nifedipine by Chitosan Based Cocrystallization Technique

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Authors’ contributions

This work was carried out in collaboration among all authors. Author Reetu designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AS and AG managed the analyses of the study. Author AY managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The objective of present study was to enhance solubility and dissolution behaviour of nifedipine by using cocrystallization method. A significant increase in solubility and dissolution rate of nifedipine has been demonstrated by solvent change method using chitosan. In this method, chitosan was precipitated on nifedipine crystals using sodium citrate as a salting out agent. An accurately weighed chitosan was dissolved in 1% acetic acid and drug was added in the chitosan solution. This resulting solution was added drop wise into 1% sodium citrate solution with continuous stirring. Sodium citrate precipitate polymer on drug crystals. FTIR, DSC, XRD, SEM, In-vitro dissolution studies, were studied for characterization of prepared cocrystals. Stability studies showed a good stability character of prepared cocrystals. Design Expert® software version 10.0 was used to develop polynomial models which were analysed to delineate the main effects for each CQA.
(critical quality attributes) through Box-Behnken design expert. Pharmacokinetic study clearly showed the enhancement of dissolution rate of cocrystals. The above investigation concluded that the significant dose reduction is possible for nifedipine with cocrystal formulation which leads to improve patient compliance.

Keywords: Cocrystallization; nifedipine cocrystal; chitosan; box behnken design; solubility.

1. INTRODUCTION

Angina pectoris is a temporary substernal chest pain occurring due to imbalance between myocardial oxygen supply and demand whereas hypertension is defined as abnormal high blood pressure (more than 120/80mmHg) in the arteries [1,2]. Nifedipine (C_{17}H_{18}N_{2}O_{6}) is a FDA approved drug for both chronic stable angina and hypertension. It is BCS class II drug classified as Ca++ channel blocking agent that reduces the inward entry of calcium ions particularly in cardiac and arterial smooth muscles, causing vasodilation and thereby improve blood supply. It has poor solubility and its bioavailability is highly dependent on the formulation type [3]. Diseases like angina require immediate drug response to manage the disease condition so, a formulation having improved aqueous solubility is essential for the enhancement of absorption and adequate oral bioavailability, but developing a new chemical entity with effective pharmacological activity is a challenging, costly and time taking procedure [4]. Recent estimates showed that the cost of developing a new drug is about US$2.8 billion so it is better to develop newer methods rather than developing a new chemical entity [5]. Cocrystals offer a low risk, low-cost, but high demanding method to formulate newer and better medicines. It could improve the physiochemical and biopharmaceutical properties by adding the suitable co-former without changing their chemical property. Hence the efforts made to increase the bioavailability of existing molecule by increasing their solubility gives an effective and economic drug formulation [6,7]. There are various practical approaches to improve the solubility of these include the use of particle size manipulation via micronization and nanonization, use of complexing agents such as cyclodextrins. The preparation of high energy drug states related to polymorphic or amorphous transformations use of co-solvents, micellar solutions and lipid based systems for lipophilic drugs are also one of the techniques to improve the solubility behaviour [8,9].

Crystal engineering is a method to design a crystalline molecular solids with the aim of impacting material properties [10]. In recent years, the advances in crystal engineering have emerged in the research for design of pharmaceutical cocrystals which consists the API and another compound (called co-former) at a given stoichiometric ratio [11]. The FDA defines cocrystals as ‘solids that are crystalline material composed of two or more molecule in the same crystal lattice’ [12]. It has advantage to transform an amorphous or hard-to- crystallize API into a readily handled, stable crystalline solid. It improves solubility of poorly soluble drugs and hence its bioavailability. It can also lowers the solubility of a highly soluble active ingredient and improved flow property [13].

Chitosan was used as a coformer which is a high molecular weight polycationic polysaccharide derived from naturally occurring chitin by alkaline deacetylation. It has favourable biological properties such as non-toxicity, biocompatibility and biodegradability. It carries a positive charge and can react with negatively charged surfaces (e.g. mucus) to improve the bioavailability of the payload. Chitosan being cationing polysaccharide in neutral and basic pH conditions, contains free amino group and hence insoluble in water. In acidic pH amino group can undergo protonation thus making it soluble in water [14].

When traditional methods of forming salts and amorphous material failed to produce a viable solid forms for continued development, the non-traditional approach of cocrystallization was used. The aim of the present study was to prepare a chitosan cocrystal of nifedipine to improve its solubility and oral bioavailability. Characterizations of cocrystal were done by FTIR, SEM, DSC and XRD. Bioavailability was measured by pharmacokinetic parameters and found to be increased by one fold.

2. MATERIALS AND METHODS

Nifedipine was obtained from HImedia as a gift sample and chitosan, sodium citrate were obtained from Methani Chitosan India. All other chemicals used in experiment were purchase
from CDH, Delhi, India. All materials used in the research were of analytical grade. Table 1 presents the chemical structure of nifedipine and chitosan.

2.1 Design of Experiments

Considering cocrystals as the target formulation for enhancing the solubility and activity of the API, among the TPP (target product profile) elements that define the key quality characteristics are: dosage form, route of administration, dosage type, pharmacokinetics, packaging and stability requirements. In order to meet the TPP, various (critical quality attributes) CQAs were identified as critical, such as drug loading, particle size and drug release taking into account that amorphous domains could be also formed during ionic cross linking.

2.2 Factor Screening Studies

A Box-Behnken design was used to study the effect of three independent factors; a) coformer concentration i.e. chitosan (A; X1), cross-linking agent i.e. sodium citrate (B; X2), and drug i.e. nifedipine (C; X3), on the three dependent variables; loading efficiency (Y1), particle size (Y2), and drug release (Y3). For analysing the response surface of the variables inside the experimental domain Stat-Ease design expert software (version no.10, MacOS) was used. Three additional experiments were done subsequently to verify the validity of the statistical experimental strategy. The statistical design provides a polynomial describing the quadratic effect, as well as the interactions of each study factor on the considered response variable. The general model corresponds to the following equation:

\[ Y = b_0 + b_1A + b_2B + b_3C + b_{12}AB + b_{13}AC + b_{23}BC + b_{123}ABC + b_{1}A^2 + b_{2}B^2 + b_{3}C^2 \]  

Where, Y is the measured response associated with each factor level combination; b0 is an intercept; b1 to b23 are the regression coefficients; and X1, X2, and X3 are the independent variables.

Design Expert® software version 10.0 (M/s StatEase, Minneapolis, USA) was used to develop polynomial models which were analysed to explain the main effects for each CQA through Box-Bhenken design model.

2.3 Preparation of Cocrystals

An accurately weighed amount of chitosan was dissolved in 1% acetic acid. Then an accurately weighed quantity of Nifedipine was added into the above solution and the resulting solution was added drop wise into 1% sodium citrate solution with continuous stirring. Sodium citrate was used as a salting out agent to precipitate chitosan as crystals on Nifedipine crystals. Crosslinking reaction between the protonated amino groups of chitosan and carboxylate group of trisodium citrate was occurred. Nifedipine chitosan co-crystals were filtered through whatman filter paper No.1 and crystals were dried at 45°C for 24hrs and passed through sieve no #40.

Table 1. Chemical structure of nifedipine and chitosan

| Name     | Structure | Chemical name                                      |
|----------|-----------|---------------------------------------------------|
| Nifedipine | ![Image](image1.png) | 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine dicarboxylic acid dimethyl ester |
| Chitosan | ![Image](image2.png) | oly-b-(1,4)-2-Amino-2-deoxy-D-glucose |
Table 2. List of dependent and independent variables in Box-Behnken design for nifedipine cocrystals

| Level used | Factor | Independent variables | Units | Low | High |
|------------|--------|-----------------------|-------|-----|------|
| A          | Chitosan (%w/v) | 1   | 8   |
| B          | Trisodium citrate (%w/v) | 10  | 20  |
| C          | Drug (%w/v) | 1   | 2   |

2.4 Characterization of Cocrystals

SEM (Scanning electron microscopy), FT-IR spectral analysis. (Differential Scanning colorimeter) DSC, XRD was done for characterization of cocrystals. It was clearly shown in results that cocrystals has been formed and provide optimum results.

2.5 In-vitro Dissolution Study of Nifedipine and Cocrystals

The in-vitro release study was done in an USP XXII dissolution apparatus. 40 mg of nifedipine cocrystals and raw drug was placed in 900 ml of gastric fluid (buffer pH 1.2) maintained at 37 ± 0.5°C, stirring speed 50 rpm. Aliquots of 5 ml were collected at regular time intervals and replaces with the same amount of fresh dissolution medium to maintain the sink condition throughout the experiment. The aliquots were filtered, further diluted suitably and estimated spectrophotometrically at 238 nm.

2.6 In-vivo Pharmacokinetic Analysis

Pharmacokinetic parameters were evaluated for determination of peak plasma concentration ($C_{\text{max}}$), time to reach maximum plasma concentration ($T_{\text{max}}$) and total area under the plasma concentration-time curve ($AUC_{0-\infty}$). Plasma concentration time profile of the reference drug and test drug was compared using six male wistar rats [15]. $C_{\text{max}}$, $T_{\text{max}}$ were calculated from each plasma concentration data. The $AUC_{0-\infty}$ was estimated by adding the area from time zero to the last sampling time ($AUC_{0-t}$) and the area from the last sampling point to infinity ($AUC_{t-\infty}$). Trapezoidal method was used for the calculation of $AUC_{0-\infty}$. $AUC_{0-\infty}$ was determined by dividing the last measurable plasma drug concentration with total elimination rate constant ($K_{\text{el}}$). Relative bioavailabilities (%) of nifedipine cocrystals was calculated using the following formulas:

$$K_{\text{el}} = \frac{\text{Slope}}{0.203}$$

$$\% \text{Relative bioavailability} = \frac{AUC_{\text{ref}}}{AUC_{\text{test}}} \times \frac{\text{Dose}_{\text{ref}}}{\text{Dose}_{\text{test}}} \times 100$$

2.7 Statistical Analysis

All measured data were expressed as mean±standard deviation (S.D.) and were analyzed using BioStat version 2009 for Windows software.

2.8 Ethical Approval

Ethical approval was taken from CPCSEA for animals used in the experiment.

3. RESULTS AND DISCUSSION

3.1 Solubility Study

The raw Nifedipine was observed having very slight solubility in distilled water, pH 1.2, 6.8 and 7.4. The solubility was found to be 0.00674±0.002 mg/ml and 0.00583±0.006 mg/ml, 0.00692±0.003 mg/ml, 0.00688±0.005 mg/ml respectively. This solubility value is in agreement with the reported literature [16]. Solubility study of optimized nifedipine cocrystals was carried out in pH 1.2 buffer. The optimized cocrystal was observed to have high solubility of 0.0157±0.006 mg/ml in pH 1.2 buffer in comparison with raw drug. There was an increase in solubility of drug in chitosan based cocrystals, the results revealed that chitosan dissolves readily in most of the acid solutions due to amine groups of the polymer become protonated, resulting in a positively charged polysaccharide (RNH$_3^+$) that are highly soluble in
nature. Moreover, chitosan is hydrophilic in nature which enhanced solubility of the nifedipine [17].

3.2 Optimization of Process Variables of Nifedipine Cocrystals

The effect of various processing parameters was studied by (Box Behnken Design) BBD on loading efficiency (Y1), particle size (Y2), and drug release (Y3). The obtained results indicated a strong dependency of dependent variables on the selected independent variables, as they show wide variation among all 17 batches (Table 3). All 17 formulations were prepared using 3^3 factorial design and evaluated. The result of evaluation for an individual response were studied by ANOVA (Table 4).

3.3 In-vitro Drug Release Study

The formulations F 1 to F 17 were formulated with different ratios of chitosan, sodium citrate and drug provided by (Box Behnken Design) BBD experimental design and evaluated for in-vitro release of all 17 formulation of nifedipine cocrystals. In-vitro study of raw drug was also done for comparison purpose. It is clearly shown in Fig. 1 that all batches have improved dissolution profile than raw drug of nifedipine.

3.4 Effect of the Process Parameters on the Loading Efficiency

The influence of the main and interactive effects of the independent variables on the LE was studied using polynomial equation and it was found to be statistically significant (P < 0.0001).

\[
\text{LE} = +78.00 - 0.6250A - 2.62B + 5.50C + 0.2500AB - 3.00AC - 4.00BC - 0.3750A^2 - 3.88B^2 - 4.62C^2
\]

The predictive ability of the model was indicated by the calculation of \( R^2 \) coefficients, which is a criterion of the model fitting. For all experimented batches, the \( Y_1 \) (LE) value showed good \( R^2 \)-squared value of 0.9976. As the equation shows, independent variables A, B, and C have ‘p’ < 0.05 that has significantly effect on the LE. The values of coefficients in the equation represent the effect of that term on the LE. The relationship between the dependent and independent variables that was further elucidated using response surface plots.

Table 3. Box-behnken experimental design (BBD) with measured responses of nifedipine cocrystals

| Run | Factor 1 | Factor 2 | Factor 3 | Response \( Y_1 \) | Response \( Y_2 \) | Response \( Y_3 \) |
|-----|----------|----------|----------|------------------|------------------|------------------|
|     | A:CS %   | B:Citrate % | C:Drug % | LE %         | Size Nm | Q2h % |
| 1   | 1        | 10       | 1.5      | 78            | 730    | 86   |
| 2   | 8        | 20       | 1.5      | 70            | 780    | 58   |
| 3   | 4.5      | 10       | 1        | 61            | 830    | 91   |
| 4   | 4.5      | 15       | 1.5      | 78            | 730    | 90   |
| 5   | 1        | 15       | 1        | 72            | 860    | 78   |
| 6   | 4.5      | 20       | 1        | 68            | 860    | 69   |
| 7   | 8        | 10       | 1.5      | 79            | 860    | 71   |
| 8   | 4.5      | 15       | 1.5      | 78            | 730    | 90   |
| 9   | 8        | 15       | 2        | 80            | 750    | 68   |
| 10  | 8        | 15       | 1        | 62            | 875    | 91   |
| 11  | 4.5      | 15       | 1.5      | 78            | 730    | 90   |
| 12  | 4.5      | 10       | 2        | 79            | 730    | 84   |
| 13  | 1        | 20       | 1.5      | 68            | 870    | 91   |
| 14  | 4.5      | 20       | 2        | 70            | 740    | 78   |
| 15  | 4.5      | 15       | 1.5      | 78            | 720    | 90   |
| 16  | 4.5      | 15       | 1.5      | 78            | 730    | 90   |
| 17  | 1        | 15       | 2        | 78            | 730    | 81   |
Table 4. ANOVA of results of the responses (LE, Size and Drug Release)

| Source        | Sum of square | d.f. * | Mean square | F value | P Value   |
|---------------|---------------|--------|-------------|---------|-----------|
| Model         | 565.22        | 9      | 62.8        | 8.26    | 0.0055    | Significant |
| A-CS          | 3.12          | 1      | 3.12        | 0.4108  | 0.542     |
| b-Citrate     | 55.12         | 1      | 55.12       | 7.25    | 0.031     |
| C-Drug        | 242           | 1      | 242         | 31.81   | 0.0008    |
| Ab            | 0.25          | 1      | 0.25        | 0.0329  | 0.8613    |
| AC            | 36            | 1      | 36          | 4.73    | 0.0661    |
| C             | 64            | 1      | 64          | 8.41    | 0.023     |
| A²            | 0.5921        | 1      | 0.5921      | 0.0778  | 0.7883    |
| b²            | 63.22         | 1      | 63.22       | 8.31    | 0.0236    |
| Response Y₂: Particle size(nm) |                      |        |             |         |           |
| Model         | 62137.28      | 9      | 6904.14     | 168.83  | < 0.0001  | Significant |
| A-CS          | 703.13        | 1      | 703.13      | 17.19   | 0.0043    |
| b-Citrate     | 1250          | 1      | 1250        | 30.57   | 0.0009    |
| C-Drug        | 28203.13      | 1      | 28203.13    | 689.68  | < 0.0001  |
| Ab            | 12100         | 1      | 12100       | 295.9   | < 0.0001  |
| AC            | 6.25          | 1      | 6.25        | 0.1528  | 0.7075    |
| bC            | 100           | 1      | 100         | 2.45    | 0.1618    |
| A²            | 9650.59       | 1      | 9650.59     | 236     | < 0.0001  |
| b²            | 4903.22       | 1      | 4903.22     | 119.9   | < 0.0001  |
| Response Y₃: Drug release (%) Q₂h |                      |        |             |         |           |
| Model         | 1279.26       | 9      | 142.14      | 2.5     | < 0.0001  | Significant |
| A-CS          | 288           | 1      | 288         | 5.06    | < 0.0001  |
| b-Citrate     | 162           | 1      | 162         | 2.85    | 0.0722    |
| C-Drug        | 40.5          | 1      | 40.5        | 0.7114  | < 0.0001  |
| Ab            | 81            | 1      | 81          | 1.42    | 0.643     |
| AC            | 169           | 1      | 169         | 2.97    | 0.004     |
| bC            | 64            | 1      | 64          | 1.12    | 0.076     |
| A²            | 221.32        | 1      | 221.32      | 3.89    | 0.028     |

Fig. 1. Drug release profile of all formulations and raw drug
3.5 Effect of the Process Parameters on the Size of the Crystals

Uniform and small size of cocrystals plays an important role in the drug release behaviour at the GIT region. Accordingly, the aim of optimizing size was considered to be the minimum value. By considering multiple linear regression analysis, the $Y_2$ equation is given as below:

\[
\text{Size} = +728.00 + 9.38A + 12.50B - 59.38C - 55.00AB + 1.25AC - 5.00BC + 47.87A^2 + 34.12B^2 + 27.88C^2
\]

The response surface plots relating cocrystals size demonstrated that increasing the CS and drug concentration led to increasing cocrystals size (Fig. 3). The variations in size and morphology of the cocrystals with different polymeric concentrations of CS and drug were due to variations in the availability of reacting/binding sites for cross-linking citrate ions. Fig. 3 reveals that increase in the average size of these cocrystals was found to be increased with the combination of drug and CS (with increasing concentration of chitosan) [18].

3.6 Effect of the Process Parameters on Drug Release

The effect of the variables on drug release is more intricate and drug release at 2 h ($Q_{2h}$) was chosen for optimization of the independent variables. The mathematical relationship in the form of a polynomial equation for the measured response, drug release $Y_3$ is given in the following Eq.:

\[
\text{Drug release} (Q_{2h}) = +90.00 - 6.00A - 4.50B - 2.25C - 4.50AB - 6.50AC + 4.00BC - 7.25A^2 - 6.25B^2 - 3.25C^2
\]

Factor A (CS) was considered almost complete release of the factor C (drug) within 2 h. This result was attributed to quick release of drugs being observed in chitosan cocrystals, due to quick degradation of the chitosan in acidic pH. Two variables (A and B) have a negative effect on drug release, which means that these factors are inversely proportional to the response, and favour drug release up to 2 h. The influence of the main and interactive effects of independent variables on the drug release was elucidated using 3D response surface plots (Fig. 4).
To get the desired optimum responses, independent variables (factors) were restricted to 
\(1 \leq A \leq 8\), \(10 \leq B \leq 20\) %, and \(1 \leq C \leq 2\); whereas the desirable ranges of responses were restricted to \(60 \leq \text{LE} \leq 80\) %, \(720 \leq \text{Size} \leq 875\) nm, and \(58 \leq Q_{2h} \leq 91\) %. In order to evaluate the optimization capability of these models generated according to the results of factorial design, optimized citrate ion-induced cocrystals containing nifedipine were prepared using one of the optimal process variable settings proposed by the design. Table 4 shown summary of ANOVA for comparison of all the three response parameters. The selected optimal process variable setting used for the formulation of optimized citrate ion induced cocrystals was \(A = 3.04\), \(B = 13.51\), and \(C = 1.67\). The cocrystals optimization was evaluated for \(\text{LE}\) (%), size (nm), and \(Q_{2h}\)(%). The predicted values of optimized cocrystals containing nifedipine showed \(\text{LE}\) of \((80 \pm 2.75)\) %, size \((707.884 \pm 6.39)\) nm, and \(Q_{2h}\)(%(90.81 \pm 7.5) %, with small error values (of 7.03, -0.236, and 2.147 respectively). This reveals that mathematical models obtained from the Box-Behnken design were well fitted. This demonstrates the reliability of the optimized procedure in predicting the operating parameters for the preparation of nifedipine cocrystals for enhancing solubility. The selected formulation was subjected to characterization and in-vitro release.

### Table 5. Optimized formula of Nifedipine cocrystals (Selected process factors with desirability (0.998))

| A:Chitosan | B:Sod. Citrate | C:Drug | Experimental values |
|------------|----------------|--------|---------------------|
| NC         | 3.04           | 13.51  | 1.67                |
|            |                |        |                     |
| LE         | 85.63±2.03     | 706.21±5.46 | 92.76±3.14         |
| Size       |                |        |                     |
| Q_{2h}     |                |        |                     |
| Predicted values | 80±2.75 | 707.884±6.39 | 90.81±7.5          |
| % Error    | 7.03           | -0.236 | 2.147               |

\(\text{Mean \pm S.D., n = 3.}\) Error (%) = \((\text{difference between observed and predicted values})/\text{predicted value} \times 100\)

### 3.7 Characterization of Optimized Formulation

#### 3.7.1 Scanning electron microscopic study

The SEM photomicrographs of nifedipine and nifedipine cocrystal are given in Fig. 5. The raw drug was characterized by crystals having comparative bigger size needle like shape with smooth surface. Whereas, nifedipine cocrystals were present in form of powder which is due to small particle size. Additionally, the cocrystals were fluffy and seemed to have porous and rough surface which might be a reason for enhanced dissolution rate as compared to raw drug.

#### 3.8 DSC Analysis

The results of DSC studies are given in Fig. 6. Raw nifedipine have a sharp endotherm at 174°C corresponding to its melting point whereas a decrease in the melting point of drug is observed (166°C) when prepared in the form of cocrystals. A noticeable reduction in the enthalpy of cocrystals was also found when compared with raw nifedipine along with a lower melting point. This reduction in melting point and enthalpy in nifedipine crystals accounts for
enhanced solubility and reduced crystallinity of nifedipine. Schultheiss et al, reported in their review that in the survey, 50 cocrystalline samples were analysed in which 26/50 (51%) cocrystals had melting points between those of the API and coformer, while 19/50 (39%) were lower than either the API or coformer, only 3/50 (6%) were higher, and 2/50 (4%) had the same melting point as either the API or coformer. These statistics clearly show that the melting point of an API can be altered through forming cocrystals, and the outcome will usually be a product having a melting point that is in between that of the API and coformer or lower than the API or conformer [19]. Observation of broadness in melting peak along with diminishes in size indicates decrease in crystallinity of nifedipine whereas melting peak disappears completely in amorphous structure [20].

3.9 XRD Analysis

The XRD patterns of the raw drug and cocrystals are shown in Fig. 7. The diffraction pattern of intact nifedipine shows that the solid drug is a highly crystalline powder and with sharp diffraction peaks at 2θ equal to 11.5, 14.1, 17.6, 19.2, 22, 24.3, 26, 32.3, 36.5, 41.2 and 54 and nifedipine cocrystal shows the diffraction peaks at the following 2θ angles 11.5, 15, 18.1, 20, 21.7, 25.2, 28, 29, and 37.8. The XRD scan of raw nifedipine have intense sharp peaks which showed crystallinity; whereas the XRD pattern of the nifedipine cocrystals are having dense peaks which showed reduction in number and intensity of peaks both when compared to raw nifedipine. This indicates decrease in crystallinity or partial amorphpization of the drug in cocrystal form [17]. This result was well corroborated with earlier studies, Mutalik et al., developed chitosan based cocrystals and the results revealed that chitosan have potential to decrease the intensity of the crystallinity of aceclofenac drug molecule [5]. Absence of intense peak in XRD diffraction pattern was observed in case of amorphous nifedipine [21].

3.10 FTIR Analysis

According to Malik A et al, any compound having covalent bonds, whether organic or inorganic absorbs various frequencies of electromagnetic radiation in the infrared region of the electromagnetic spectrum. The molecules absorb selected frequencies of infra red radiation. FT-IR spectrum of nifedipine exhibits relatively low C=O (doublet centred at 1680 cm\(^{-1}\) ) and N-H (3327 cm\(^{-1}\)) wavenumbers. This is due to strong intermolecular hydrogen bond interactions between the ester carbonyl and the NH group of the adjacent nifedipine molecule. The FT-IR spectrum of amorphous nifedipine shows a distinct difference to the crystalline nifedipine in the sharpness and definition of peaks, specifically in the 1700 cm\(^{-1}\) region. The (N-H) band was also shifted to a higher wavenumber indicating less or weaker intermolecular hydrogen bond interactions in amorphous nifedipine. Amorphous nifedipine shows a single broad peak at 754 cm\(^{-1}\), whereas crystalline nifedipine shows two peaks, 744 cm\(^{-1}\) and 762 cm\(^{-1}\) [20]. Obtained peak in IR spectra of nifedipine cocrystals confirms that the obtained product is in a crystalline form and not in a
amorphous form. Raw nifedipine showed major peaks at wavenumbers 3536.81 cm⁻¹ (free O–H stretching vibrations); 3058.55 and 2923.56 cm⁻¹ (C–H stretching vibrations); and 1693.19 cm⁻¹ (stretching vibration of ester and lactone carbonyl functional groups); 1600.63, 1454.06 and 1268.93 etc., cm⁻¹ (C-O stretching of esters and anhydrides). Chitosan physical mixture showed different peaks from drug which showed a compatibility between drug and chitosan [22,23].

3.11 In vivo Pharmacokinetics Evaluation Study

The objective of preparing nifedipine cocrystals was to improve the drug oral bioavailability. So, the evaluation of bioavailability of drug in animal is an important parameter to consider when preparing new dosage forms. The plasma drug levels after a 5 mg/kg single dose of raw drug and nifedipine cocrystals are shown in Fig. 9. A significant increase in the peak plasma concentration (C_max) was observed for nifedipine cocrystals (1.47 µg/ml) compared to that of raw nifedipine (0.96 µg/ml). The time of peak plasma concentration (T_max) was observed for nifedipine cocrystals and raw nifedipine at 2 hr. A significant increase in the AUC was observed for nifedipine cocrystals (6.0275µg.hr/ml) compared to that of raw nifedipine (4.015 µg.hr/ml).

3.12 Stability Studies

Stability studies were done for three months as per ICH guidelines. At time intervals (30 days), samples were evaluated for physical appearance, % drug content and % drug release. There was no major change in the evaluation parameters (Table 6).

Fig. 6. DSC thermogram of (A) raw nifedipine and (B) nifedipine cocrystals
Fig. 7. XRD patterns of (A) raw nifedipine (B)nifedipine cocrystals

Fig. 8. FTIR Spectroscopy study of A) drug, B) chitosan, C) cocrystals
Table 6. Stability studies

| Formulation       | Conditions         | Time Interval (month) | Colour | Assay (%) | In-vitro drug release (%) |
|-------------------|--------------------|-----------------------|--------|-----------|---------------------------|
| Nifedipine cocrystals | 25±2°C/60±5% RH    | 0                     | White  | 100       | 85.84                     |
|                   |                    | 3                     | White  | 99.33     | 84.28                     |
|                   | 40±2°C/75±5% RH    | 0                     | White  | 100       | 85.84                     |
|                   |                    | 3                     | White  | 99        | 83.55                     |

4. CONCLUSION

The enhancement in dissolution rate of nifedipine was achieved by cocrystallization method. Presence of intense peak in XRD, sharp melting point in DSC and some new band formation in FTIR studies confirm the formation of cocrysalts. The pharmacokinetic study for oral bioavailability of nifedipine in wistar rats resulted from cocrystals was increased by one fold compared with the marketed nifedipine formulation. The significant dose reduction is possible for nifedipine with cocrystal formulation leads to improve patient compliance.

Hence, it can be concluded that cocrystallization is a promising approach to improve the solubility and dissolution behaviour of Nifedipine.

ETHICAL APPROVAL

Ethical approval was taken from CPCSEA for animals used in the experiment.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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