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RESEARCH ARTICLE

Enhancement of Oral Bioavailability of Nimodipine using Quercetin Dihydrate: A Natural Cytochrome P450 Inhibitor

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Abstract

Background: Cytochrome P450 enzymes are responsible for the low bioavailability of many drugs. Nimodipine is a Biopharmaceutical Classification System class II dihydropyridine calcium channel blocker. Nimodipine's oral bioavailability is limited to 3-13% due to poor water solubility and cytochrome P450 enzymes. **Aim:** The main aim of this study is to develop a bilayer tablet to improve the oral bioavailability of nimodipine using a cytochrome P3A4 inhibitor. **Methods:** Solid dispersion of quercetin dihydrate was prepared by the solvent evaporation technique, and the direct compression method was used to prepare a bilayer tablet. Solid dispersion of quercetin dihydrate and Polyvinylpyrrolidone K30 was prepared in a 1:3 ratio. **Results:** Various post-compression parameters were evaluated for the developed bilayer tablet and optimised by Design of Experiments. The effect of experimental factors such as polymer concentration, super disintegration concentration, and main compression force on % quercetin dihydrate release, % drug release, friability, and hardness remains to be investigated to get an optimised formulation. The NQ4 formulation was optimised batch to give 97.80% quercetin dihydrate release in 2 h and 98.74% nimodipine drug release in 12 h with 8.5 kg/cm^2 hardness and 0.22 % W/W friability. The *in vivo* pharmacokinetic study data proved that the developed bilayer tablet enhances the oral bioavailability of nimodipine when compared to the nimodipine sustained release tablet. The physicochemical stability of optimised bilayer tablets during storage under accelerated conditions was also validated. **Conclusion:** The present research proved the development of a nimodipine bilayer tablet formulation with quercetin dihydrate as a CYP3A4 inhibitor in the immediate release form to increase nimodipine oral bioavailability.

Major Findings: The oral bioavailability of nimodipine was increased with quercetin dihydrate as a CYP3A4 inhibitor in bilayer tablet formulation compared to the nimodipine sustained release tablet.

Keywords: Bilayer Tablet, Cytochrome P3A4 Inhibitor, Nimodipine, Oral Bioavailability, Quercetin Dihydrate

Abbrevations: AUC: Area Under Curve; BCS: Biopharmaceutical Classification System; CI: Carr's Index; cm: centimeter; CYP450: Cytochrome P450; CYP3A4: Cytochrome P3A4; Cmax: Maximum plasma concentration; CQAs: Critical Quality Attributes; °C: Degree Celsius; DOE: Design of Experiments; FDA: Food and Drug Administration; FIP: International Pharmaceutical Federation; g: Gram; h: Hour; HPLC: High Performance Liquid Chromatography; ICH: International Council for Harmonisation; kg: kilogram; KN: Kilonewton; µM: Micromole; µL: Microliter; mg: Milligram; ml: Milliliter; mm: Millimeter; m: Minute; OECD: Organisation for Economic Cooperation and Development; QbD: Quality by Design; QTPP: Quality Target Product Profile; RA: Risk Assessment; Rpm: Revolution per minute; S: Seconds; USP: United States Pharmacopoeia; UV: Ultraviolet.

1. Introduction

Bioavailability is defined as "the rate and extent to which the active drug excipients are absorbed from a drug product and become available at the site of drug action. The rate and extent of drug content and the rate of gastrointestinal tract and presystemic drug metabolism are the factors which affect the bioavailability. Humans are continuously affected by drugs, food additives and various environmental chemicals, which affect human

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health. Cytochrome P450 (CYP450) enzymes are the human health defence system to protect organisms against toxic compounds[2]. Due to the properties of membrane-bound hemoproteins, the enzymes called CYP450 are responsible for xenobiotic detoxification of xenobiotics and endobiotics. CYP450 enzymes are present in the liver and intestine and are responsible for the metabolism of more than 90% of drugs[2'3]. Among them, 95% of Food and Drug Administration (FDA) approved drugs are metabolised by six isoforms: CYP1A2, CYP2B6, CYP2C9, CYPC19, CYPD6 and CYP3A4[4]. Inhibition or induction of CYP450 enzymes leads to drug-drug interactions. CYP450 inhibition may lead to an increase or decrease in the bioavailability of the drug by increasing or decreasing the elimination rate[5]. Grapefruit juice and dietary polyphenols are CYP3A4 inhibitors. Quercetin inhibits 55-65% of CYP3A4 enzyme at 100 µM concentration[6]. Grapefruit juice reduces the activity of CYP3A4 enzymes by a reversible and an irreversible mechanism[7]. Grapefruit juice enhances nimodipine bioavailability by 51% with a 24% Cmax by inhibiting CYP3A4[7].

Nimodipine is classified as a Biopharmaceutical Classification System (BCS) class II dihydropyridine calcium channel blocker drug[8'9]. Nimodipine is used to treat stroke, migraine, hypertension, and Alzheimer's disease. Nimodipine, introduced by Bayer in 1982, has been found to widen cerebral arteries and enhance cerebrovascular flow[10]. It has a limited oral bioavailability (3-13%) due to its low water solubility and substantial first-pass metabolism in the liver via CYP3A4. As a result, nimodipine is administered intravenously rather than orally to achieve better bioavailability. The many strategies for improving BCS Class II drug solubility include solid dispersion, self-emulsification, particle size reduction, and nanotechnology[11]. The solid dispersion approach produces good outcomes in clinical and preclinical formulation development for delivering insoluble substances by influencing specific surface area and solubility. Solid dispersion is produced via melting (fusion), hot melt extrusion, spray drying, melting-solvent, freeze-drying, and co-evaporation[10].

The most preferred route for many drugs is oral solid dosage form administration. Due to the patient compliance and its suitability for large-scale production, the majority of research studies focus on it[12]. The administration of a large number of prescribed medicines is reducing patient compliance[13]. The limitation of the single-layer tablet is overcome by preparing a bilayer tablet formulation[14]. Modified release bilayer tablets are more likely to be consumed by patients due to their lower frequency of dosing or enhanced therapeutic efficacy[14,15]. The first layer of a bilayer tablet is designed for sustained release of the drug for a long period of time, while the second layer provides immediate release to inhibit CYP3A4 isoenzymes. Manufacturing of bilayer tablets is problematic due to mechanical properties, compression characteristics, mechanical disparity of the layers, and layer mixing. If these variables are not managed, they will have an impact on the quality of the bilayer tablet formulation. Material properties, compression force, lubricants, layer ratio, bilayer tablet characterisation, and other factors must be considered during bilayer drug formulation[12].

The purpose of this study is to formulate a nimodipine-containing CYP3A4 inhibitor bilayer tablet to enhance nimodipine oral bioavailability by inhibiting the CYP3A4 enzyme and improving patient compliance by lowering the dose or enhancing the drug's effectiveness. The first layer of the bilayer tablet consists of a nimodipine for sustained release, while the second layer contains a CYP3A4 inhibitor for immediate release.

2. Materials and Methods

2.1 Materials

Nimodipine was obtained from West-Coast Pharmaceutical Works Limited (Ahmedabad, Gujarat, India). Quercetin dihydrate was purchased from Sisco Research Laboratories Private Limited (Mumbai, Maharashtra, India). Methocel K100 premium LVCR was supplied by Colorcon Asia Private Limited (Verna, Goa, India). Polyvinylpyrrolidone (PVP K30), microcrystalline cellulose 101, crospovidone XL 10, sodium lauryl sulfate, colloidal silicon dioxide and magnesium stearate were obtained from Chemdyes Corporation (Vadodara, Gujarat, India). Nitrendipine was purchased from Tokyo Chemical Industry Co., Limited (Chuo-ku, Tokyo, Japan). High-purity organic solvents for High Performance Liquid Chromatography (HPLC) analysis (HPLC grade, Sisco Research Laboratories Private Limited, Mumbai, Maharashtra, India) were also utilised.

2.2 Preparation of Bilayer Tablet

The sustained release blend was prepared by the dry granulation method. Nimodipine, along with Methocel K100 premium LVCR and microcrystalline cellulose 101, was weighed and passed through a sieve number 40. Dispense the magnesium stearate and colloidal silicon dioxide that pass through sieve number 60. The mixture was blended with colloidal silicon dioxide and magnesium stearate for 5 min. The solvent evaporation technique was used to prepare a solid dispersion of quercetin dihydrate with PVP K30[16]. Quercetin dihydrate was mixed with PVP K30 in a 1:3 ratio and dissolved in ethanol using a magnetic stirrer at 800 rpm to produce clear yellow solutions. The prepared solution was evaporated at room temperature in the dark conditions, and the dried mass was passed through a sieve number 40. The solid dispersion was stored in a firmly sealed container at 25°C and kept away from light until further research. The immediate release blend is prepared using a pre-prepared solid dispersion. Solid dispersion was passing through sieve number 40 with crospovidone XL 10 and sodium lauryl sulfate. After passing through sieve number 40, dispense the microcrystalline cellulose 101 and mix it with the above mixture. Dispense the magnesium stearate and colloidal silicon dioxide that were thoroughly passed through a sieve number 60. The mixture was blended with colloidal silicon dioxide and magnesium stearate for 5 m. The bilayer tablet was prepared with a 10.5 mm round, biconvex punch tool using the direct compression method[15,17]. Initially, the sustained release blend (240 mg) was poured into the die cavity and compressed at a compression force of 2.0 KN. Consequently, the immediate release blend (140 mg) was poured over the pre-compressed sustained release blend, and by improving compression force, tablets were compressed using a compression machine.

2.3 Pre-compression Parameter

2.3.1 Bulk and Tapped Density

A powder sample was poured into a graduated measuring cylinder (100 ml). Bulk density is estimated by dividing the mass by the volume of untapped powder. After determining bulk density, place the graduated measuring cylinder into a density tester to determine tapped density. The powder was tapped 1250 times, and the tapped volume was measured. Tapped density was calculated by dividing the powder's mass by its tapped volume. The measurements were taken in triplicate and complied with United States Pharmacopoeia (USP) <616> bulk density and tapped density of powders[18-20].

2.3.2 Flowability

The powder's flowability was determined using Carr's index (CI). The CI was calculated using the following equation (1):

$$CI = 100\% \times \left(1 - \frac{Bulk\ \ Density}{Tapped\ \ Density}\right) \quad (1)$$

The measurements were taken in triplicate and complied with USP <1174> powder flow[18-20].

Table 1. Factors and responses for bilayer tablet optimisation

Factors	Level		
	Low (-1)		High (+1)
A: Methocel K100 LVCR (mg)	80.0		100.0
B: Crospovidone XL10 (mg)	2.0		4.0
C: Compression Force (KN)	8.0		12.0
Responses	Acceptable Range		Target
	Low Limit	High Limit	
R1: Nimodipine drug release at 2 h (%)	20.0	30.0	In range
R2: Nimodipine drug release at 6 h (%)	45.0	55.0	In range
R3: Quercetin Dihydrate release at 30 min (%)	80.0	---	Maximise
R4: Hardness (kg/cm^2)	5.0	15.0	In range
R5: Friability (%W/W)	---	1.0%	In range

2.3.3 Optimisation of Bilayer Tablet

The same established procedure was used in the bilayer manufacturing process. As a result, except for the main compression force, all tablet manufacturing parameters were assigned low risk by risk assessment (RA). As a result, the parameters for the bilayer tablet mentioned in Table 1 were optimised using Design of Experiments (DOE) via the Quality by Design (QbD) technique[21]. The experiments were designed using the Box-Behnken with quadratic model[22,23]. Fifteen experiments were carried out for three variables at three levels, and the central point was examined three times. The main compression force, polymer concentration, and superdisintegrating agent concentration were selected as factors, whereas nimodipine drug release at 2 h, nimodipine drug release at 6 h, quercetin dihydrate release at 30 min, hardness and friability were selected as responses.

2.3.4 Critical Quality Attributes (CQAs) for Bilayer Tablet

The CQAs for drug products were selected from the Quality Target Product Profile (QTPP) analysis for use in the initial risk assessment (Table 2), with the assumption that the CQAs must meet the stated parameters to ensure the desired quality of the drug product. CQAs were identified as physical appearance, hardness, friability, % drug content, % quercetin dihydrate content, drug dissolution, and inhibitor dissolution. The formulation variables and process stages of bilayer tablet manufacture could change the CQAs.

2.3.5 Risk Evaluation for Bilayer Tablet Formulation Factors and Process Parameters

Risk assessment determines the effects of formulation or process factors on CQAs. The risk assessment was performed for this purpose in order to identify high-risk factors for formulation and manufacturing processes that can significantly impact the CQAs of bilayer tablets and to determine risk levels. Formulation variables and main compression force were considered as high risk for various CQAs, including hardness, friability, % drug content, % quercetin dihydrate content, dissolution of the drug and inhibitor. Therefore, an updated RA for the impact of formulation variables and manufacturing processes was done to determine the concentration and parameters for the DOE study based on the QbD

Table 2. Critical quality attributes of bilayer tablet

Drug product quality characteristics		Objective	Is its CQA?	Cause
Physical charact eristics	Appearance	The shape and colour of the tablet are acceptable to the patient. There were no visible tablet defects observed.	Yes	The appearance of the tablet has no direct impact on patient safety or product quality. But it can confirm that the tablets are properly manufactured. As a result, it is a significant characteristic.
	Odor	No unpleasant odour	No	The odour and size of the tablet are negligible because they have no impact on product quality.
	Size	10.50 mm	No	
	Friability	NMT 1.0 %W/W	Yes	Friability minimises the post-market complaints related to appearance. Hence, it is an important attribute.
	Hardness	5.0-15.0 kg/cm^2	Yes	The hardness of the tablet can directly impact the dissolution of the drug and the appearance of the tablet. As a result, it is a significant characteristic.
Drug content	Nimodipine	95.0-105.0 % of label claim	Yes	Fluctuations in the drug content of tablets can directly impact the efficacy and safety of the drug product. As a result, drug product assessment is necessary.
	Quercetin dihydrate	95.0-105.0 % of label claim		
Dissolution	Nimodipine	% Drug release 20.0-30.0 % at fixed time point (2 h) % Drug release 45.0-55.0 % at fixed time point (6 h)	Yes	Dissolution directly impacts the efficacy and bioavailability of the drug. The determination of dissolution helps to identify whether a drug is continuously released or not. Dissolution can be affected by the main compression force. So, the evaluation of dissolution is required throughout the development.
	Quercetin dihydrate	% Drug release 80.0 % at fixed time point (30 m)		

technique (Table 3). Based on the risk assessments mentioned above, the following formulation variables and process parameters were identified as high risk for CQAs: (i) Polymer concentration was considered to be a significant risk factor for dissolution and hardness. (ii) Superdisintegrating agent concentration was considered a high risk for disintegration time for immediate release and dissolution. (iii) The main compression force was also considered a high risk factor in terms of appearance and hardness.

2.4 Post-compression Parameter of Bilayer Tablet

2.4.1 Physical Appearance

The appearance of the tablet was checked visually by identifying the colour variation between the two layers[24].

2.4.2 Weight Variation

The average weight of the 20 tablets was measured using an analytical balance and was within the USP guidelines[25]. Calculate the standard deviation by individually weighing the tablets.

2.4.3 Hardness

The hardness of a tablet is defined as the pressure applied to the tablet to break the tablet in the specific plane[26]. The Monsanto hardness tester was used to measure the hardness of the tablets.

2.4.4 Thickness

A vernier calliper was used for the measurement of the tablet thickness. Thickness was an essential component in recreating the appearance of the tablet and packaging.

2.4.5 Friability

The mechanical strength of a bilayer tablet was measured through this parameter. An approximately 6.5g weighted tablet was placed in the Roche friabilator and rotated at 25 rpm (100 rotations) for 4 min. The tablets were reweighed, and the percentage loss was calculated using the equation (2) shown below[24,27]:

$$\%Friability = \left(\frac{Initial\,Weight - Final\ Weight}{Initial\ Weight} \right) \times 100 \quad (2)$$

2.5 Drug Content of Nimodipine and Quercetin Dihydrate

The tablet was crushed using a motor pestle, and an accurately weighed amount of tablet powder was transferred into a 100 ml volumetric flask to dilute up to 100 ml with methanol to prepare a 1000 µg/ml solution. The prepared 1000 µg/ml concentration solution was further diluted with methanol for preparation of a 10 µg/ml solution, and the absorption of nimodipine and quercetin dihydrate was measured using an Ultraviolet (UV) visible spectrophotometer[28,29].

2.6 *In Vitro* Dissolution Study

The *in vitro* dissolution study was carried out using a paddle-type apparatus (USP apparatus II). In the vessel, 900 ml of 0.1 N Hydrochloric acid (HCl) was poured and allowed to equilibrate to a temperature of 37 °C ± 0.5 °C. The rotational speed of the paddle was set at 50 rpm. The tablet was placed in the vessel, and the sample was drawn at regular intervals to determine the concentration of nimodipine and quercetin dihydrate. After 2 h, the 0.1 N HCl was replaced with pH 6.8 phosphate buffers. At definite intervals, 5 ml of sample

Table 3. Risk evaluations for the manufacturing of bilayer tablets

Drug product CQAs	Formulation variables		Process parameter			
	Polymer concentration	Super-disintegrating agent concentration	Main compression force	Design of tooling	Force of ejection	Environment temperature and RH
Appearance	Medium	Low	High	Low	Low	Low
Friability	High	Low	High	Low	Low	Low
Hardness	High	Low	High	Low	Low	Low
% Drug content	Low	Low	Low	Low	Low	Low
Dissolution	High	High	High	Low	Low	Low

Low risk suggests that it is generally accepted and there is no need for additional investigation.
Medium indicates that the risk is acceptable, but further investigation may be necessary to reduce the risk.
A high score signifies that the risk is unacceptable and further investigation is required to reduce the risk.

was drawn, filtered, and 5 ml of receptor fluid was replaced. Suitable dilutions of receptor fluid were made and evaluated using a UV spectrophotometer[30'31].

2.7 Stability Study

An accelerated stability study was performed as per International Council for Harmonisation (ICH) recommendation, at 40℃ ± 2℃/75%RH ± 5%RH for 6 months. This investigation is intended to discover chemical degradation and changes in drugs by using exaggerated storage conditions as part of a formal stability testing procedure[32].

2.8 Pharmacokinetic Study

2.8.1 Animal Handling

The Committee, for control and supervision of experiments on animals, was authorised to use the protocol for the pharmacokinetics studies (Project proposal number: PIPR 984/2022/02/16), and the studies were carried out in accordance with Organisation for Economic Co-operation and Development (OECD) guideline 417[33]. The bilayer tablet's *in vivo* performance was compared to nimodipine sustained release tablets. A total of eight rats were separated into two groups (n = 4 per group) and fasted for 24 h prior to the start of the investigation. The formulation was administered orally by a gastric gavage tube. After dosing, blood samples were taken into a heparinised tube at 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, and 24.0 h and for extraction of plasma from the blood sample centrifuged at 13,000 rpm for 5 min at 4 °C. Plasma samples were collected and kept at -20 °C until analysis[34'35].

2.8.2 Sample Preparation

Nitrendipine was utilised as an internal standard. Briefly, 50 µL of nitrendipine and 50 µL of ethyl acetate were added to 0.5 ml of the plasma samples. After stirring for 10 min, the mixture was centrifuged for 10 min at 3000 rpm. The obtained supernatant was transferred to a clean test tube and allowed to evaporate at 40 °C under a nitrogen stream. Then the residue was dissolved in 300 µL of 65% methanol and centrifuged at 3000 rpm for 10 min. Finally, HPLC analysis was performed on the samples[34].

Table 4. Evaluation of the experimentally designed bilayer tablet

Batch number	Factors					Responses		
	A Methocel K100 LVCR (mg)	B Cros povidone XL 10 (mg)	C Com pression force (KN)	R1 Nimodipine drug release at 2 h (%)	R2 Nimodipine drug release at 6 h (%)	R3 Quercetin dihydrate drug release at 30 min (%)	R4 hardness (kg/cm^2)	R5 % friability (%w/w)
NQ1	90	4	8	33.88	74.27	88.75	8.0	0.23
NQ2	90	3	10	27.81	52.62	87.19	9.0	0.21
NQ3	90	3	10	28.32	53.41	87.32	9.0	0.21
NQ4	90	3	10	29.02	54.80	87.42	8.5	0.22
NQ5	90	2	12	19.64	48.90	88.50	9.5	0.18
NQ6	90	2	8	33.21	71.49	88.50	8.0	0.22
NQ7	90	4	12	20.64	49.20	88.42	9.5	0.16
NQ8	80	4	10	35.88	75.90	88.38	8.5	0.18
NQ9	80	3	12	30.12	67.91	88.21	9.5	0.14
NQ10	80	3	8	36.21	79.20	88.51	7.5	0.25
NQ11	80	2	10	35.52	75.72	88.34	8.5	0.20
NQ12	100	3	12	12.88	40.18	88.48	10.0	0.18
NQ13	100	2	10	14.98	46.80	88.82	9.5	0.24
NQ14	100	4	10	15.64	47.98	88.88	9.0	0.20
NQ15	100	3	8	21.67	51.98	88.98	8.0	0.29

3. Results and Discussion

3.1 Factors Affecting the Bilayer Tablet

The RA was used to optimise the polymer concentration, super disintegrating agent concentration, and main compression force of the bilayer tablet. The experiments were designed using a Box-Behnken design with a quadratic model. 15 runs were tested with independent variables and corresponding responses, which are shown in Table 4. The dependent variables (CQAs) were hardness, friability, nimodipine dissolution at 2 h and 6 h, and quercetin dihydrate dissolution at 30 m. In this study, a central composite design for three variables was adopted, with each component having three levels. This design replicated the experimental space's centre point and used a multidimensional cube to define the interesting area. The experimental design was validated using multiple statistical parameters. P-value, lack of fit, R^2 value and adjusted R^2 value are shown in Table 5, which has been obtained using statistical analysis data.

3.2 Effect on Physical Appearance of Bilayer Tablet

As shown in Figure 1, it was determined that the bilayer tablet had an acceptable appearance of a smooth surface with no layer separation or defects and was within the range of bilayer tablet characteristics used in the study.

3.3 Effect on % Drug Content, % Quercetin Dihydrate Content, Disintegrating Time, Friability, and Hardness

During several experimental runs, the % drug content of nimodipine ranged from 97.82% to 99.18%, while the % quercetin dihydrate content ranged from 98.02 % to 99.71%. The developed bilayer tablet's thickness ranged from 4.54 to 4.66 mm, and its hardness ranged from 6.5 to 11.5 kg/cm^2. Friability was found to be between 0.14 and 0.29% W/W, which is less than 1% W/W according to USP criteria. As shown in Figure 2, the hardness and friability of the bilayer tablet increased with increasing polymer concentration. Hardness and friability were inversely proportional to the main compression force. The disintegration time of the immediate release layer

Table 5. Summary of model fitting and statistical analysis

Response	Model p-value	Lack of Fit	R^2	Adjusted R^2
R1: Nimodipine rug release at 2 h (%)	<0.0001	0.0624	0.9441	0.9288
R2: Nimodipine drug release at 6 h (%)	<0.0001	0.0509	0.9121	0.8882
R3: Quercetin dihydrate release at 30 min (%)	0.0004	0.5684	0.9860	0.9607
R4: Hardness (kg/cm^2)	<0.0001	0.8661	0.9375	0.9205
R5: Friability (%W/W)	<0.0001	0.0956	0.8467	0.8049
R1 = + 26.36 – 9.07 A + 0.3363 B – 5.21 C				
R2 = + 59.36 – 13.97 A + 0.5550 B – 8.84 C				
R3 = + 87.31 + 0.2150 A + 0.0338 B – 0.1412 C + 0.0050 AB – 0.0500 AC – 0.0825 BC + 0.6487 A^2 + 0.6463 B^2 + 0.5863 C^2				
R4 = + 8.80 + 0.3125 A – 0.0625 B + 0.8750 C				
R5 = + 0.2073 + 0.0175 A – 0.0087 B – 0.0412 C				

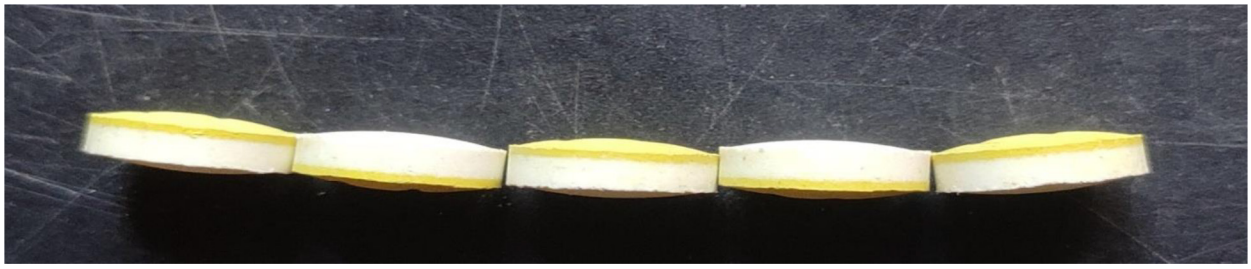


Figure 1. Physical appearance of bilayer tablet.

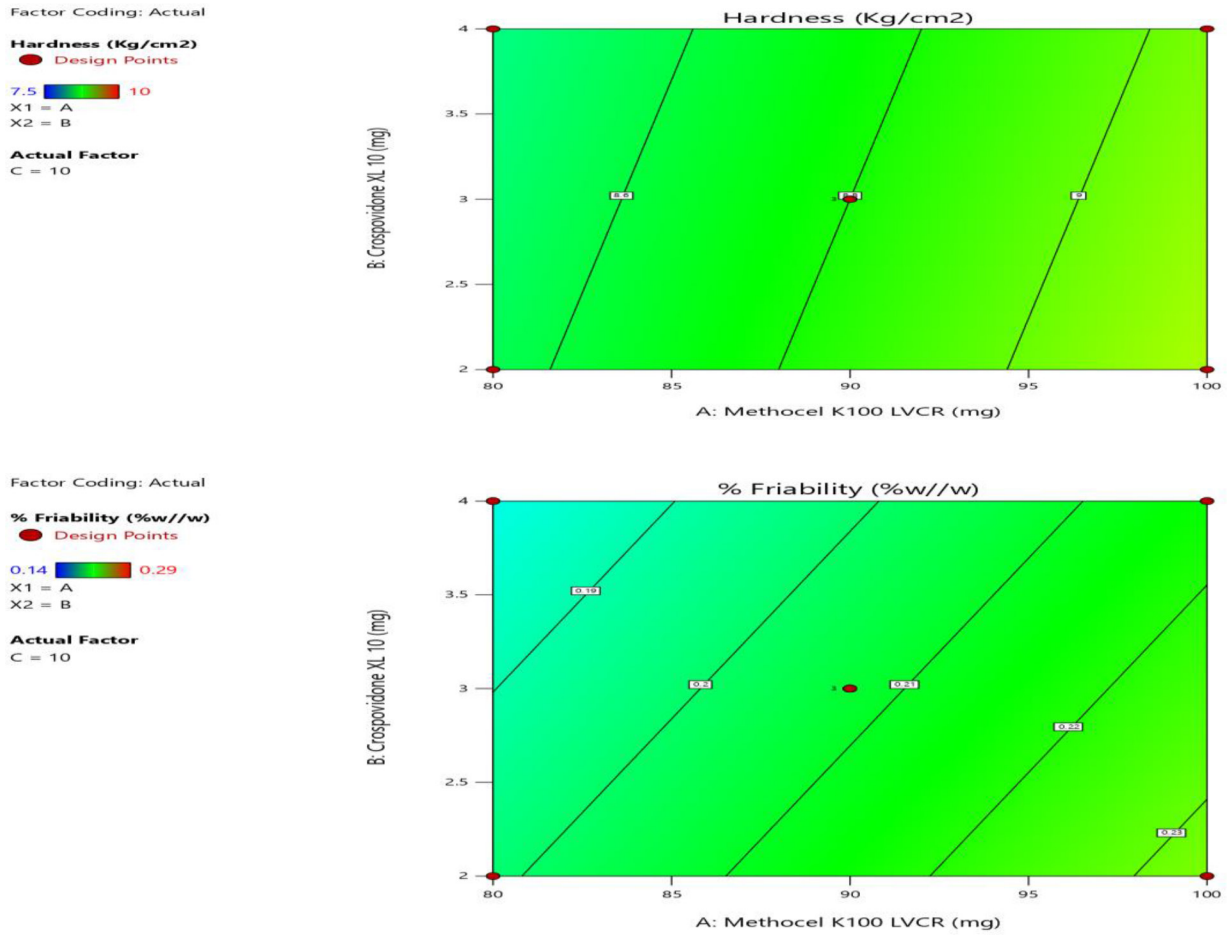


Figure 2. Influence of polymer concentration and superdisintegrating agent concentration on hardness and friability.

was observed at 12 to 38 s for informational purposes only.

3.4 Effect on Dissolution Profile

It was found that the dissolution of nimodipine from the sustained release layer is influenced by polymer concentration and the main compression force. The drug release was slowing down with an increase in the main compression force and polymer concentration. The release of quercetin dihydrate increased as the polymer concentration increased and was inversely related to the main compression force. In several studies, it has been reported that compression pressure and dissolution are inversely proportional to each other. According to the International Pharmaceutical Federation (FIP) specifications, modified release formulation must include at least three time points of dissolution. To prevent dose dumping, at least 20-30% of the labelled drug should be dissolved within the first one or two hours. The second time point should be around 50% drug release to establish the dissolution pattern. The dissolution limit should be at least 80% drug release at the third time point to achieve practically quantitative release[36]. In this case, the NQ4 batch gives satisfactory results and adheres to the requirements for dissolution at the specified time point for immediate and sustained release. As indicated in the table, the NQ4 batch provided 29.02% drug release at 2 h and 54.80% drug release at 6 h. It demonstrated that it follows the modified release and immediate release guidelines by releasing 98.74% nimodipine from the sustained release layer at 12 h and 98.12% quercetin dihydrate release from the immediate release layer at 2 h. As shown in Figure 3, the release of quercetin dihydrate from the immediate release layer slowed as the compression force increased.

3.5 Pharmacokinetic Study

Figure 4 shows the plasma levels of nimodipine after oral administration of nimodipine (30 mg/kg) to rats in the presence or absence of quercetin dihydrate, and Table 6 shows the associated pharmacokinetic characteristics. The nimodipine pharmacokinetic parameters are significantly altered by quercetin dihydrate. According to the results of pharmacokinetic studies, nimodipine with quercetin dihydrate increased the area under the plasma concentration-time curve ($AUC_{0-\infty}$), and the peak plasma concentration (C_{max}) of nimodipine was significantly higher than the control group. The cytochrome P450 (CYP3A4) enzyme is responsible for nimodipine metabolism in the liver and small intestine, and the P-glycoprotein efflux pump in the intestinal mucosa decreased nimodipine absorption[37]. The increased AUC and C_{max} of nimodipine might be attributed to the inhibition of the CYP3A4 enzyme and the P-glycoprotein efflux pump. As a result of these findings, the optimised bilayer tablet formulation indicated more therapeutic potential when compared to a nimodipine sustained-release tablet.

3.6 Stability Study

An accelerated stability study was conducted for the optimised bilayer tablet for 6 months, and samples were investigated for hardness, % drug content, % quercetin dihydrate content, and *in vitro* release of

Table 6. Pharmacokinetic parameters of an optimised bilayer tablet in rats

Pharmacokinetic parameters	Reference formulation	Test formulation
C_{max} (µg/ml)	440	790
T_{max} (h)	6.0	6.0
$t_{1/2}$ (h)	6.8	6.8
AUC (µg. h/ml)	4539.5	9473.5

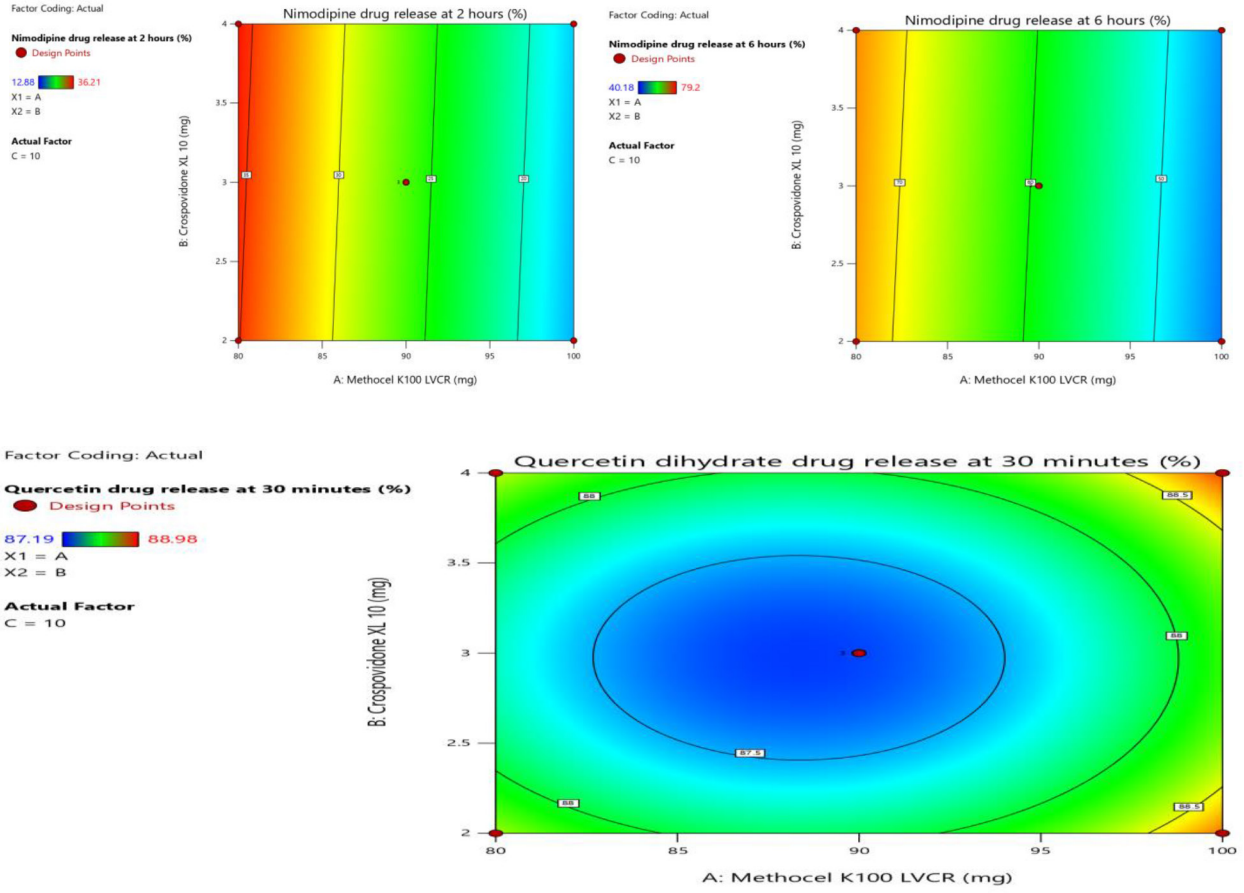


Figure 3. Effect of polymer and super disintegrating agent concentrations on nimodipine and quercetin dihydrate release.

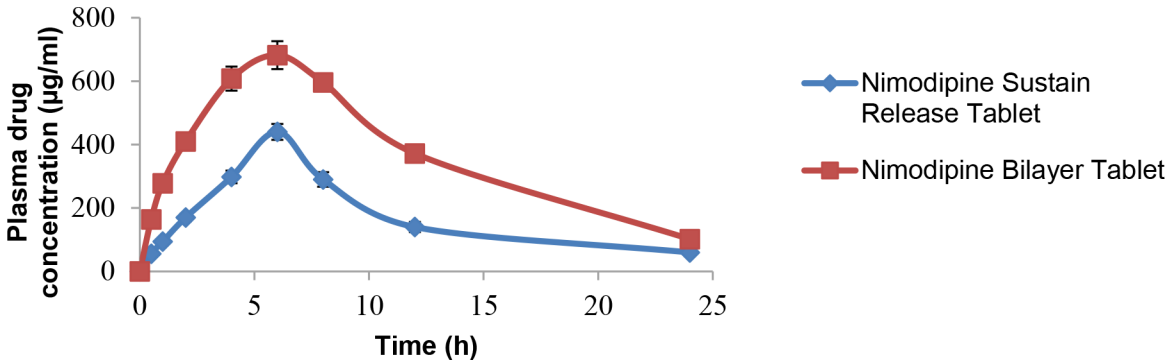


Figure 4. Plasma concentration-time profile of nimodipine in rats (n = 5).

Table 7. Results of optimum formulation stability studies

Time (month)	Evaluation parameters				
	Hardness (kg/cm^2)	Nimodipine content (%)	Quercetin dihydrate content (%)	Nimodipine CDR (%) at 12 h	Quercetin dihydrate CDR (%) at 2 h
0	9.0	98.60	99.05	98.39	97.78
1	9.0	98.42	99.19	97.52	97.08
3	9.0	98.52	99.13	97.29	96.59
6	9.0	98.56	99.08	97.72	97.19

nimodipine and quercetin dihydrate. According to the results provided in Table 7, no significant variation was detected for any of the mentioned parameters.

4. Conclusion

The present research proved the development of a nimodipine bilayer tablet formulation with quercetin dihydrate as a CYP3A4 inhibitors in the immediate release form to increase nimodipine oral bioavailability. We conducted *in vitro* drug release research to evaluate the bilayer tablet, and the drug release profile of the tablet was exhibited for 12 h. The pharmacokinetic results demonstrated that the bioavailability of the developed nimodipine bilayer tablet was higher than that of the nimodipine sustained release tablet. The main aim of the prepared formulation was achieved.

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6. Author Contributions

A Suthar: Methodology, investigation, data curation, writing-original draft preparation; D Patel: Writing-review and editing, supervision. All authors have read and agreed to the published version of the manuscript.

8. References

1. Allam AN, Gamal SSE, Naggar VF. Bioavailability: A pharmaceutical review. J Novel Drug Deliv Tech. 2011; 1(1):77-93.
2. Palrasu M, Nagini S. Cytochrome P450 structure, function and clinical significance: A review. Curr Drug Targets. 2018; 19(1):38-54. https://doi.org/10.2174/1389450118666170125144557 PMid:28124606
3. Fasinu P, Choonara YE, Khan RA, Toit L-CD, Kumar P, Ndesendo V-MK, *et al*. Flavonoids and polymer derivatives as CYP3A4 inhibitors for improved oral drug bioavailability. J Pharm Sci. 2013; 102(2):541-555. https://doi.org/10.1002/jps.23382 PMid:23188647
4. Kato H. Computational prediction of cytochrome P450 inhibition and induction. Drug Metab Pharmacokinet. 2020; 35(1):30-44. https://doi.org/10.1016/j.dmpk.2019.11.006 PMid:31902468

5. Pelkonen O, Turpeinen M, Hakkola J, Honkakoski P, Hukkanen J, Raunio H. Inhibition and induction of human cytochrome P450 enzymes: Current status. Arch Toxicol. 2008; 82(10):667-615. https://doi.org/10.1007/s00204-008-0332-8 PMid:18618097
6. Ho PC, Saville DJ, Wanwimolruk S. Inhibition of human CYP3A4 activity by grapefruit flavonoids, furanocoumarins and related compounds. J Pharm Pharm Sci. 2001; 4(3):217-227.
7. Sica DA. Interaction of grapefruit juice and calcium channel blockers. Am J Hypertens. 2006; 19(7):763-773. https://doi.org/10.1016/j.amjhyper.2005.11.003 PMid:16814135
8. Zhao Y, Xin T, Ye T, Xinggang Y, Weisan P. Solid dispersion in the development of a nimodipine delayed-release tablet formulation. Asian J Pharm Sci. 2014; 9(1):35-41. https://doi.org/10.1016/j.ajps.2013.11.006
9. Liu X, Shang W, Liqing C, Dong Z, Yinghua S, Lu X, *et al*. A two-step strategy to design high bioavailable controlled-release nimodipine tablets: The push-pull osmotic pump in combination with the micronization/solid dispersion techniques. Int J Pharm. 2014; 461(1-2):529-539. https://doi.org/10.1016/j.ijpharm.2013.12.023 PMid:24374219
10. Huang S, Haung Z, Fu Z, Shi Y, Dai Q, Tang S, *et al*. A novel drug delivery carrier comprised of nimodipine drug solution and a nanosuspension: Preparation, characterization, *in vitro*, and *in vivo* studies. Int J Nanomed. 2020; 15:1161-1172. https://doi.org/10.2147/IJN.S226591 PMid:32110014 PMCid:PMC7036601
11. Ghareeb MM, Nehmah AJ. Formulation and characterization of nimodipine nanoemulsion as ampoule for oral rout. Int J Pharm Sci Res. 2017; 8(2):591-602.
12. Admassu A, Ilgaz A, Omar S, Niranjan K, Alberto MC. Review of bilayer tablet technology. Int J Pharm. 2014; 461(1-2):549-558. https://doi.org/10.1016/j.ijpharm.2013.12.028 PMid:24370841
13. Dong HW, Heejun P, Ha ES, Kim HH, Jang SW, Kim MS. Optimization of bilayer tablet manufacturing process for fixed dose combination of sustained release high-dose drug and immediate release low-dose drug based on Quality by Design (QbD). Int J Pharm. 2021; 605:1-19. https://doi.org/10.1016/j.ijpharm.2021.120838 PMid:34197909
14. Akhtar M, Jamshaid M, Zaman M, Mirza AZ. Bilayer tablets: A developing novel drug delivery system. J Drug Deliv Sci Technol. 2020; 60:1-10. https://doi.org/10.1016/j.jddst.2020.102079
15. Fu Q, Su X, Hou Y, Li M, Li J, Sun J, *et al*. Once-daily amoxicillin immediate- and extended-release bilayer tablets. Pow Technol. 2016; 301:405-411. https://doi.org/10.1016/j.powtec.2016.06.030
16. Nikghalb LA, Singh G, Singh G, Kahkeshan KF. Solid dispersion: Methods and polymers to increase the solubility of poorly soluble drugs. J App Pharm Sci. 2012; 2(10):170-175.
17. Navamanisubramanian R, Nerella R, Duraipandian C, Seetharaman S. Quality by design approach for optimization of repaglinide buccan tablets using Box-Behnken design. Future J Pharm Sci. 2018; 4:265-272. https://doi.org/10.1016/j.fjps.2018.10.002
18. Shah RB, Tawakkul MA, Khan MA. Comparative evaluation of flow for pharmaceutical powders and granules. AAPS Pharmscitech. 2008; 9(1):250-258. https://doi.org/10.1208/s12249-008-9046-8 PMid:18446489 PMCid:PMC2976911
19. United States Pharmacopeia (USP). United States Pharmacopeia (USP) 32, General Chapter <1174> Powder Flow. The United States Pharmacopeial Convention, Inc., Rockville, MD; 2019a.
20. United States Pharmacopeia (USP). United States Pharmacopeia (USP) 32, General Chapter <616> Bulk Density and Tapped Density of Powders. The United States Pharmacopeial Convention, Inc., Rockville, MD; 2019b.
21. Fakuda IM, Pinto CFF, Moreira CDS, Saviano AM, Lourenco FR. Design Of Experiments (DOE) applied to pharmaceutical and analytical Quality by Design (QbD). Braz J Pharm Sci. 2018; 54:1-16. https://doi.org/10.1590/s2175-97902018000001006
22. Roy H, Rahman SA. Box-Behnken design for optimization of formulation variables for fast dissolving tablet of urapidil. Asian J Pharm. 2018; 12(3):S946-S954.
23. Monton C, Wunnakup T, Suksaeree J, Charoenchai L. Impact of compression force, croscarmellose sodium, and microcrystalline cellulose on black pepper extract tablet properties based on design of experiments approach. Sci Pharm. 2023; 91(30):1-15. https://doi.org/10.3390/scipharm91030030
24. Balaji M, Mudavath H, Nakkala RK, Jhawat V, Rawat P, Alam A *et al*. Formulation and evaluation of gastro-retentive floating bilayer tablet for the treatment of hypertension. Heliyon 2020; 6(11):1-12. https://doi.org/10.1016/j.heliyon.2020.e05459 PMid:33241144 PMCid:PMC7674293
25. United States Pharmacopeia (USP). United States Pharmacopeia (USP) 32, General Chapter <905> Uniformity of Dosage Units. The United States Pharmacopeial Convention, Inc., Rockville, MD; 2019c.
26. United States Pharmacopeia (USP). United States Pharmacopeia (USP) 32, General Chapter <1217> Tablet Breaking Force. The United States Pharmacopeial Convention, Inc., Rockville, MD; 2019d.
27. United States Pharmacopeia (USP). United States Pharmacopeia (USP) 32, General Chapter <1216> Tablet Friability. The United States Pharmacopeial Convention, Inc., Rockville, MD; 2019e.
28. Rathinaraj BS, Rajveer C, Choudhury PK, Sheshrao GB, Shinde GV. Studies on dissolution behaviour of sustained release solid dispersions of nimodipine. Int J Pharm Sci Rev Res. 2010; 3(1):77-82.

29. He Y, He Z, He F, Wan H. Determination of quercetin, plumbagin and total flavonoids in *Drosera peltata* Smith var. glabrata Y.Z. Ruan. Pharmacogn Mag. 2012; 8(32):263-267. https://doi.org/10.4103/0973-1296.103649 PMid:24082628 PMCid:PMC3785162
30. Dara H, Reddy NY. Formulation of time dependent controlled release tablet of nimodipine and its evaluation using linear regression analysis. J Pharm Res. 2015; 4(1):1-8.
31. Chaudhari S, Hande S, Nikam S. Formulation and evaluation of allopurinol and quercetin pulsatile tablet using central composite design. Eur Chem Bull. 2023; 12(10):7510-7526.
32. ICH Guidelines. Stability testing of new drug substances and products; 2003. Available from: https://www.ich.org/page/quality-guidelines
33. OECD guideline for the testing of chemicals. Test no. 417: Toxicokinetics; 2010. Available from: https://www.oecd.org/env/test-no-417-toxicokinetics-9789264070882-en.htm
34. Choi JS, Burm JP. Enhanced nimodipine bioavailability after oral administration of nimodipine with morin, a flavonoid, in rabbits. Arch Pharm Res. 2006; 29(4):333-338. https://doi.org/10.1007/BF02968580 PMid:16681041
35. Choi JS, Piao YJ, Kang KW. Effects of quercetin on the bioavailability of doxorubicin in rats: Role of CYP3A4 and P-gp inhibition by quercetin. Arch Pharm Res. 2011; 34(4):607-613. https://doi.org/10.1007/s12272-011-0411-x PMid:21544726
36. Friedel HD, Brown CK, Barker AR, Sperry DC, Buhse LF, Keitel S, *et al*. FIP guidelines for dissolution testing of solid oral products. J Pharm Sci. 2018; 107(12):2995-3002. https://doi.org/10.1016/j.xphs.2018.08.007 PMid:30148985
37. Cho YA, Choi JS, Burm JP. Effects of the antioxidant baicalein on the pharmacokinetics of nimodipine in rats: A possible role of P-glycoprotein and CYP3A4 inhibition by baicalein. Pharmacol Rep. 2011;63(4):1066-1073. https://doi.org/10.1016/S1734-1140(11)70624-7 PMid:22001996