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ORIGINAL ARTICLE

In vitro and in vivo evaluation of hydrophilic and hydrophobic polymers-based nicorandil-loaded peroral tablet compared with its once-daily commercial sustained-release tablet

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Abstract

Context: Hydrophilic and hydrophobic polymer-based nicorandil (10 mg)-loaded peroral tablets were prepared using the wet granulation technique. The influence of varying amounts of hydroxypropyl methylcellulose (HPMC) (30–50 mg), ethylcellulose (2–4 mg), microcrystalline cellulose (5–20 mg) and Aerosil® (5–12 mg) in conjunction with the constant amounts (3 mg) of glidant and lubricant (magnesium stearate and talc) on the in vitro performances of the tablets (hardness, friability, weight variation, thickness uniformity, drug content, and drug release behavior) were investigated. *Objective*: The objectives of this study were (i) to select a nicorandil-loaded peroral tablet that matched the in vitro dissolution profile of once-daily commercial sustained-release tablet, and (ii) to compare the in vivo sustaining/controlling efficacy of the selected peroral tablet with that of its commercial counterparts. *Results and Discussion*: Because the nicorandil (10 mg)-loaded tablet prepared based on F-IX composition (50 mg HPMC, 4 mg ethylcellulose, 10 mg MCC and 3 mg glidant and lubricant) showed a release profile comparable to that of the Nikoran® OD SR tablet release profile, the tablet with this composition was considered to be the optimized/selected formulation and, therefore, was subjected to stability study and in vivo study in rabbits. Despite of the higher C_{max} and *AUC* values obtained with the optimized tablet, there was no sign of difference between the optimized- and Nikoran® OD SR- tablets following a single-dose crossover oral administration into rabbit. *Conclusion*: The optimized tablet could be used as an alternative to the commercial once-daily tablet.

Key words: Ethylcellulose, in vivo bioavailability, nicorandil, peroral tablet

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Introduction

Nicorandil, a nicotinamide derivative with a nitrate side chain, belongs to the class of potassium channel activators that are characterized by their arterio- and veno-dilating properties and represents a novel type of compound in the treatment of angina pectoris[1]. Indeed, an investigation that studied the impact of nicorandil in angina demonstrated significant improvement in patients with coronary heart disease and nonfatal myocardial infarction[2]. However, when administered through oral route, this drug is subjected to hepatic first-pass metabolism and therefore, it's systemic bioavailability is about 75% only. In addition, because of its short elimination half-life (1 hour), this drug has to be administered frequently (5–40 mg taken 2–4 times a day), which can lead to patient noncompliance.

To reduce the frequency of administration and to improve patient compliance, a once-daily sustained-release formulation of nicorandil is desirable. Because nicorandil is freely soluble in water, a judicious selection of release-retarding excipients is necessary to achieve a constant in vivo input rate of the drug. The use of a

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combination of hydrophilic and hydrophobic polymers to prepare the peroral tablets is a well-known practice for achieving a desired drug release pattern over a prolonged time period. As far as the nicorandil peroral tablets preparation is concerned, Reddy et al.[3] have already shown the synergistic effect when using the hydrophilic polymers such as hydroxypropyl methylcellulose (HPMC), sodium carboxy-methylcellulose, polyvinylpyrrolidone and sodium alginate, in combination with the hydrophobic polymers such as ethylcellulose, Eudragit RL-100 and Eudragit RS-100 in a specific proportion to achieve a drug release over 24 hours. These authors used 80 mg of nicorandil to prepare the once-daily sustained-release tablet. However, commercially available nicorandil sustained-release tablet contains only 10 mg of the drug (Nikoran® OD SR, Torrent Labs (P) Ltd., Ahmedabad, India).

The peroral tablets investigated in this study, however, differ significantly from the once-daily peroral tablets developed by Reddy et al.[3] in several aspects. In the interest of increasing the uniform supply of drug to the hypertensive patient or the patient with angina and at the same time, reducing the number of polymers used to prepare such peroral tablets, only the HPMC in conjunction with the ethylcellulose polymer was selected. The drug amount was also reduced from 80 mg to 10 mg for all of the developed tablets. HPMC is mixed alkyl hydroxyalkyl cellulose ether containing methoxyl and hydroxypropyl groups. The hydration rate of HPMC depends on the nature of these substituents and specifically, the hydration rate of HPMC increases with an increase in the hydroxypropyl content. In addition, the solubility of HPMC is pH independent[4]. Most importantly, HPMC K4M was used because it forms a strong viscous gel on contact with aqueous media, which may be useful in sustained delivery of highly water-soluble drugs. Furthermore, to control the drug release in the initial hours, besides making the formulation to release a high cumulative amount of drug at the end of 24 hours, ethylcellulose was the only one hydrophobic polymer to be included in the preparation of the nicorandil-loaded peroral tablets.

The major objectives of the current study were (i) to prepare hydrophilic and hydrophobic polymer-based nicorandil (10 mg)-loaded peroral tablets using a wet granulation technique, (ii) to select a peroral tablet that matched the in vitro dissolution profile of once-daily commercial sustained-release tablet, and (iii) to compare the in vivo sustaining/controlling efficacy of the selected peroral tablet with that of its commercial counterparts. Specifically, the influence of varying amounts of HPMC (30–50 mg), ethylcellulose (2–4 mg), microcrystalline cellulose (5–20 mg) and Aerosil® (5–12 mg) in conjunction with the constant amounts (3 mg) of glidant and lubricant (magnesium stearate and talc) on the in vitro performances of the tablets (hardness, friability, weight variation, thickness uniformity, drug content, and drug release behavior) were also investigated.

Materials and methods

Materials

Nicorandil was a gift from Wokhardt Pharmaceuticals (Mumbai, India). HPMC K4M was purchased from BDH Chemicals (Mumbai, India). Ethylcellulose (14 cps) and microcrystalline cellulose were purchased from SD Fine Chemicals Ltd (Mumbai, India). Aerosil® was procured from R.K. Chemicals (Mumbai, India). Magnesium stearate and talc were obtained from Scientific India (Akola, India). All the other chemicals used were of high analytical grade.

Methods

Preparation of peroral tablets

Different tablet formulations were prepared using wet granulation technique (Formulation FI-FX, Table 1). All the powders were passed through ASTM (American Society of Testing and Materials) 80 mesh. Required quantities of drug and polymer were mixed thoroughly, and a sufficient volume of granulating agent (dichloromethane solution of ethylcellulose) was added slowly. After enough cohesiveness was obtained, the mass was sieved through 22/44 mesh. The granules were dried at 40°C for 12 hours and thereafter kept in a desiccator for 12 hours at room temperature. Once dry, the granules retained on 44 mesh were mixed with 15% of fines (granules that passed through 44 mesh). Talc and magnesium

Table 1. Composition of nicorandil-loaded tablets (80 mg).

Ingredients	Formulation codes and compositions									
(mg)	F-I	F-II	F-III	F-IV	F-V	F-VI	F-VII	F-VIII	F-IX	F-X
Nicorandil	10	10	10	10	10	10	10	10	10	10
HPMC	40	42	40	40	30	32	40	50	50	50
Ethylcellulose	na	na	2	3	3	2	4	4	4	4
Microcrystalline cellulose	12	10	10	10	20	20	10	5	10	na
Aerosil®	12	12	12	11	11	10	10	5	na	10
Magnesium stearateTalc	33	33	33	33	33	33	33	33	33	33
Dichloromethane	qs	qs	qs	qs	qs	qs	qs	qs	qs	qs

HPMC, hydroxypropyl methylcellulose; na, not added; qs, quantity sufficient.

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stearate were finally added as glidant and lubricant. The practical weight of tablets was calculated based on the drug content of the granulations, and the tablets were compressed (6 mm diameter, oval shape punches) using a single-punch tablet compression machine (Cadmach, Ahmedabad, India). The total weight of each tablet was kept constant at 80 mg, which includes the weights of nicorandil and other pharmaceutical ingredients as listed in Table 1. The prepared tablets were packed with following specification: 10 tablets were packed in Alu-Alu Blister packing. Before the compression, the granules were evaluated for several tests.

Evaluation of granules

Angle of repose. The angle of repose of granules was determined by the funnel method and the angle of repose was calculated using the following equation[5]:

$$\tan\theta = h/r \tag{1}$$

where h and r are the height and radius of the powder cone.

Bulk density. Both loose bulk density (LBD) and tapped bulk density (TBD) were determined according to the standard procedure and formulas given by Shah et al.[6]:

$$\text{LBD} = \text{weight of the powder} / \text{volume of packing} \tag{2}$$

$$\text{TBD} = \text{weight of the powder} / \text{tapped volume of the packing} \tag{3}$$

Compressibility index. The compressibility index of the granules was determined by Carr's compressibility index[7]:

$$\text{Carr's index } (\%) = [(\text{TBD} - \text{LBD}) \times 100]/\text{TBD} \tag{4}$$

Total porosity. Total porosity was determined by measuring the volume occupied by a selected weight of a powder (V_{bulk}) and the true volume of granules (the space occupied by the powder exclusive of spaces greater than the intermolecular space, V)[8]:

$$\text{Porosity } (\%) = [V_{\text{bulk}} - (V/V_{\text{bulk}})] \times 100 \tag{5}$$

Drug content. An accurately weighed amount of powdered nicorandil granules (50 mg) was extracted with water and the solution was filtered through 0.45 micromembrane (Nunc, New Delhi, India). After suitable dilution with water, the absorbance was measured at 262 nm in a diode array UV-visible spectrophotometer (Hewlett-Packard, Agilent Technologies) fitted with Chemstation software (Agilent Technologies) against water as blank.

Evaluation of tablets

Physical characterizations. Although the hardness (Pfizer hardness tester) and friability (Roche Friabilator) tests were conducted according to the procedure mentioned in the *United States Pharmacopoeia*[9], the weight variation (Precisa, XB-600 MC, Geneva, Switzerland) and thickness uniformity tests were performed following the method described in the *Indian Pharmacopoeia*[10]. The following formula was used to calculate the percentage friability of the peroral tablets.

$$\%\, F = [1 - (W/W_0)] \times 100 \tag{6}$$

where $\%\, F$ = friability in percentage, W_0 = initial weight of tablet, W = weight of tablet after the friability test.

In vitro dissolution. A previously reported method by Adelbary and Tadros[11] for nicorandil extended-release matrix tablets was simply followed in the current in vitro dissolution studies. The dissolution tests were performed using the basket method (Apparatus 1) in a USP Dissolution Test Apparatus (Campbell Electronics, Mumbai, India) at 37 ± 0.5°C. The baskets were rotated at a speed of 50 rpm. The prepared peroral tablets, in addition to commercially available Nikoran® OD SR tablet, were placed in the baskets and then submerged into 900 mL of 0.1 N HCl solution (pH 1.2) for 2 hours. These were then transferred to 900 mL of phosphate buffer pH 7.4 from 3 to 24 hours. Aliquots of 5 mL were withdrawn at different time intervals and filtered through cellulose acetate membrane (0.45 μm). At each time of withdrawal, 5 mL of fresh corresponding buffer solution (pH 1.2 or pH 7.4) was replaced into the dissolution flask. After suitable dilution with water, the content of nicorandil in each of the collected sample was determined spectrophotometrically at a wavelength of 262 nm using water as blank, as mentioned before. The amount/percentage of drug released at each time point was calculated after taking the actual drug content of the formulations into consideration, and the cumulative amount/percent was then determined. The dissolution tests were conducted in triplicates and the mean values were plotted versus time.

Stability studies of the tablets. Stability studies were conducted on the selected/optimized tablet (prepared based on F-IX composition) to assess drug stability with respect to physical appearance and drug degradation after storing the alu-alu packed tablet in drug stability testing chambers (Campbell Electronics) at two different conditions for up to 12 weeks. Typical stresses are 25°C at 75% relative humidity to represent temperate conditions and 38°C at 90% relative humidity to represent tropical conditions[12]. Drug stability testing chambers containing a saturated aqueous solution in contact with an excess of a definite solid phase at a given temperature to maintain constant humidity in an enclosed space were used. The saturated salt solutions and temperatures used in this study were NaCl at 25°C and ZnSO4.

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7H2O at 38°C to represent 75% and 90 ± 5% relative humidity, respectively.

Thin-layer chromatography

Qualitative thin-layer chromatography (TLC) was performed using 10 × 10 cm precoated silica gel 60 aluminum-backed TLC sheets (Merck (India) Limited, Mumbai, India) with layer thickness of 0.25 mm. A dichloromethane solution of an accurately weighed amount of nicorandil and an equivalent amount of nicorandil present in the selected/optimized tablet and commercial sustained-release once-daily tablet was applied, using a sample applicator (Camag Nanomet 11 with 1-µl capillary and holder, CAMAG, Berlin, Germany), directly onto the TLC sheet, leaving 2 cm between this area and the edge. The sheet was developed with a chloroform-methanol-2.61 M NH_3 (2.5:1.5:0.4v/v/v) system[13] system in a Camag chamber for 20 minutes. After development, the sheet was air-dried and spot was detected in iodine chamber. From the following relationship, the R_f value was calculated and the experiment was duplicated under identical conditions.

$$R_f = \text{Distance traveled by samples} / \text{Distance traveled by solvent front} \quad (7)$$

In vivo bioavailability study

The bioavailability study was carried out on white albino rabbits (2–2.5 kg) in a randomized, single-dose cross-over design. The animal experiments are conducted in full compliance with the Institutional Review Board resolutions on the use of animals in research, Arulmigu Kalasalingam College of Pharmacy, Krishnankoil, and C. L. Baid Metha College of pharmacy, Chennai, India (Ethical committee research numbers: MD-58.17-8 & MD-158.21-9, respectively).

The rabbits were divided into two equal groups (designated as Group 1 and 2, $n = 3$). In the first experiment, Group 1 received the commercial sustained-release tablet whereas the other group (Group 2) allocated the selected/optimized tablet formulation. A dose of 80 µg/kg body weight was converted into animal dose and administered to each group with the help of a standard technique used for the administration of solid oral dosage form to rabbits. The rabbits were freely allowed to take their usual food and water ad labium throughout the experiment. Blood sample (2 mL) was directly withdrawn from the ear vein at different time intervals of 0, 1, 2, 4, 6, 8, and 24 hours after oral administration. Blood samples were transferred into vials containing heparin solution as anticoagulant. The blood samples were centrifuged at 2500 rpm (i.e., 700 × *g* with a table modle centrifuge) for 10 minutes to separate the plasma. The separated plasma were collected in Eppendorf tubes and stored in a deep freezer until the sample was analyzed using HPLC method.

After a wash out period of 15 days, the second experiment was initiated by changing the nicorandil dosage forms among the groups. The single oral dose of the selected/optimized tablet formulation was allocated to rabbits in Group 1 whereas the rabbits in Group 2 received the single oral doses of commercial sustained-release tablet. Sampling time points and blood sample treatments were the same as those in the first experiment.

Estimation of nicorandil in plasma

In this study, plasma nicorandil concentration in rabbits was determined using an HPLC method of Ojha and Pargal[14] as adopted by Adelbary and Tadros[11].

The Kontron HPLC system consisted of a Kontron 420 pump, a UV detector 332, and an autosampler 360 (Kontron Instruments, Zurich, Switzerland) equipped with a Rheodyne sample injector with a 50-µl sample loop. The column was a reverse-phase microparticulate Bondapak C18, particle size 10 m, 25 cm × 4.6 mm (Waters Corp., Milford, MA, USA). The flow rate was 0.8 mL/min. Peak areas were determined with a C-R6A chromatopac Shimadzu integrator. The mobile phase consisted of acetonitrile : phosphate buffer (40:60, v/v) (premixed). The mobile phase was filtered through a 0.22 µm-sized membrane and degassed using an ultrasonicator. Metformin (obtained from Central Drug Laboratory, Calcutta, India) was adopted as an internal standard.

Primary standard solutions (100 µg/mL) of nicorandil and metformin were prepared in methanol. Blank plasma samples were spiked with the nicorandil stock solution to contain 5–300 ng/mL. To each tube, 1 mL of metformin stock solution was added. Aliquots of 100 µL of spiked plasma samples were mixed with 250 µL of acetonitrile and the mixtures were vortexed for 30 seconds and centrifuged at 3000 × *g* for 10 minutes. A portion of the supernatants were transferred to HPLC tubes and 50 µL was injected onto the HPLC column. The eluent was detected by the UV detector at 260 nm, and the sensitivity range of the detector was set at 0.0001 AUFS (absorption units full scale). Under the described conditions, the retention time of nicorandil and metformin were 4.8 and 7.2 minutes, respectively.

A standard curve was constructed by plotting the peak area ratio of nicorandil to metformin against nicorandil concentrations in plasma. Ratios were fitted by least-squares regression to a linear calibration model. A good linear relationship ($r^2 = 0.9996$) was observed between the peak area ratio and the plasma concentration of nicorandil in the range of 5–300 ng/mL. The lower detection limit was determined to be 5 ng/mL. The interday and intraday variation was found to be less than 2.8% coefficient of variation for the HPLC method. Recovery of nicorandil was found to be 96.8–99.3%. All assays were performed in triplicate.

The plasma samples derived from the six rabbits after receiving the single oral doses of the selected/optimized

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tablet formulation and commercial sustained-release tablet were assayed as described above without the addition of nicorandil. The unknown sample concentration was calculated from the following formula:

$$Q = [(R \pm B)/A] \times \text{Dilution Factor} \quad (8)$$

where Q is nicorandil concentration, R is the peak area ratio (drug/internal standard), A is the slope of the standard curve, and B is the y-intercept.

Pharmacokinetic parameters

The pharmacokinetic parameters of the two experiments were estimated for each rabbit by using a computer program; WinNonlin® (version 1.5, Scientific consulting, Inc., Cary, NC, USA), adopting noncompartmental analysis. The maximum drug concentration (C_{max}) and the time to reach C_{max} (T_{max}) were obtained from the individual plasma concentration–time curves. The area under the curve from 0 to α, ($AUC_{(0-\alpha)}$), was calculated using the trapezoidal rule method.

Statistical analysis

Results are expressed as mean values of 6 rabbits ± SD. A two-way analysis of variance was performed for the untransformed data derived from the pharmacokinetic Parameters, C_{max}, T_{max}, and $AUC_{(0-\alpha)}$, using the software SPSS 14.0 (SPSS Inc., Chicago, IL, USA), to investigate the statistical significance among groups. The level of significance was α = 0.05. A P-value < 0.05 was considered statistically significant.

Results

Granule characterizations

The nicorandil-loaded granules of different formulations were evaluated for angle of repose, LBD, TBD, compressibility index, total porosity, and drug content (Table 2). The results of angle of repose and compressibility index (%) ranged from 21.45 ± 0.02 to 30.09 ± 0.01, and 13.11 ± 0.03 to 18.55 ± 0.02, respectively. For LBD and TBD, the obtained values were confined from 0.294 ± 0.03 to 0.640 ± 0.01 and 0.346 ± 0.03 to 0.745 ± 0.02, respectively. Whereas the percentage porosity of the granules ranged from 25.53 ± 0.02 to 35.92 ± 0.03, the drug content in a weighed amount of granules of all formulations was found to be from 90.69 ± 0.05 to 99.87 ± 0.04%.

Tablet characterizations

The thickness of the nicorandil-loaded peroral tablets ranged from 2.96 ± 0.02 to 3.02 ± 0.02 mm. The average weight percentage deviation of 20 tablets of each formula was less than ± 5%. The hardness and percentage friability of the tablets of all batches ranged from 5.5 ± 0.29 to 6.7 ± 0.15 kg/cm^2 and 0.239 ± 0.03 to 0.501 ± 0.05%, respectively (Table 3). Note that the percentage friability value obtained with tablet prepared based on F-IX composition was lesser than the value obtained with tablet prepared based on F-X composition (0.239 ± 0.03 vs. 0.337 ± 0.04). The thickness, hardness, and drug content values of the commercial sustained-release tablet were 7.4 ± 0.06 mm, 4.7 ± 0.32 kg/cm^2, and 98.69 ± 0.10%, respectively. With an exception of tablets prepared based on F-I and F-II compositions/formula, the drug content values for the remaining tablets were around 98.69 ± 0.06 to 99.87 ± 0.08%. Note that the tablets prepared based on F-I and F-II compositions contained the drug content values of only 91.69 ± 1.15% and 92.46 ± 0.05%, respectively. This indicated that these two tablets (F-I and F-II) did not pass the acceptable drug content % variation limit, i.e., ±5%.

In vitro drug release

Figure 1 shows in vitro release characteristics of developed nicorandil-loaded tablets and commercial once-daily nicorandil tablet in pH 1.2 (up to 2 hours) and pH 7.4 buffer (from 3 to 24 hours) solutions. The tablets prepared based on F-I to F-III compositions showed a rapid drug release that ranged from 30.10 ± 2.61 to 57.18 ± 1.88 % within 2 hours of dissolution in pH 1.2. However, the tablets that were prepared based on F-IV to F-X

Table 2. Physicochemical properties of nicorandil-loaded granules.

Formulation code	Angle of repose (°), $\theta = \tan^{-1}h/r$	Loose bulk density (LBD) (g/mL)	Tapped bulk density (TBD) (g/mL)	Compressibility index (%)	Total porosity (%)	Drug content (%)
F-I	28.56 (±0.03)	0.562 (±0.03)	0.690 (±0.03)	18.55 (±0.02)	26.92 (±0.03)	90.69 (±0.05)
F-II	30.09 (±0.01)	0.640 (±0.01)	0.745 (±0.02)	14.09 (±0.03)	35.92 (±0.03)	92.56 (±0.04)
F-III	25.46 (±0.02)	0.305 (±0.03)	0.351 (±0.03)	13.11 (±0.03)	27.12 (±0.03)	98.75 (±0.03)
F-IV	24.98 (±0.01)	0.317 (±0.02)	0.367 (±0.01)	13.63 (±0.01)	28.02 (±0.01)	99.32 (±0.01)
F-V	24.23 (±0.03)	0.310 (±0.02)	0.360 (±0.02)	13.89 (±0.02)	30.18 (±0.04)	98.55 (±0.03)
F-VI	25.09 (±0.01)	0.318 (±0.01)	0.378 (±0.03)	15.87 (±0.03)	25.53 (±0.02)	99.87 (±0.04)
F-VII	23.78 (±0.02)	0.294 (±0.03)	0.346 (±0.03)	15.02 (±0.03)	29.32 (±0.03)	98.36 (±0.03)
F-VIII	25.56 (±0.01)	0.307 (±0.03)	0.360 (±0.03)	14.72 (±0.03)	32.15 (±0.01)	98.69 (±0.01)
F-IX	22.98 (±0.03)	0.311 (±0.03)	0.368 (±0.01)	15.21 (±0.02)	33.54 (±0.03)	99.12 (±0.02)
F-X	21.45 (±0.02)	0.302 (±0.03)	0.356 (±0.02)	15.17 (±0.02)	32.54 (±0.01)	99.08 (±0.01)

Figures in the parenthesis indicate standard deviation (±SD, n = 5).

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Table 3. Physicochemical properties of the nicorandil-loaded peroral tablets (80 mg) and commercial sustained-release tablet (Nikoran® OD SR).

Formulation code	Average thickness (mm) (mean ± SD, $n = 5$)	Hardness (kg/cm^2) (mean ± SD, $n = 6$)	Deviation in weight variation test (%) (mean ± SD, $n = 20$)	Percentage friability (%) (mean ± SD, $n = 6$)	Drug content (%)
F-I	3.01 (±0.06)	5.8 (±0.32)	3.895 (±0.03)	0.310 (±0.06)	91.69 (±1.15)
F-II	3.06 (±0.01)	5.5 (±0.29)	2.658 (±0.04)	0.492 (±0.04)	92.46 (±0.05)
F-III	3.0 (±0.02)	6.5 (±0.27)	2.264 (±0.02)	0.501 (±0.05)	98.70 (±0.08)
F-IV	2.97 (±0.02)	6.5 (±0.49)	4.025 (±0.01)	0.433 (±0.06)	99.52 (±0.11)
F-V	2.96 (±0.02)	6.4 (±0.24)	3.026 (±0.03)	0.478 (±0.05)	99.55 (±0.09)
F-VI	3.00 (±0.06)	6.5 (±0.21)	3.895 (±0.03)	0.242 (±0.04)	99.87 (±0.08)
F-VII	3.02 (±0.02)	6.4 (±0.22)	2.987 (±0.02)	0.414 (±0.05)	98.82 (±0.05)
F-VIII	3.0 (±0.08)	6.7 (±0.15)	3.263 (±0.05)	0.417 (±0.02)	98.69 (±0.06)
F-IX	3.0 (±0.03)	6.4 (±0.24)	4.125 (±0.13)	0.239 (±0.03)	99.52 (±0.12)
F-X	2.97 (±0.02)	6.5 (±0.20)	3.915 (±0.06)	0.337 (±0.04)	99.32 (±0.09)
Commercial tablet	7.4 (±0.06)	4.7 (±0.32)	3.991 (±0.01)	ND	98.69 (±0.10)

ND-not determined.

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compositions showed the nicorandil release in the range between 16.78 ± 2.55 and 37.89 ± 1.09% in pH 1.2 for up to 2 hours dissolution time period. Note that the lowest cumulative % release (16.78 ± 2.55%) was observed with the tablet prepared based on F-IX composition and the highest cumulative % release (37.89 ± 1.09%) was being the tablet prepared based on F-V composition. Interestingly, the commercial sustained-release once-daily tablet was also shown the lowest and highest cumulative % release values (15.72 ± 2.15 and 19.62 ± 1.75%, respectively) that were closer to the lowest and highest cumulative % release values (10.65 ± 0.95 and 16.78 ± 2.55%, respectively) obtained with the tablet prepared based on F-IX composition.

When changing the medium to basic side, that is, phosphate buffer pH 7.4 solution, all the developed tablets (based on F-I to F-X compositions) together with the commercial tablet continued to release the nicorandil almost completely (97.23 to 99.72%) in the dissolution time period of 3–24 hours. Except the tablet prepared based on F-I composition, which released the drug completely around 9 hours of dissolution time period in pH 7.4 (98.32 ± 1.09%), all of the remaining tablets including the commercial one attained complete drug release only at the 24-hour dissolution time period. This indicated that basic pH does not speed up release for the majority of the formulations. Furthermore, irrespective of the dissolution medium (pH 1.2 or pH 7.4), the tablet prepared based on F-IX and F-X compositions showed the drug release profile that were almost in a similar manner to the drug release profile obtained with the commercial once-daily tablet (16.78 ± 2.55 and 22.12 ± 12 vs. 19.62 ± 1.75% at 2 hours in pH 1.2 and 97.23 ± 2.10 and 98.54 ± 2.84 vs. 99.02 ± 0.62% at 24 hours in pH 7.4, respectively) (Figure 1).

In vivo bioavailability

Figure 3 shows the obtained mean plasma concentration of nicorandil after oral ingestion into rabbits in two different occasions with tablet prepared based on F-IX composition and commercial nicorandil once-daily tablet. T_{max} for these two formulations occurred at 2 hours post-ingestion time period, and the C_{max} values of 44.07 ± 5.25 and 41.07 ± 6.05 µg/mL were observed, respectively, for the selected/optimized tablet and the commercial tablet. Table 4 presents the various pharmacokinetic parameters obtained with these two tablets. The *AUC* value obtained for commercial tablet was always less than the selected/optimized tablet (175 vs. 205). This indicates that at the same T_{max} value of 2 hours, the optimized/selected tablet produced the higher C_{max} and *AUC* in comparison to the commercial once-daily nicorandil tablet.

Table 4. Summary of pharmacokinetic parameters of orally administered nicorandil tablet formulations*.

Parameters	Commercial once-daily tablet	Tablet prepared based on F-IX composition
C_{max} (µg/mL)	41.1 ± 2.10	44.1 ± 1.68
T_{max} (h)	2.0 ± 0.40	2.0 ± 0.12
Ke (µg/h)	0.32 ± 0.01	0.27 ± 0.01
T_{half} (h)	2.15 ± 0.09	2.55 ± 1.02
$AUC_{(0-\infty)}$ (µg/h/mL)	175 ± 25.08	205 ± 9.56

AUC-area under the concentration-time curve; *Values indicate mean ± SD, $n = 6$.

Discussion

The use of hydrophilic and hydrophobic polymers to prepare the peroral tablets seems to provide a desired drug release profile over a prolonged period of time. The synergistic effect (i.e., release retardation) provided by the hydrophilic and hydrophobic polymers combination depends both on the nature of the polymers used and on the drug molecule selected. When preparing the once-daily sustained-release tablets, Reddy et al.[3] previously

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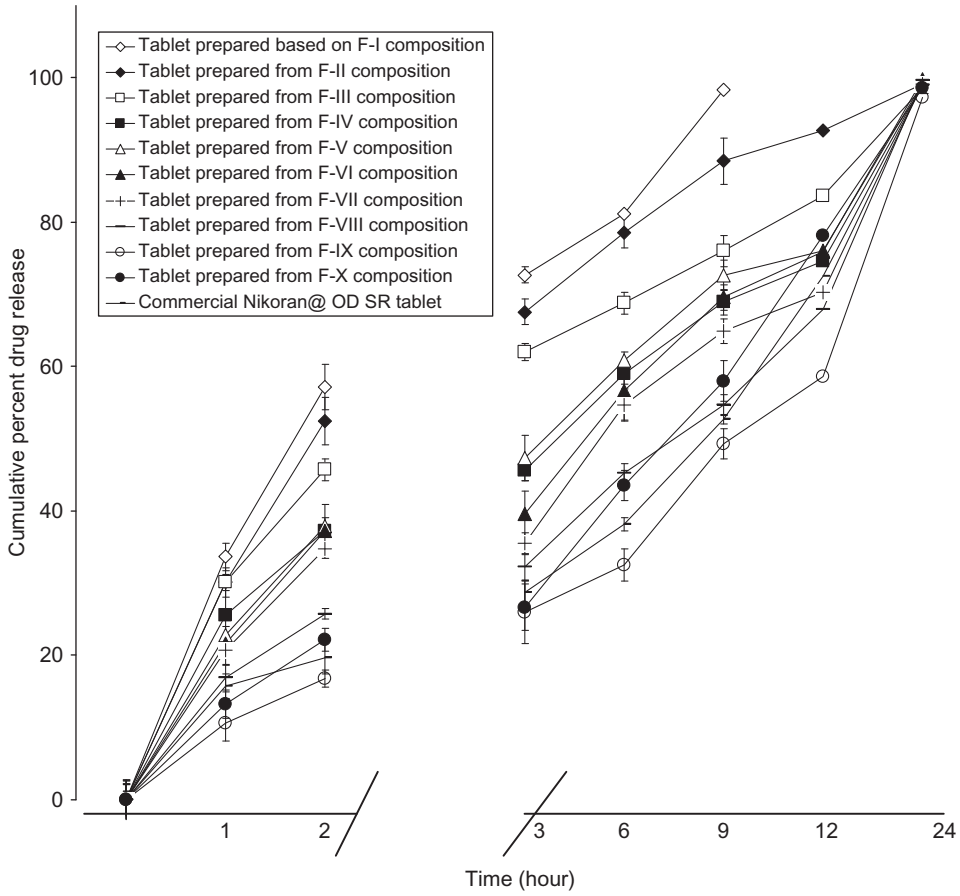

Figure 1. In vitro dissolution of developed nicorandil-loaded peroral tablets (80 mg) and commercial sustained-release tablet (Nikoran® OD SR) in two different dissolution media [initial 2 hours in pH 1.2 (0.1 N HCl) and from 3 to 24 hours in phosphate buffer of pH 7.4].

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showed that the HPMC and ethylcellulose polymers combination along with the nicorandil drug molecule was the most successful formulation in terms of total drug release pattern achieved over 24 hours. However, these authors prepared the tablets using 80 mg of nicorandil according to the theoretical release profile calculation made from the following equation[15] using available pharmacokinetic data[16,17].

$$D_t = Dose \times (1 + 0.693 \times t/t_{1/2}) \quad (9)$$

where D_t = total dose of drug; *Dose* = dose of the immediate release part (5.92 mg); t = time (hours) during which the sustained release is desired (24 hours); $t_{1/2}$ = half-life of the drug (1.33 hours). On substituting all these values on eq. (9), it produces

$$D_t = 5.92\,[1 + (0.693 \times 24)/1.33] \equiv 80.0 \text{ mg} \quad (10)$$

In this study, the drug amount was reduced significantly from 80 mg to only 10 mg, and HPMC and ethylcellulose amounts were also decreased significantly in comparison to the amounts used by Reddy et al.[3]. Furthermore, the total weight per tablet was also diminished from >400 mg[21] to only 80 mg. If the total weight per tablet is decreased, then, it should shrink the packaging cost that should add further benefits in terms of patients' tablet-purchasing capacity as well as tablet ingestion capability of the cardiac patients. Taking the cardiac patient economic and patient compliance points into consideration, the currently developed nicorandil-loaded peroral tablets differ significantly from the tablets prepared by Reddy et al.[3] and at the same time, the developed tablets also agree closely to the total weight and drug amount of the commercial nicorandil sustained-release tablet. Therefore, it becomes necessary to show how far the developed tablet is superior to the commercial tablet in terms of in vitro release and in vivo bioavailability. Keeping this perspective in mind, the granules for tablet preparation were made by the HPMC and ethylcellulose blend along with other tablet ingredients.

All the granules prepared based on F-I to F-X compositions were shown the angle of repose values below 30 indicating the good flow properties of the granules[7,8]. Generally, compressibility index values up to 15% result in good to excellent flow properties[7]. Granules prepared based on F-II, F-III, F-IV, F-V, and F-VIII compositions

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were shown less than 15% compressibility index values (Table 2). However, bulk densities of granules prepared based on F-I and F-II compositions were found to be moderately higher than those of other granules. This may be due to the presence of more fines in the granules, as the higher HPMC amount alone could not provide sufficient binding to the granules. This indicates the need of a hydrophobic polymer such as ethylcellulose to get better binding granules. The percentage porosity values of the granules ranged from 25.53 ± 0.02 to 35.92 ± 0.03%, indicating that the packing of the granules may range from close to loose packing and also further confirming that the particles are not of greatly different sizes[8]. The drug content in the weighed amount of granules of all formulations was found to be uniform (~98–99%) with an exception of the granules prepared based on F-I and F-II compositions (90.69 ± 0.05 and 92.56 ± 0.04%, respectively). All these results indicate that the granules prepared based on F-VI, F-VII, F-IX, and F-X compositions were possessed satisfactory flow properties, compressibility, and drug content.

The tablets prepared based on F-I to F-X compositions were subjected to various evaluation tests, such as thickness, hardness, uniformity of weight, friability, and drug content (Table 3). All the formulations showed uniform thickness. In a weight variation test, the average percentage deviation of all tablet formulations was found to be within the ±5% limit, and hence all formulations passed the test for uniformity of weight as per official requirements[10]. The percentage of drug content was more than 95% with an exception of tablets prepared based on F-I and F-II compositions. These two tablets (prepared based on F-I and F-II compositions) also showed a less hardness value (~5.5–5.8 kg/cm^2) compared with the other developed tablets. This could be due to the absence of ethylcellulose polymer. It appears that the presence of ethylcellulose provided a more hardness values observed with other tablets. The low hardness value observed with F-I- and F-II-based tablets may also be due to the presence of higher amount of HPMC, which generally decreases the hardness of tablets[18]. In this study, the percentage friability for all the formulations was below 1%, indicating that the friability is also within the prescribed limits[19]. But the tablet prepared based on F-X composition showed somewhat higher percentage friability value compared with the tablet prepared based on F-IX composition (0.337 ± 0.04 vs. 0.239 ± 0.03). Taking this particular point into consideration, it seems that it would be better to work with F-IX-based tablet for further stability and in vivo studies.

From figure 1, the pH-independent drug release was observed for all of the developed tablets including the commercial tablet. When ethylcellulose was absent in the tablets, there was a initial burst release (~52–57% release within 2 hours dissolution in pH 1.2) as observed especially with the tablets prepared based on F-I and F-II compositions. Presence of ethylcellulose (2–4 mg) gradually reduced the initial burst release as noticed from the tablets prepared based on F-III to F-X compositions. Even the tablet prepared based on F-IX composition released the drug in an almost similar manner as that of the drug released from the commercial tablet (Figure 1) over 24 hours dissolution time periods.

To ascertain/justify that these two tablets had a similar drug release profiles even at each one of the tested dissolution time periods, it is pertinent to perform a more stringent statistical analysis involving both a difference factor (F1) and a similarity factor (F2), which originates from simple model independent approach [20–22]. Hence, comparison between dissolution profiles was done using the F1/F2 tests. The difference factor calculates the percent difference between the two curves at each time point and is a measurement of the relative error between the two curves:

$$F1=\left\{\left[\sum_{t=1}^{n}\left|R_t-T_t\right|\right]\Big/\left[\sum_{t=1}^{n}R_t\right]\right\}\times 100 \qquad (11)$$

where n is the number of time points, R_t is the dissolution value of the reference batch at time t, and T_t is the dissolution value of the test batch at time t. The similarity factor is a logarithmic reciprocal square root transformation of the sum squared error and is a measurement of the similarity in the percent dissolution between the two curves.

$$F2=50\times\log\left\{\left[1+(1/n)\sum_{t=1}^{n}(R_t-T_t)^2\right]^{-0.5}\times 100\right\} \qquad (12)$$

Generally, F1 values up to 15 (0–15) and F2 values greater than 50 (50–100) ensure sameness or equivalence of the two curves[20]. This study showed a F1 value of 10 and a F2 value of 87. In addition, the calculated correlation coefficient value was also within the acceptable limit (r^2 = 0.9937) to show the linearity between these two curves. Therefore, the juxtapositioning/superimposition of the drug release profiles indicates that the tablet prepared based on F-IX composition was equivalent, in vitro, with the commercial sustained-release once-daily tablet (Figure 2). It should be noted that the drug release profile obtained with tablet prepared based on F-X composition was also plotted in figure 2.

Nicorandil-loaded tablets may meet different thermal, photolytic, hydrolytic, and oxidative conditions before reaching the patient. All these stress conditions are good enough to induce degradation of the drug within the tablet itself. To find out this type of drug degradation because of environmental conditions, the selected/optimized tablet along with the commercial tablet was stored at two different temperatures over 12-week time periods. Tablets stored at higher temperature over 12 weeks

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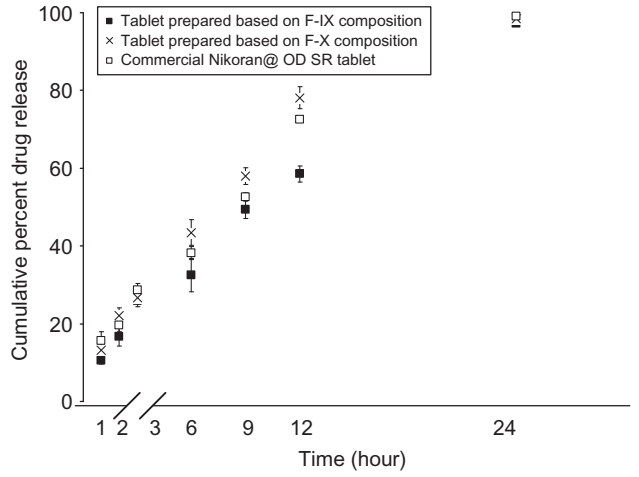

Figure 2. Comparison of nicorandil release in two different dissolution medium [initial 2 hours in pH 1.2 (0.1 N HCl) and from 3 to 24 hours in phosphate buffer of pH 7.4].

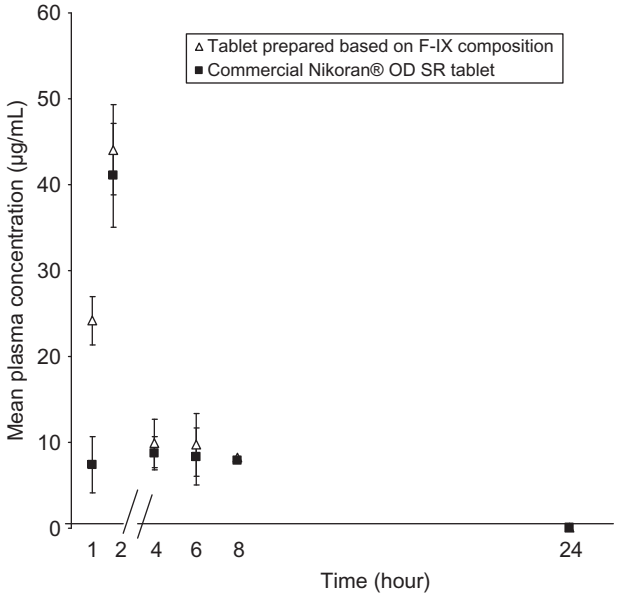

Figure 3. Mean plasma concentrations of nicorandil in white albino rabbits following single oral administration of 80 mg nicorandil-loaded tablet prepared based on F-IX composition (×) and 80 mg commercial sustained release once-daily (Nikoran® OD SR) tablet (Δ). Vertical bars represent mean ± SD ($n = 3$).

showed a slight color change (from white to pale yellow) on its physical surface indicating the possibility of degradation of drug or tablet ingredients. On the other hand, the tablets stored in lower temperature did not show any change from their original color. Regardless of the lower and higher temperature storage conditions, qualitative TLC was conducted on the samples collected from both the selected/optimized and the commercial tablets. The R_f value of the drug obtained from these two tablet dosage forms was the same as that of the pure drug (0.675 vs. 0.68). The obtained R_f values for nicorandil is also in agreement with the value obtained for nicorandil by other author[23]. The qualitative TLC results thus revealed that the drug was compatible with the formulation excipients, and neither decomposition of the drug nor drug-excipient interaction occurred in any tested formulation.

From the mean plasma concentration–time curve (Figure 3), it was seen that the absorption rate for the selected/optimized tablet and commercial once-daily tablet were slow to attain a maximum and slow to fall. However, there was a burst release at 1-hour post-ingestion time period following oral administration of the selected/optimized tablet into rabbit compared with the commercial once-daily tablet (24.17 ± 2.81 vs. 7.40 ± 3.25 µg/mL). This result is contrary to the in vitro release data. Therefore, the dosage form does not show an in vivo sustained drug release profile and the in vitro–in vivo correlation is poor. Despite that, the plasma levels remained above the minimum effective concentration, 40–60 ng/mL[24,25], for at least >8 hours after ingestion of the commercial and optimized tablet formulations. This may be due to the result of more slow absorption/release from these formulations. This indicates the continuing therapeutic equivalency of these two tablet formulations, although the C_{max} and AUC values obtained with the optimized tablet were found to be higher than the values observed with the commercial tablet.

Conclusion

This work revealed that nicorandil-loaded peroral tablets could be prepared based on the F-IX composition through the conventional wet granulation technique. The duration and intensity of nicorandil released, in vitro, from the optimized and commercial Nikoran® OD SR tablets were almost identical in the dissolution apparatus used. After a burst release at 1-hour post-ingestion time period, the plasma levels of optimized tablet remained above the minimum effective concentration for at least of >8 hours compared with that of the Nikoran® OD SR tablet.

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Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of this paper.

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