Review

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Enhanced oral bioavailability of nisoldipine-piperine-loaded poly-lactic-co-glycolic acid nanoparticles

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Abstract

Background: Piperine helps in the improvement of bioavailability through pharmacokinetic interaction by modulating metabolism when administered with other drugs. Nisoldipine is a substrate for cytochrome P4503A4 enzymes. The study was undertaken to assess the influence of piperine on the pharmacokinetics and pharmacodynamics of nisoldipine nanoparticles in rats.

Methods: Optimization studies of nanoparticles were performed using Taguchi L_9 orthogonal array, and the nanoparticles were formulated by the precipitation method. The influence of piperine and nanoparticles was evaluated by means of *in vivo* kinetic and dynamic studies by oral administration in rats.

Results: The entrapment efficiency, drug loading, ζ potential, and average particle size of optimized nisoldipine-piperine nanoparticles was 89.77 ± 1.06%, 13.6 ± 0.56%, –26.5 mV, and 132 ± 7.21 nm, respectively. The *in vitro* release in 0.1 N HCl and 6.8 pH phosphate buffer was 96.9 ± 0.48% and 98.3 ± 0.26%, respectively. Pharmacokinetic studies showed a 4.9-fold increase in oral bioavailability and a >28.376 ± 1.32% reduction in systemic blood pressure by using nanoparticles as compared to control (nisoldipine suspension) in Wistar rats.

Conclusion: The results revealed that piperine being an inhibitor of cytochrome P4503A4 enzymes enhanced the bioavailability of nisoldipine by 4.9-fold in nanoparticles.

Keywords: bioenhancer; CYP3A4; nisoldipine; optimization; piperine; PLGA.

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1 Introduction

Poly-lactic-co-glycolic acid (PLGA)-based nanocarriers have been extensively explored as drug delivery systems. PLGA is considered to be appropriate for most administration routes [1]. It is approved by the Food and Drug Administration and the European Medicines Agency for application in drug targeting [2, 3]. PLGA, due to its adaptive physical properties, gives flexibility to formulate and accomplish an anticipated dosage form by modifying the molecular weight and lactide/glycolide ratio. Moreover, the metabolism and the kinetics of the active ingredient can be regulated [4–6].

Enhancement of drug bioavailability is always strived for. One of the approaches for enhancing bioavailability is to co-administer drugs with a bioenhancer. Bioenhancers are defined as compounds that themselves are not therapeutic agents but potentiate the therapeutic effect of the co-administered drugs [7]. A number of natural compounds and herbal extracts have the ability to boost the bioavailability by inhibiting metabolism and/or improving absorption [8]. Piperine, obtained from *Piper nigrum*, has been reported to be an excellent bioenhancer [9]. Piperine improves the bioavailability of co-administered drugs by modulating metabolism. It is reported to downregulate or inhibit phase II enzymes like cytochrome P450 isoforms, UDP-glucuronyltransferase, hepatic arylhydrocarbon hydroxylase, and the glucuronidation process in the liver [10–12]. Shoba et al. [13] in 1998 showed a remarkable 2000% increase in curcumin bioavailability by piperine.

Nisoldipine is a second-generation long-acting calcium channel blocker. The vascular selectivity of nisoldipine is 10 times more than that of felodipine, isradipine, and nicardipine, and 100 times more than that of amlodipine and nifedipine [14]. The absolute bioavailability of nisoldipine is about 5% due to high presystemic metabolism in the gut wall and intestine [15]. Cytochrome P4503A4 enzymes are supposed to play a foremost role in the metabolism of nisoldipine [16].

A variety of experimental design methods, like the Taguchi and response surface methodologies, have been

successfully used for optimization of process parameters [17]. The main purpose of the design of experiments is to lessen the experimental runs required for optimization. The Taguchi design is based upon fractional factorial as provided by a standard orthogonal array. The Taguchi method uses array designs to take into account noise factors (outer) and design factors (inner), which estimate the effect of factors on the response mean and variation. An orthogonal array means the design is well adjusted so that factor levels are weighted equally and each factor can be evaluated autonomously of all the other factors. This allows assessment of the effect of one factor without the interference of effects of other factors [18]. This helps in the reduction of time and cost associated with the experiment when fractionated designs are used [19, 20].

The basic study objective of the current study was to determine the pharmacokinetic and pharmacodynamic changes and bioavailability of nisoldipine using nisoldipine-piperine nanoparticles after oral administration in rats. To test this hypothesis, the nisoldipine-piperine PLGA nanoparticles were formulated and evaluated for *in vivo* pharmacokinetic and dynamic changes using rats.

Table 1: Dependent variables and their respective levels used in the experiments.

Variable	Levels		
	1	2	3
Polymer (%) (A)	50 mg	100 mg	150 mg
Piperine (%) (B)	5 mg	10 mg	20 mg
Solvent (ml) (C)	25 ml	50 ml	75 ml

Table 2: Taguchi's L_9 orthogonal array of experiment for preparing piperine-loaded nisoldipine nanoparticles.

Experiment number	Polymer (%)	Piperine (%)	Solvent (ml)
1	1	1	1
2	1	2	2
3	1	3	3
4	2	1	1
5	2	2	2
6	2	3	3
7	3	1	1
8	3	2	2
9	3	3	3

2 Materials and methods

2.1 Materials

Orchid Pharma (Chennai, India) provided the nisoldipine and PLGA as gift samples. Piperine, methylprednisolone acetate, was procured from Sigma-Aldrich. PVP-K30, Tween 80, sodium lauryl sulfate (SLS), polyvinyl alcohol (PVA), and acetone were procured from Ranbaxy (India). All other chemicals and reagents used in the study were of AR grade.

2.2 Methods

2.2.1 Experimental design and analysis

The Taguchi design method was used to optimize various parameters for preparing nisoldipine and piperine-loaded nanoparticles. The various variables involved in preparing nisoldipine and piperine-loaded nanoparticles were categorized as dependent and independent variables. In this study, three factors namely polymer concentration (coded as A), piperine concentration (coded as B), and ratio of solvent (coded as C) were considered as variable factors affecting the process performance in terms of encapsulation efficiency (Y1) and nanoparticle size (Y2). The three levels of each of the dependent variables are given in Table 1. Nine experiments were performed to find out the factors and their optimized level ranges having a prominent effect on the proficiency of formulation based on Taguchi's L_9 orthogonal array (Table 2). The run involved the corresponding combination of levels to which the factors in the experiment was set. All nine experiments were performed in triplicate to minimize experimental errors.

The responses of signal-to-noise (*S/N*) ratio effect plots and analysis of variance (ANOVA) of each response, individually as well as simultaneously, were determined to assess significant optimized levels of factors (Table 3). The optimum conditions were determined to yield an improved performance with the minimum possible influence of the noise factor. The first step was to select the factor/level combination to maximize the response. The second step was to find the condition for attaining optimal desirability. All calculations and statistical analysis of the results were carried out by ANOVA to determine the factors having a statistically significant effect on the response parameters (Table 4).

2.2.2 Fabrication of nisoldipine and piperine-loaded nanoparticles

PLGA-based nanoparticles were prepared using a modified precipitation process [21]. Briefly, 100 mg PLGA

Table 3: The responses of various factors and output levels of observed vs. predicted levels on nine formulations.

Experiment number	EE (%, mean)	PS (Nm, mean)	*S*/*N* ratio		Fitness (%)	Levels		Predicted "better to best" level
			Actual	Predicted		Coded	Fitted	
1	87.77	135.2	−25.25	−24.82	89.77		A1B1C1	Nominal is best
2	88.14	132.7	−27.09	−25.69	−0.004	A1B2C2		
3	88.47	129.5	−27.15	−24.98	89.80		A1B3C3	
4	88.1	131.3	−27.19	−24.73	89.90		A2B1C2	
5	88.33	129.4	−27.16	−24.53	89.77	A2B2C3		
6	89.5	134.5	−26.59	−26.18	−0.004	A2B3C1		
7	89.23	128.9	−26.87	−26.76	−0.0005	A3B1C3		
8	89.77	132.01	−26.52	−25.81	89.77		A3B2C1	
9	89.67	130.1	−26.19	−25.27	89.70		A3B3C2	
Mean value	88.78	131.69	−26.6	−25.4	–			

The responses interpreted were fitted value of observed vs. predicted *S*/*N* ratios and the optimized level formula was A2B3C1.

Table 4: Analysis of variance responses.

Source	A	B	C	Residual error	Total
Combined effect on response Y1 and Y2 (EE in % and particle size in nm); nominal is best					
Degree of freedom	2.000	2.000	2.000	2.000	8.00
Seq SS	1.755	0.244	6.139	0.0539	8.19
F-value	32.59	4.540	114.0		
p-Value	0.030	0.181	0.009		
Rank of levels	2.000	3.000	1.000		A2B3C1
Individual effect on response Y1; larger is better					
Degree of freedom	2	2	2	2	8
Seq SS	0.028	0.003	0.00004	0.0005	0.0324
F-value	0.460	2.410	4.730		
p-Value	0.407	0.294	0.174		
Rank of levels	1.000	2.000	3.000		A1B2C3
Individual effect on response Y2; smaller is better					
Degree of freedom	2	2	2	2	8
Seq SS	0.0143	0.005	0.225	0.002	0.246
F-value	8.02	2.55	126.51		
p-Value	0.111	0.282	0.008		
Rank of levels	2.0000	3.0000	1.000		A2B3C1

(50:50, Mw = 40,000–75,000) polymer, 10 mg nisoldipine, and 20 mg piperine were dissolved in 25 ml acetone at room temperature to make a clear solution. Then, this solution was added dropwise with continuous magnetic stirring in 25 ml PVA (1%; Mw = 30,000–70,000) for 30 min at room temperature. The organic solvent was evaporated at 26°C first by stirring (2029 *g* using rotary evaporation; Rotavapor R-124, Buchi, Switzerland) at atmospheric pressure for 6 h and then at reduced pressure for 2 h. Thereafter, nanoparticle suspension was centrifuged (REMI, Mumbai, India) for 25 min at 10,956 *g* at 4°C to remove the polymer aggregates and washed twice with deionized water to remove acetone and PVA residues, and were freeze dried at −40°C (Eclipse 400; RS Biotech, UK) and stored in a vacuum desiccator at 4°C for further use. The percentage yield was >78%.

2.2.3 Preparation of nisoldipine suspension (control)

The nisoldipine suspension was prepared according to the method of Sandhya et al. [22] with modifications, by dissolving 10 mg nisoldipine in 3.0 ml of ethanol. Mixture 1 containing 30 mg PVP-K30, 0.3 ml Tween 80, and 1.0 ml of 0.01% SLS in 30 ml water was prepared and kept on a magnetic stirrer for uniform mixing. The drug solution was slowly added dropwise to mixture 1 and magnetic stirring was continued for 30 min. Then, the solution was

kept in a sonicator for 30 min. The suspension was formed and preserved for further use.

2.2.4 Entrapment efficiency and loading capacity

The entrapment efficiency of the prepared nanoparticles was determined by an indirect method [23]. The concentration of nisoldipine in the supernatant after isolation of nanoparticles was determined spectrophotometrically at 238 nm. The percent entrapment efficiency (EE%) was calculated by the following equation:

$$\text{EE}\,(\%) = [N_{\text{total}} - N_{\text{supernatant}}/N_{\text{total}}] \times 100,$$

where N_{total} is the total amount of nisoldipine and $N_{\text{supernatant}}$ is the free nisoldipine in the supernatant.

For calculating drug loading, the nanoparticles were dissolved in a mixture of acetone and ethanol (10:1) and centrifuged at 1677 *g* for 15 min. The precipitate was then extracted with acetone. The acetone was evaporated and percent drug loading [24] was calculated by the following equation:

$$\text{Drug loading}\,(\%) = [\text{amount of nisoldipine extracted/ weight of nanoparticles}] \times 100.$$

2.2.5 Characterization of nanoparticles

The characterization of nanoparticles was achieved using transmission electron microscopy (TEM). Photon correlation spectroscopy (NanoZS90; Malvern Instruments, Worcestershire, UK) was used to measure the particle size, polydispersity index, and zeta potential (ZP) of the prepared formulation. All samples were diluted with ethanol to achieve sufficient concentration before measurement. All parameters were analyzed in triplicate and data are represented as mean ± standard deviation (SD). Thermal [differential scanning calorimetry (DSC)] and spectroscopic [Fourier transform infrared (FTIR)] analyses were used to assess the chemical interaction of the drug with excipients.

2.2.6 Dissolution and release studies

In vitro release of nisoldipine from nanoparticles was studied in 0.1 N HCl (pH 1.2) and in phosphate buffer (pH 6.8) using a type II paddle apparatus (DS 8000; Lab India Ltd., Mumbai, India) at 37 ± 0.5°C and 60 rpm for comparing the release pattern at the two different pHs. The nisoldipine release profile is influenced by pH; therefore, the two different pHs were selected to comparatively evaluate the effect on the release profile of nanoparticles, as some researchers claimed it to be independent of pH. Aliquots of 5 ml were withdrawn from the apparatus at specific intervals. Nisoldipine concentration was measured in the withdrawn aliquots. The dissolution medium volume was kept constant by adding the same amount of fresh medium. Drug dissolution and cumulative drug release was determined for nisoldipine-piperine nanoparticles and conventional nisoldipine formulation at 2, 4, 6, 8, 12, 18, 24, 36, 48, and 60 h.

2.2.7 Antihypertensive and pharmacokinetic studies

The study was carried out in Wistar rats of either sex weighing 150–200 g. The animals were maintained under standard laboratory conditions of temperature (22 ± 2°C) and 12 h light/dark cycle. All animals were kept in quarantine for 1 week prior to experiments. Rats were fed with standard laboratory chow and water *ad libitum*. All experimental procedures were reviewed and approved by the Institutional Animal Ethical Committee of Chandigarh College of Pharmacy, Landran (IAEC/FEB16/018). The animals were fasted overnight before the experiment. Before experimentation, the animals were trained to stay in the restrainer. This ensured that the rats were calm and unaggressive during blood pressure (BP) measurements.

Hypertension was induced by subcutaneous injection of methyl prednisolone acetate (20 mg/kg/week) for 2 weeks [25]. The rats were then randomly divided into three groups with six animals in each group. The groups were methyl prednisolone + saline (methyl prednisolone group), methyl prednisolone + nisoldipine (nisoldipine group), and methyl prednisolone + nisoldipine-piperine nanoparticles (nanoparticle group). In addition, a normal control group treated with saline alone was also included. Drug administration was started 30 min after the last methylprednisolone injection. Nisoldipine was administered orally once in a dose of 0.9 mg/kg and nanoparticles were also administered orally once in a dose of 0.45 mg/kg in solution form by using a cannula attached to a syringe into the mouth of animals. The dose of nisoldipine used was selected based upon the approved clinical dose in humans [26]. This dose was then converted to the equivalent rat dose on the basis of body surface area formula. A non-invasive tail cuff method was used to measure systolic BP [27]. BP in all groups was measured up to 120 h after drug administration starting from 0 h. At each time

point, three readings were taken for each animal and the mean was calculated.

The pharmacokinetic profiles of nisoldipine nanoparticles and conventional nisoldipine formulation were also evaluated after oral administration of drugs. Parameters such as peak serum concentration (C_{max}), time for peak serum concentration (t_{max}), area under the curve (AUC, 0– ∞), half-life ($t_{1/2}$), and mean residence time (MRT) were calculated. The blood samples were withdrawn from the tail vein at 0, 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose. About 0.1 ml of blood sample was withdrawn in Eppendorf tubes and centrifuged at 3000 rpm for 30 min. The serum was transferred to another Eppendorf tube and stored at –20°C until further analyzed by high-performance liquid chromatography (HPLC) using a C-18 analytical column (250 mm × 4.6 mm and 5 μm in diameter; Merck, Mumbai, India). The mobile phase consisted of acetonitrile-water (78:22) at a flow rate of 1.1 ml/min and detection at λ max of 237 nm. The injection volume was 10 μl [28]. A stock solution was prepared by dissolving accurately weighed 10 mg of nisoldipine in 10 ml volumetric flask to obtain a 1 mg/ml solution using HPLC-grade methanol. Kinetica software (version 5.0; Innaphase Corporation, Philadelphia, PA, USA) was used to calculate the pharmacokinetic parameters. The values are expressed as mean ± SD. The data from BP experiments and pharmacokinetic data are presented as mean ± SD. The data were analyzed by unpaired Student's t-test. A value of $p \leq 0.05$ was considered statistically significant.

2.2.8 Accelerated stability studies

Nanoformulation stability analysis was done according to International Council on Harmonization [29] guidelines at temperature of 25 ± 2°C and relative humidity of 5%. The nanoparticle formulation samples were collected at prearranged intervals (30, 60, and 90 days) and tested for particle size, shape, appearance, and DSC analysis. Drug content stability and drug entrapment of nanoparticles were also determined by storing the nanoparticles at 4.0 ± 1°C in a refrigerator and 40 ± 2°C in a stability testing chamber for 3 months.

3 Results and discussion

The Taguchi L_9 orthogonal array optimized design was used to prepare nanoparticles of PLGA loaded with nisoldipine and piperine by the precipitation technique, to increase the bioavailability and to obtain a sustained release of drug. Nanoparticles were prepared to study the effect of polymer concentration, amount of bioenhancer, and ratio of solvent on the response variables of nanoparticles. Nine formulations were developed and the calculated values of response variables are shown in Tables 3 and 4. The main effect plots were analyzed to define optimal levels of factors independently and also simultaneously using ANOVA.

The experimental vs. predicted S/N ratio was obtained, and the obtained effect of each factor alone as well as at combined level on responses were plotted (Figure 1A–C). The main responses of S/N ratio plots confirmed the significant factor levels and were used further in the development and characterization of formulation.

It was concluded that mean particle size (nm) increased with the increase in polymer-drug ratio, whereas it decreased with the increase in piperine concentration. Drug entrapment efficiency (% w/w) was increased with the increase in polymer-drug ratio and piperine concentration, while it remained unaffected by solvent ratio. The optimized formulation had a mean particle size of 132 ± 7.21 nm, drug entrapment efficiency of 89.77 ± 1.06% w/w, drug loading of 13.6 ± 0.56% w/w, and ZP of –26.5 mV. The factors most affecting formulation were polymer ratio and piperine concentration.

3.1 Characterization of nanoparticles

The FTIR spectral analysis showed the characteristic peaks of nisoldipine at 3320 cm^{-1}, N-H stretching at 3001 cm^{-1}, C-H stretching and peak at 1701 cm^{-1}, and esterified carbonyl group stretch. There was also a peak at 1555 cm^{-1} (aryl nitro group) and at 1230 cm^{-1} (ether absorption) (Figure 2). Similar peaks were observed in the spectra of nisoldipine nanoparticles without any remarkable change in their positions. Hence, it may be concluded that there was no chemical interaction between the drug and the polymer.

3.2 DSC

If the drug was present in a molecular dispersion or solid solution state in the polymeric nanoparticles, then no detectable endotherm was observed. In the absence of any interaction, the thermogram of a formulation will show patterns corresponding to those of the individual components. Pure nisoldipine shows an endothermic melting peak at 150°C indicating its crystalline nature,

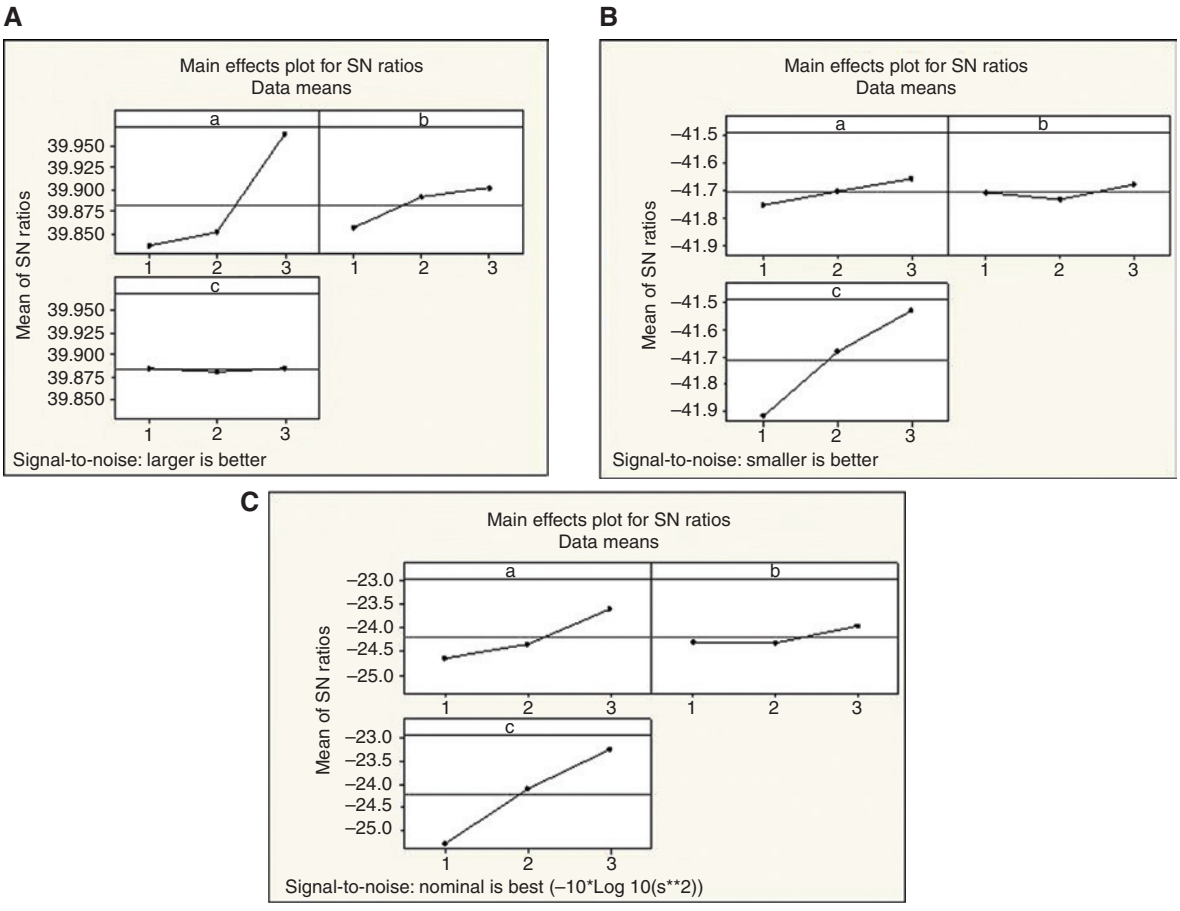

Figure 1: Taguchi analysis of significant factors individually: (A) main effect plots on entrapment efficiency (Y1); (B) particle size (Y2); (C) combined effects on Y1 and Y2.

piperine shows a peak at 128°C, while PLGA shows an endothermic peak at 59°C corresponding to its glass transition temperature. The thermogram of nanoparticles showed broadening of the characteristic endothermic peak corresponding to nisoldipine and piperine, while the PLGA peak shifted to 54°C, suggesting a decrease in its glass transition temperature. This deviation may be attributed to physical interactions such as hydrogen bonding between the carbonyl group of PLGA and the NH group of nisoldipine. Hence, considering the fact that the drug peak was broadened and the polymer peak was shifted in the drug-loaded nanoparticles, it could be due to physical interaction or H-bonding between the carbonyl groups of PLGA and the NH groups of nisoldipine (Figure 3).

3.3 Morphological considerations

TEM was used for morphological analysis of nanoparticles. Discrete spherical structures were seen without aggregation (Figure 4). It was observed that the particle sizes were uniform with a narrow size distribution range (average 132 ± 1.21 nm). Additionally, the surface charge of optimized nanoparticles was obtained as –26.5 mV (Zetasizer Nano ZS90). The nanoparticles were dispersed in dilutions of PBS at pH 6.8 (ionic strength 1.5×10^{-5} m) at 25°C.

3.4 Drug release studies

The *in vitro* release behavior of conventional nisoldipine formulation was rapid, consistent, and completed within 12 h (92.5 ± 2.17% for pH 6.8 and 90.4 ± 1.77% for pH 1.2). In 0.1 N HCl (pH 1.2), the cumulative percentage of release from the nanoformulation was 96.9 ± 0.48% over a period of 60 h (Figure 5). In phosphate buffer (pH 6.8), the cumulative percentage of release was 98.3 ± 0.26% in 60 h (Figure 5). No difference was observed in drug release from nanoparticles in phosphate buffer (pH 6.8) and 0.1 N HCl. Thus, the release and solubility of

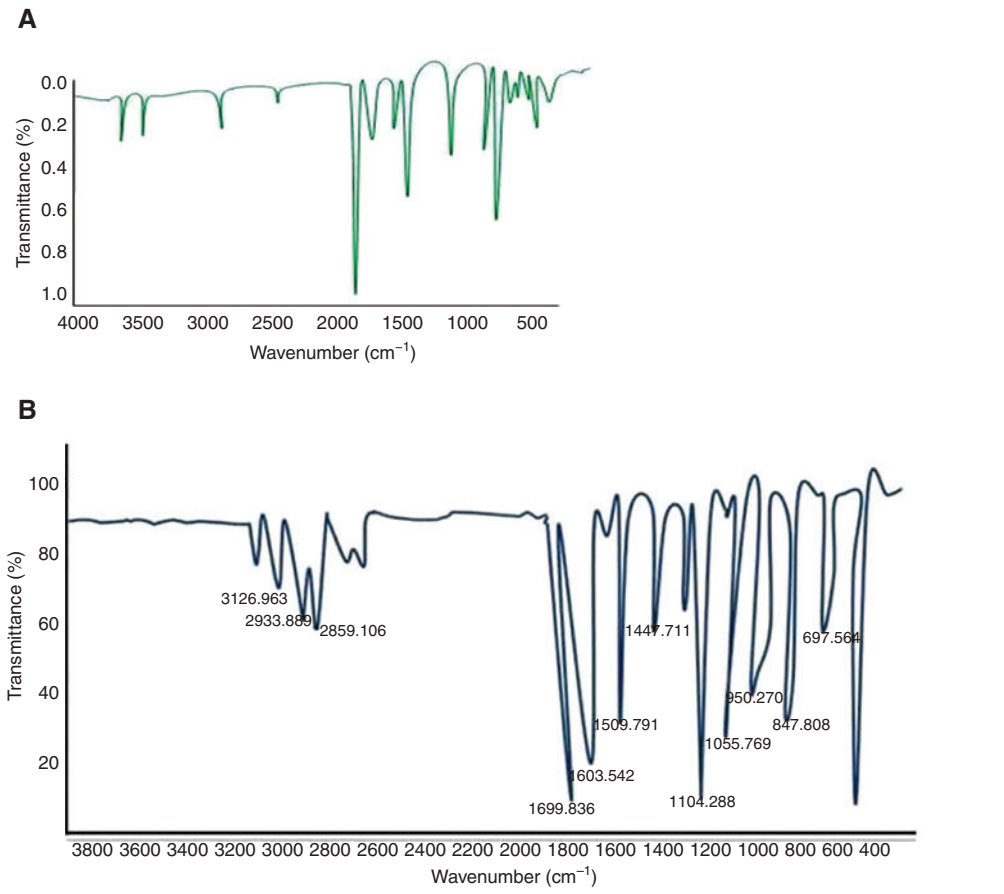

Figure 2: (A) The Fourier transforms spectra of nisoldipine. (B) The Fourier transforms spectra of nanoparticles.

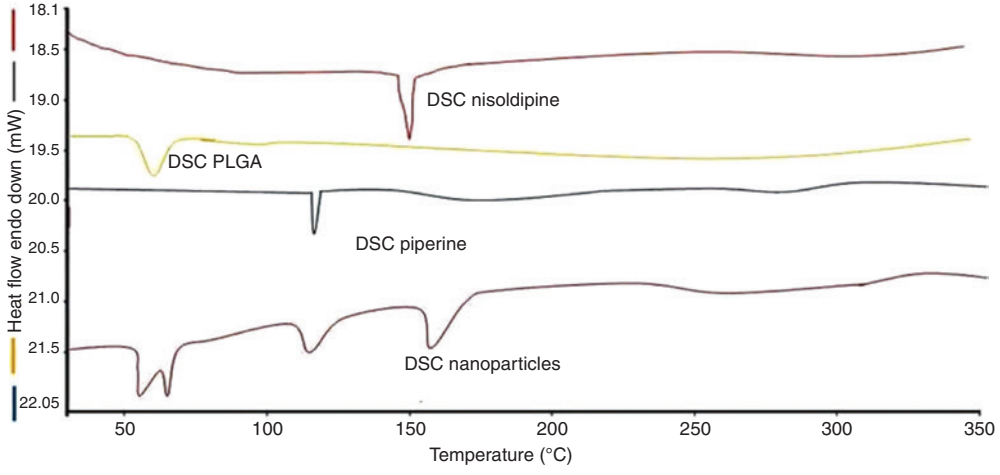

Figure 3: Comparison of DSC of ingredients and formulation.

nisoldipine from nanoparticles were independent of pH. Nanoparticles showed a biphasic release behavior of drug. Biphasic release consisted of an initial burst effect up to 6 h followed by a sustained release phase up to 60 h. The initial burst effect was characterized by approximately 32.13 ± 2.3% drug release within 6 h. The initial burst phase was attributed to the dissipation and diffusion of drug adhered to the surface of polymeric nanoparticles or entrapped poorly in polymer matrix. The explosion effect was favorable and helped in realizing

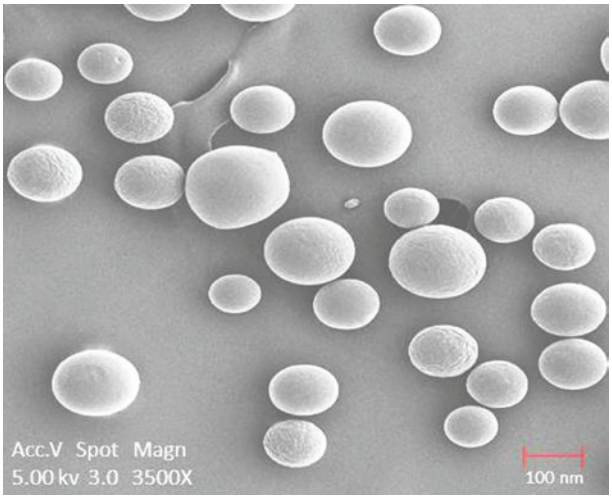

Figure 4: TEM analysis of nanoparticles.

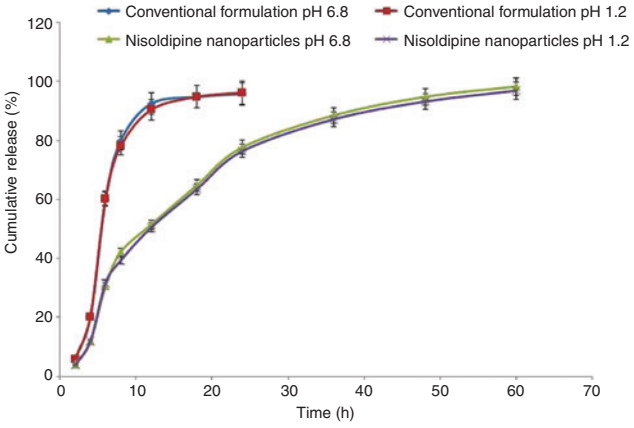

Figure 5: Percentage of cumulative release of nisoldipine from conventional and nanoparticles.

the therapeutic concentration of drug in comparatively lesser time [30].

3.5 Release kinetics and stability

The *in vitro* release data indicated first-order and Higuchi-order release kinetics of nanoparticles, signifying the role of the diffusion principle in drug release. The n-value (0.86–0.33) obtained for the Peppas release model proposed the "non-Fickian diffusion transport" mechanism supported diffusion and relaxation mechanism of controlled drug release (Table 5). The formulation was stable, as no noticeable alteration was noted in terms of particle size and drug content in the tested storage conditions.

Table 5: Value of regression obtained from different kinetic models.

Formulation	R^2				
	Zero order	First order	Higuchi model	Korsmeyer-Peppas model	n-Value
Nanoparticles	0.8267	0.9317	0.9369	0.9976	0.86–0.33

3.6 Antihypertensive study

The antihypertensive effect of nisoldipine-piperine nanoparticles was studied and compared to conventional nisoldipine formulation. Systolic BP was measured and results are given in Table 6. In the normal control group, normal systolic BP was observed. Oral administration of the conventional formulation significantly controlled the hypertension initially, with the maximum effect at 2 h; however, afterwards, the BP gradually increased and stabilized. In contrast, the oral administration of nisoldipine-piperine nanoparticles resulted in a steady decrease of BP. The maximum effect was observed at 36 h, and this effect was maintained up to 72 h. In the hypertensive control group, the systolic BP was significantly higher compared to the normal control group. The 22.30% reduction in BP by nisoldipine nanoparticles as compared to 16.24% reduction by conventional formulation clearly indicated that the nisoldipine nanoparticle formulation gradually released the drug over a period of 72 h. As the administration of nisoldipine-piperine nanoparticles led to sustained and continued drug release, it was proficient in overcoming the limitations of oral administration of conventional nisoldipine formulation [31, 32].

3.7 Pharmacokinetic study

The appropriate pharmacokinetic factors were calculated for the prepared nanoformulation and compared with conventional nisoldipine formulation (Table 7). The C_{max} value (22.30 ± 0.56 µg/ml) for nisoldipine-piperine nanoparticles was statistically significant at $p < 0.0001$ with respect to conventional formulation (12.10 ± 0.29 µg/ml). The AUC (0–∞), which symbolizes the magnitude of absorption, was also significantly ($p < 0.05$) higher for nanoparticles (255.54 ± 5.92 µg/ml/h) compared to conventional formulation (52.09 ± 3.76 µg/ml/h). The t_{max}, $t_{1/2}$, and MRT values were also higher for nisoldipine-piperine nanoparticles as compared to conventional formulation, resulting in a 4.9-fold improvement in oral bioavailability. The reported literature suggests the role of particle

Table 6: Antihypertensive effect of optimized nisoldipine-piperine nanoparticles and conventional formulation.

Time (h)	Group 1 Normal control	Group 2 Hypertensive control	Group 3 Nisoldipine conventional	Group 4 Nisoldipine-Piperine nanoparticles
Initial	100.23 ± 0.57	165.31 ± 0.38	164.12 ± 0.51	167.13 ± 0.32
1	110.11 ± 0.42	164.91 ± 0.24	150.82 ± 0.26	158.11 ± 0.35
2	105.54 ± 0.31	164.43 ± 0.34	110.36 ± 0.22	153.17 ± 0.27
4	98.61 ± 0.28	164.11 ± 0.38	112.61 ± 0.37	148.24 ± 0.21
8	110.01 ± 0.33	163.73 ± 0.61	114.58 ± 0.39	142.68 ± 0.41
12	106.82 ± 0.42	163.47 ± 0.29	116.57 ± 0.42	136.41 ± 0.45
18	107.14 ± 0.44	161.92 ± 0.28	119.67 ± 0.23	128.54 ± 0.34
24	112.03 ± 0.29	160.18 ± 0.35	123.29 ± 0.37	119.13 ± 0.29
36	103.71 ± 0.36	159.55 ± 0.41	128.37 ± 0.25	104.19 ± 0.33[a]
48	101.96 ± 0.41	158.37 ± 0.43	132.61 ± 0.34	105.72 ± 0.42[a]
60	103.05 ± 0.52	158.12 ± 0.48	137.34 ± 0.55	106.79 ± 0.22[a]
72	105.11 ± 0.31	157.15 ± 0.33	139.17 ± 0.61	106.97 ± 0.28[a]
84	109.44 ± 0.27	156.37 ± 0.27	143.01 ± 0.38	106.55 ± 0.31[a]
96	100.76 ± 0.45	148.44 ± 0.55	145.27 ± 0.21	108.31 ± 0.36[a]
108	99.61 ± 0.39	142.91 ± 0.41	147.64 ± 0.39	108.81 ± 0.51[a]
120	102.48 ± 0.24	142.56 ± 0.43	149.38 ± 0.24	109.62 ± 0.47[a]

Data are presented as mean ± SD, n = 6. [a]p-Value <0.05 compared to conventional formulation.

Table 7: Pharmacokinetic profile of nisoldipine-piperine nanoparticles and nisoldipine conventional formulation.

Parameters studied	Nisoldipine conventional	Nisoldipine-Piperine nanoparticles
C_{max}	12.1 ± 0.29	22.3 ± 0.56[a]
t_{max}	2.0	6.0[a]
$AUC_{0-\infty}$	52.09 ± 3.76	255.54 ± 5.92[a]
$t_{1/2}$	6.25	17.74[a]
MRT	4.63	14.19[a]

Data are presented as mean ± SD. [a]p-Value <0.05 when compared to conventional formulation. All values are means of three readings.

size reduction in improvement of bioavailability by 1.55–2.22 times. Due to the nano size of the formulation, the effective surface increased consequently, increasing the contact time of the nanoparticles and oral bioavailability by 2.17 [33]. The designing of the nisoldipine-loaded self-nanoemulsifying drug delivery system increased the bioavailability by 2.22 times [34]. Similarly, an increase of 1.55-fold bioavailability of nisoldipine was reported in a hydrogel formulation [35]. An additional increase in bioavailability of the prepared nanoparticles was due to the CYP3A4 enzyme inhibitory activity of piperine, which reduces the first-pass metabolism of the drug. Hence, it could be concluded that the enhanced oral bioavailability of nisoldipine-piperine nanoparticles was due to the contribution of both of these factors.

4 Conclusion

Nanotechnology-based delivery systems for antihypertensive agents are a promising approach in resolving some constraints of antihypertensives. Targeted nanoparticles can effectively take antihypertensives to their site of action, and chronotherapeutics along with nanotechnology can effectively control high BP by not only modifying the release pattern of the drug but also by increasing the bioavailability of the drug. The nisoldipine-piperine-loaded nanoparticles demonstrated good encapsulation efficiency and sustained release behavior. The results obtained from the study are encouraging, as drug-piperine nanoparticulates resulted in a 4.9-fold increase in the bioavailability by nanonization and inhibition of CYP3A4 enzymes. Hence, nisoldipine doses may require special consideration if used along with piperine-containing preparations to avoid complications resulting from drug-drug interactions, leading to alterations of bioavailability.

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