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RESEARCH PAPER

Improving the Dissolution and Bioavailability of Nifedipine Using Solid Dispersions and Solubilizers

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ABSTRACT

Nifedipine (NF) is a poorly water-soluble drug, of low and irregular bioavailability after oral administration. Although some reports have attempted to improve the dissolution of NF using solid dispersions and solubilizers, little literature information is available on the in vivo performance of such preparations. The aim of the present work was to improve the therapeutic efficacy of NF via incorporation into different types of carriers, and to investigate their in vitro dissolution and bioavailability in rabbits. Nifedipine solid dispersions were prepared by fusion, solvent, and freeze-drying methods with polyethylene glycol (PEG) 6000 and PEG monomethylether 5000 (PEG MME 5000). Complexation of NF with β-cyclodextrin (β-CyD) and solubilization by sodium lauryl sulfate (SLS) have also been studied. The dissolution was determined by the flow-through cell in order to maintain perfect sink conditions. The solid dispersions resulted in a significant increase in the dissolution rate as compared to pure drug. The highest NF dissolution rate was obtained from solid dispersions containing 95% PEG 6000 prepared by the solvent method. While, unexpectedly, the highest absorption in rabbits was obtained from 95% PEG 6000 prepared by the fusion method. Compared to SLS, β-CyD gave higher in vitro and in vivo values. Differential scanning calorimetry (DSC) and

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powder x-ray diffractometry indicated that NF in solid dispersions is homogeneously distributed, and no drug crystallized out of the system. The DSC thermograms of NF-β-CyD complex and NF/SLS solid mixture showed a decrease in the NF endothermic peak. The x-rays showed different diffraction patterns of pure NF and pure carrier, suggesting the formation of a new solid form.

Key Words: *Bioavailability; Complexation; Flow-through cell; Nifedipine; Solid dispersion; Solubilizer*

INTRODUCTION

Together with the permeability, the solubility behavior of a drug is a key determinant of its oral bioavailability. In recent years, the number of poorly soluble drug candidates has risen sharply, and the formulation of poorly soluble compounds for oral delivery now presents one of the interesting challenges to formulation scientists in the pharmaceutical industry.[1]

Nifedipine (NF) is a calcium channel blocker which belongs to the dihydropyridine derivatives. It exhibits poor dissolution characteristics due to its poor wettability and dispersibility in body fluids. Therefore, a number of attempts, such as decreasing particle size, the use of wetting agents, co-precipitation, and preparation of solid dispersions, have been made to modify the dissolution characteristics and thereby improve the absorption rate.[2–15]

Little literature information is available on the in vivo performance of NF solid dispersions,[9,16] NF-cyclodextrin complexes,[16,17] and NF solubilization with surfactants.[15] In addition, the in vivo evaluation of NF-β-cyclodextrin (NF-β-CyD) complex and NF/sodium lauryl sulfate (NF/SLS) has not yet been reported. The different methods used for the preparation of NF/polyethylene glycol (NF/PEG) solid dispersions need to be examined in vivo, considering the processing effect on the formulation performance. The aim of the present work was to compare the efficiency of several methods, excipients, and drug-to-excipient ratios, in improving NF dissolution and bioavailability. This was accomplished by: formulating solid dispersions of NF/PEG 6000 and NF/PEG monomethylether (MME) 5000, at different concentrations, NF-complexation with β-CyD, and solubilization with SLS, using different preparation methods. On the basis of the in vitro dissolution behavior, the selected preparations were subjected to bioavailability testing in rabbits.

MATERIALS AND METHODS

Because of the NF photosensitivity,[18] all experimental studies were conducted under yellow light.

Materials

Nifedipine powder (Arzneimittel Werk Dresden, Germany, B.N. 9608013), nitrendipine (Sigma, USA), PEG 6000 (Laboratory Rasayan, India), PEG MME 5000 (Fluka, Switzerland), β-CyD (Merck, Germany), SLS (Adwic, Egypt), chloroform (Adwic, Egypt), distilled water (Milli RO plus 10, Millipore purification systems, USA), ethylacetate (HPLC grade, Romil, UK), acetonitrile (HPLC grade, Merck, Germany), methanol (HPLC grade, Merck, Germany), acetic acid 96% (Adwic, Egypt), deionized water (Milli Q, Millipore, USA), ethylene diamine tetra acetic acid (EDTA) (Sigma, USA).

Preparation of NF Solid Dispersions with PEG 6000 and PEG MME 5000

Solid dispersions of NF in PEG 6000 (85%, 90%, and 95%) were prepared by fusion, 95% by solvent, and 90% by freeze-drying methods. Meanwhile NF with PEG MME 5000 (70%, 85%, 90%, and 95%) was prepared by fusion and solvent methods.

Fusion Method

The calculated amounts of NF and the carriers were weighed and mixed together in a mortar. The physical mixture was then heated directly on a hot plate at 80–85°C with continuous stirring till complete melting. The fused mixture was then solidified by rapid cooling in an ice bath[10] and sieved (150–90 μm).

Solvent Method

The calculated amounts of NF and the carriers were weighed and mixed together. The physical mixture was then dissolved in an adequate amount of chloroform[19] followed by evaporation under vacuum in a rotatory evaporator at 35°C. The solid dispersions prepared by either of the above methods were further dried by storage in a vacuum oven at room temperature. The dried mass was pulverized, uniformly mixed in a mortar, and sieved into defined particle size fractions (150–90 μm).

Freeze-Drying Method

A 50% solution of PEG 6000 was prepared in distilled water. Nifedipine was added to the concentrated PEG 6000 solution and stirred overnight. The solution was then freeze-dried (VirTis, USA). The freeze-dried solid dispersion was pulverized and sieved (150–90 μm).

Preparations of NF with β-CyD

NF-β-CyD Kneaded Complex

Solid complex of NF-β-CyD in a molar ratio of 1:1 was prepared by kneading.[6] The kneaded complex was dried at room temperature in a vacuum oven until complete dryness then sieved (150–90 μm).

NF-β-CyD Physical Mixture

Physical mixture of NF-β-CyD in a molar ratio of 1:1 was prepared by mixing in a mortar[6] and sieved (150–90 μm).

NF-β-CyD Freeze-Dried Complex

Physical mixture of NF-β-CyD in a molar ratio of 1:1 was added to 500 mL distilled water and stirred for 5 days. The suspension was freeze-dried (VirTis, USA), and the obtained freeze-dried complex was pulverized and sieved (<38 μm).

Preparation of NF/SLS Solid Mixture

A mixture of NF and SLS in a weight ratio of 1:1 was prepared by kneading method. The NF/SLS mixture was then dried under vacuum at room temperature in a vacuum oven until complete dryness, obtaining the solid mixture, and then sieved (150–90 μm).

Determination of NF Content

The content of NF in each preparation was determined using high-performance liquid chromatography (HPLC). An accurately weighed preparation was dissolved in acetonitrile by sonication, then filtered (Millex 0.45 μm). This was analyzed by HPLC using a mobile phase of acetonitrile–methanol–deionized water–acetic acid (30:30:45:1), pH 3.1, flow rate of 1 mL/min, a reversed phase column, and detection wavelength at 340 nm (details given later). Each preparation was tested in triplicate. The developed HPLC method has proved capable of detecting the four known degradation products of NF.[18] The peaks of these products could be formed upon exposure of the NF solutions to daylight. Throughout this study, HPLC analysis was also used to guarantee NF stability in the preparations.

In Vitro Dissolution

The dissolution study was carried out using the open system of the flow-through cell apparatus (Dissolutest® CE-6, connected with a piston pump CY 7, SOTAX AG, Basel, Switzerland), representing the USP apparatus #4 (USP XXIII, NF, 1995). An amount of each preparation equivalent to 20 mg NF was placed into 22.7 mm diameter cell. A pre-filtered (0.45 μm) and degassed simulated gastric fluid (pH 1.2) was pumped at a laminar flow rate of 8 ± 0.2 mL/min at 37°C. A built-in filtration system (0.7 μm Whatman GF/F and GF/D, and glass wool) was used. Fractions were collected at specified time intervals and analyzed for NF content by UV spectrophotometer at λ_{max} 336 nm (Beckman DU 650, USA). Each preparation was tested in three cells and the mean values were calculated.

Statistical Comparisons

The dissolution profiles were compared using two parameters: D_{24} (the percentage of NF dissolved at 24 min) and D_{156} (the percentage of NF dissolved at 156 min). The comparisons were made among the methods and carriers by analysis of variance (ANOVA) and least squares difference at $p < 0.05$.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) of some selected preparations compared with the plain NF was carried out. About 70 mg of the powdered sample (particle size 150–90 μm) was placed in a platinum crucible. The DSC (Perkin-Elmer, DTA7, USA) thermograms were recorded at a heating rate of 5°C/min from 50 to 300°C.

X-Ray Diffraction Analysis

X-ray diffraction analysis of the selected preparations (particle size 150–90 μm) compared with the plain NF was investigated by measuring the 2θ range from 4 to 50° with reproducibility of $\pm 0.001°$ on a diffractometer (Siemens, D-500, Germany). The x-ray diffraction patterns were recorded automatically using a rate meter with time constant of 2×10^2 pulse/sec and scanning speed of 2° (2θ)/min.

Bioavailability Study

HPLC Method for Determination of NF in Rabbit Plasma

NF Standard Plasma Samples

A stock solution of NF was prepared in acetonitrile at a concentration of 200 μg/mL. This solution was diluted with acetonitrile to yield the appropriate working solutions for the preparation of the calibration standards. These standards were prepared by adding a definite volume of a suitable standard solution to 0.5 mL of drug-free plasma to obtain NF concentrations in the range of 0.05–5 μg/mL.

Internal Standard Solution

A stock solution of 200 μg/mL nitrendipine in acetonitrile was prepared and diluted to 50 μg/mL with acetonitrile. Ten microliters of this solution was spiked into the plasma to yield an internal standard concentration of 1 μg/mL.

Sample Treatment

Each of the test or standard plasma samples was treated as follows. 0.5 mL plasma was spiked with 1 μg/mL nitrendipine as internal standard; 4 mL dry ethyl acetate was added and the extraction was done by vortex for 1 min, followed by centrifugation for 5 min at 7000 rpm; 3 mL of the ethyl acetate layer was separated, transferred to a series of screw-cap vials, and evaporated to dryness. The residue was reconstituted with 200 μL of the mobile phase, and vortexed for 1 min. Then a 50-μL aliquot was injected onto the HPLC column (Lichrosorb 5 RP-Phenomenex, size 250×4.60 mm, 5 micron) protected by a guard pack pre-column module with Bondapack C18 inserts (Waters Assoc., USA).

Chromatographic Conditions

The mobile phase consisted of acetonitrile–methanol–deionized water–acetic acid (30:30:45:1) at pH 3.1. The mobile phase was filtered on Millipore membrane filter 0.45 μm and degassed. The flow rate was 1.5 mL/min (Waters 600 E Multi Solvent Delivery System with a model U6K injector), the column temperature was kept at 45°C (TCM, Waters Assoc.), and the detection wavelength was 340 nm (484 Tunable UV Detector, Waters, USA).

Chromatograms and Calibration

The HPLC chromatograms revealed that NF and the internal standard were eluted at 5.50 and 9.38 min, respectively. No interfering peaks were detected from the blank rabbit plasma, which indicated a good resolution and selectivity. A calibration curve of NF was constructed by plotting NF concentrations in the range of 0.05 to 5.00 μg/mL against the peak area ratio. The linear least-square regression line of the constructed standard curve was computed using the Baseline 810 computer program. The correlation coefficient, slope, and intercept were found to be 0.9980, 1.5743, and −0.0954, respectively. The percentage recoveries were found to be 87.4, 90.2, 105, 102.5, 100, 100.5, and 93.4% for 0.05, 0.1, 0.2, 0.4, 1.0, 2.0, and 5.0 μg/mL, respectively. The percentage coefficient of variation was 9.09 and 3.64% for 0.1 and 5.0 μg/mL, respectively. These data prove that the adopted method was sensitive, accurate, and precise.

Animal Treatment

Protocol

The study design was: single dose, fasting, five treatment, five period. Each rabbit was administered the five preparations with a one-week washout period between each preparation. Four rabbits were involved for each treatment.

Drug Administration

One capsule containing an amount of each preparation equivalent to 20 mg NF was administered to male albino rabbits weighing 2–2.5 kg, fasted for two nights before dosing and during the study with free access to water.

Blood Sampling

Blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8 and 10 hr from the eye vein using heparinized capillary tubes. The blood samples were collected in evacuated blood collection tubes and spiked with a trace of EDTA. Following centrifugation, the plasma was separated and immediately frozen at −20°C till analysis. The procedure was performed as described previously.

Pharmacokinetic Analysis

The individual pharmacokinetic parameters of NF were derived by non-compartmental analysis[20] using the WinNonLin-Pro 2.1 computer program (Pharsight, NC, USA). The following parameters were derived: the peak plasma concentration (C_{max}), the time to reach peak plasma concentration (t_{max}) (both observed values), (AUC_{0-10}) by the linear trapezoidal rule.

RESULTS AND DISCUSSION

Dissolution Study

Before determining the dissolution rate studies, the content of NF in each preparation was assayed by HPLC. The assay values were between 93% and 98% of the theoretical values.

Because of the low aqueous solubility of NF, it would not be possible or appropriate to evaluate the dissolution profiles of different types of NF preparations using the classical dissolution systems with the limited volume of dissolution media. Therefore, comparison of dissolution characteristics of different types of formulations would be difficult.[21] A flow-through cell dissolution system represented by USP apparatus # 4[22] offers a unique advantage by which large volumes of fresh dissolution medium can be used, to ensure perfect sink conditions. However, only one published report[23] has utilized this approach to evaluate NF in some oral marketed formulations. Accordingly, this technique has been adopted to evaluate the proposed dissolution-enhanced preparations.

The amounts of NF dissolved in simulated gastric fluid (pH 1.2) from solid dispersions containing PEG 6000 prepared by fusion and solvent methods were significantly higher when compared to the amount dissolved from pure NF (Fig. 1). The trend observed for solid dispersions prepared by fusion is that an increase in PEG 6000 percentage resulted in a significant increase in the dissolution rate (Fig. 1A). The maximum increase was found in case of 95% PEG 6000. The solid dispersions prepared by solvent method showed a significantly higher D_{24} and D_{156} than those prepared by fusion method (Table 1 and Fig. 1A and B). There was an approximately 9- and 12-fold increase in D_{156} over plain NF in the case of fusion and solvent methods, respectively. The high dissolution values obtained at 95% carrier may be

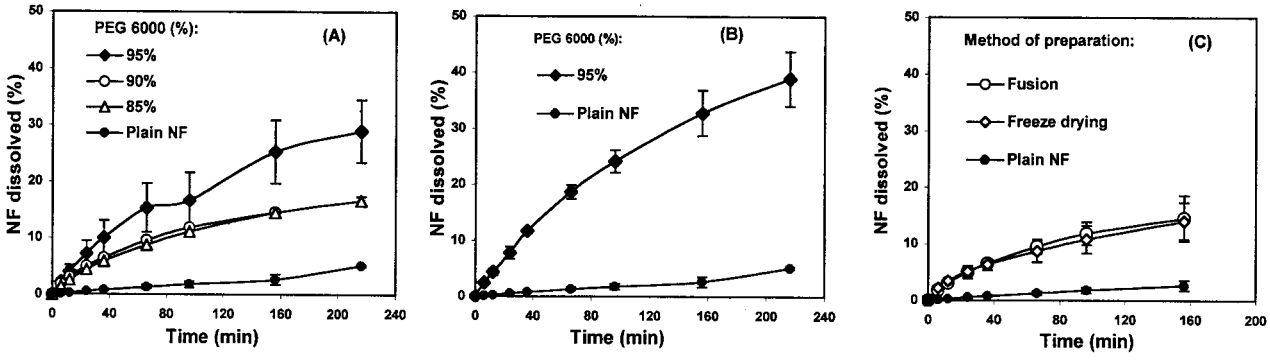

Figure 1. NF solid dispersions: effect of PEG 6000 content and method of preparation on the dissolution behavior using the flow-through cell. (A) Fusion; (B) solvent; (C) freeze-drying and fusion methods.

Table 1

Effect of Formulation Variables on Dissolution of Nifedipine from Different Preparations at 24 Min (D_{24}) and 156 Min (D_{156})

Carrier	Carrier (Conc.)	Method of Preparation	D_{24} NF Dissolved (%) Mean ± SD	D_{156} NF Dissolved (%) Mean ± SD
PEG 6000	95%	Fusion	7.25 ± 2.28	25.28 ± 5.59
	90%		4.99 ± 0.10	14.57 ± 0.23
	85%		4.41 ± 0.45	14.55 ± 0.59
	95%	Solvent	7.82 ± 1.11	32.83 ± 4.05
	90%	Freeze-drying	5.16 ± 0.59	14.09 ± 3.29
PEG MME 5000	95%	Fusion	3.83 ± 0.13	14.97 ± 2.43
	90%		3.47 ± 1.19	12.82 ± 1.37
	85%		3.14 ± 0.80	15.36 ± 3.69
	70%		1.80 ± 0.33	6.93 ± 1.50
	95%	Solvent	7.98 ± 0.11	26.99 ± 5.33
	90%		6.67 ± 0.01	23.31 ± 0.86
	85%		8.18 ± 1.14	28.40 ± 3.98
	70%		3.34 ± 0.50	9.91 ± 1.26
β-CyD	1:1 (molar ratio)	Physical mixture	1.84 ± 0.18	7.20 ± 0.16
	1:1 (molar ratio)	Kneaded complex	6.10 ± 0.30	31.13 ± 1.54
	1:1 (molar ratio)	Freeze-dried complex	3.45 ± 0.64	18.04 ± 1.37
SLS	1:1 (weight ratio)	Solid mixture	5.06 ± 0.13	13.23 ± 0.07
Plain NF		Pure drug	0.58 ± 0.13	2.67 ± 0.93

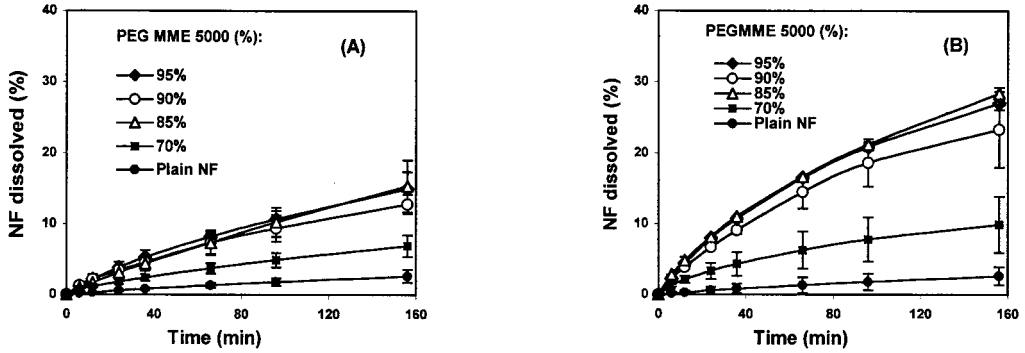

Figure 2. NF solid dispersions: effect of PEG MME 5000 content and method of preparation on the dissolution behavior using the flow-through cell. (A) Fusion; (B) solvent methods.

due to the formation of a critical mixture in a metastable form at the saturation point, the point at which a system exhibits maximum solubility.[10,24] Preparation of NF in 90% PEG 6000 by freeze-drying method (cf. Table 1 and Fig. 1C) reveals that there was no significant difference in the dissolution rate between the freeze-drying and fusion methods.

Solid dispersions of NF in 70% to 95% PEG MME 5000 have been prepared by fusion, and solvent methods. Figure 2 shows that relatively higher amounts of NF dissolved from the solid dispersions prepared by the two methods compared to the plain NF. Table 1 reveals that the method of preparation had no significant effect on D_{24} and D_{156} in case of NF/70% PEG MME 5000; while upon increasing

the carrier percentage the method of preparation showed significant differences in D_{24} and D_{156}. On the other hand, there were no significant differences between the effects of PEG MME 5000 at 85%, 90%, and 95% on D_{24} and D_{156}. This might indicate that 85% of NF/PEG MME 5000 is the optimum ratio, in which a complete absence of crystallinity of NF and thereby enormous increases in the solubility and dissolution rate of the drug has taken place.

A comparison between NF/95% PEG 6000 and NF/95% PEG MME 5000 reveals that there was a significant difference in D_{156} in case of the fusion and solvent methods (cf. Table 1). This might be due to the higher molecular weight of PEG 6000, causing more drug to be dissolved than by PEG MME 5000, leading to a greater percentage of drug in the molecularly dispersed form. In addition, the higher viscosity of the PEG 6000 hindered precipitation of the drug following dissolution of the carrier.[1]

Figure 3 shows that incorporation of NF in a solubilizer such as SLS led to enhancement of NF dissolution rate compared to the plain powder. The NF/SLS solid mixture exhibited a significantly higher D_{24} and D_{156} over the plain NF (cf. Table 1). The enhancement of the dissolution rate might be attributed to the fact that SLS results in a decrease in the surface tension, and an increase in the wettability and solubility of NF.[25]

Figure 4 shows that inclusion complexes of NF in β-CyD enhanced the dissolution rate of NF. According to the method of preparation, the enhancement effect was arranged in the following descending order: kneaded complex > freeze-dried complex > physical mixture > plain drug. Table 1 shows that there was a significant difference in D_{24} and D_{156} between the plain NF and all NF-β-CyD preparations. Also, there was a significant difference among the three NF-β-CyD preparations. It was found that the major particle size of NF-β-CyD prepared by freeze-drying method was less than 38 μm. However, the complex prepared by the kneading method, with a particle size of 150–90 μm, showed the most improvement in the dissolution rate. This might be attributed to the fact that the formation of an inclusion complex, which involves molecular encapsulation of the guest molecule by the cyclodextrin molecule,[26] was more pronounced in case of the kneading method than freeze-drying. Meanwhile, in case of the physical mixture, the

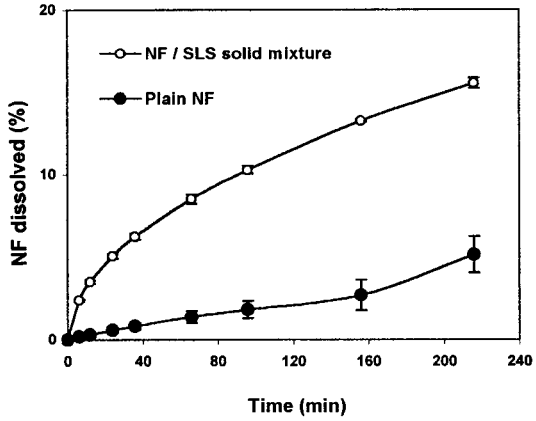

Figure 3. NF/SLS solid mixture: dissolution profile of NF using the flow-through cell.

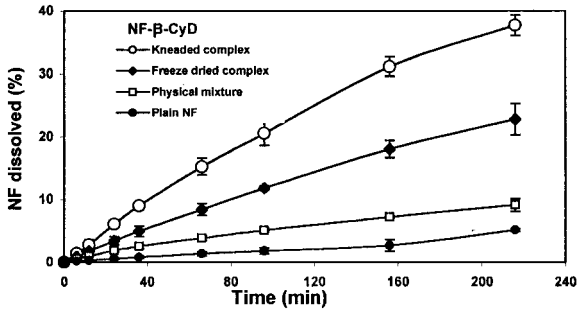

Figure 4. NF-β-CyD complex: dissolution profile of NF using the flow-through cell.

enhancement in the dissolution rate was due to the reduction in particle size during preparation of the mixture, and to the dilution of NF in the mixture.[26]

On comparing D_{24} and D_{156} of NF/SLS and NF-β-CyD, it was found that β-CyD was a better solubilizer for NF than SLS (cf. Table 1 and Figs. 3 and 4).

DSC Study

Figure 5 shows the DSC thermograms of a solid dispersion of NF in 95% PEG 6000 prepared by the fusion and solvent methods. In case of NF/PEG 6000 prepared by the fusion method, the characteristic endothermic peak of PEG 6000 at 71.77°C was recorded with ΔH of 237.71 J/g, which was lower than that of the pure polymer ($\Delta H = 282.69$ J/g).

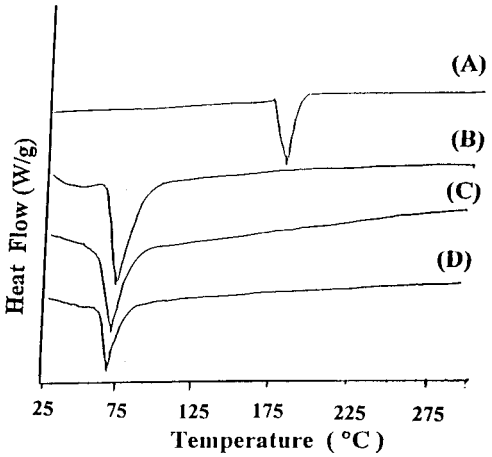

Figure 5. DSC thermograms of (A) plain NF; (B) PEG 6000; (C) NF/95% PEG 6000 solid dispersion by fusion method; (D) NF/95% PEG 6000 by solvent method.

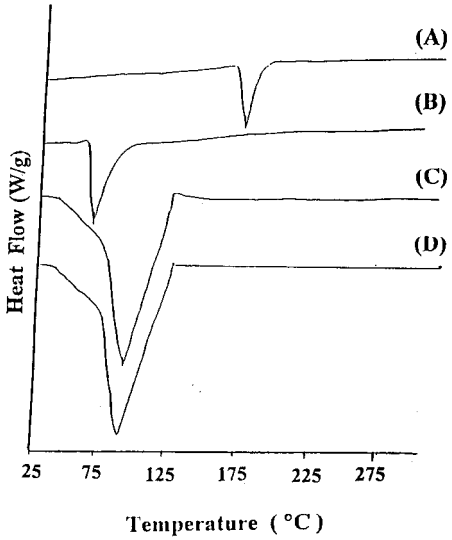

Figure 6. DSC thermograms of (A) plain NF; (B) PEG MME 5000; (C) NF/95% PEG MME 5000 solid dispersion by fusion method; (D) NF/95% PEG MME 5000 by solvent method.

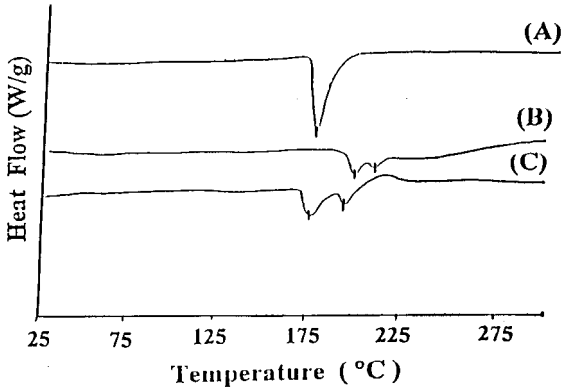

Figure 7. DSC thermograms of (A) plain NF; (B) SLS; (C) NF/SLS solid mixture.

The disappearance of the endothermic peak of NF demonstrates that NF could be dispersed homogeneously in an amorphous state and that no NF crystallizes out of the dispersion. The lower the ΔH value, the more amorphous the product is, and this agrees with the enhancement of the dissolution rate obtained. Almost the same relation was obtained in case of the solvent method.

For solid dispersions of NF in 95% PEG MME 5000 prepared by fusion and solvent methods (Fig. 6), the endothermic peak was shifted from 73.16 to 80.65°C and 81.81°C for fusion and solvent methods, respectively. The endothermic peak was highly broadened, more than that of the pure PEG MME 5000, with an increase of ΔH value from 290.82 to 566.81 and 517.51 J/g for the fusion and solvent methods, respectively. The higher value of ΔH for solid dispersions prepared from PEG MME 5000 accounted for the high crystallinity of the system, and hence the lower dissolution rates than the corresponding preparation using PEG 6000. The disappearance of the endothermic peak of NF from the thermogram indicates that NF is distributed homogeneously in an amorphous state within the solid dispersion.

The thermogram of SLS shows two endothermic peaks (Fig. 7). The incorporation of NF with SLS results in a slight shifting of the two endothermic peaks, and the endothermic peak of NF at 178°C disappeared (Fig. 7C). This might suggest that the enhancement of NF dissolution was attributed to the conversion of NF to an amorphous state (cf. Fig. 3).

The thermograms of β-CyD and NF-β-CyD kneaded complex are presented in Fig. 8. The NF-β-CyD complex showed that the intensity of the sharp endothermic peak of NF was markedly decreased compared to plain NF, however, the peak did not disappear completely. The value of ΔH was found to be 27.49, compared to 143.34 J/g for the plain NF. The reduction of the endothermic peak

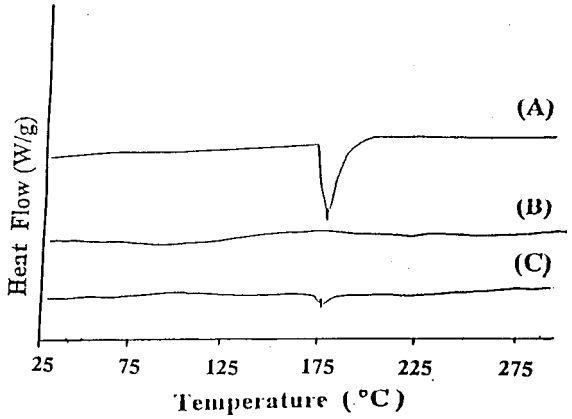

Figure 8. DSC thermograms of (A) plain NF; (B) β-CyD; (C) NF-β-CyD kneaded complex.

of NF might indicate the formation of an inclusion complex of NF in β-CyD. Therefore, the endothermic peak of NF was decreased since the crystalline drug molecule was contained within the cavity of the ring molecule of β-CyD.

X-ray Study

The x-ray diffraction pattern of NF (Fig. 9) reveals high crystallinity of the drug with major sharp diffraction peaks of high intensities and other peaks of lower intensities. Solid dispersions of NF in 95% PEG 6000 prepared by the fusion and solvent methods were nearly identical to that of PEG 6000, and the sharp crystalline peaks of NF were reduced.

Figure 10 shows the diffraction pattern of a solid dispersion of NF in 95% PEG MME 5000 prepared by fusion and solvent methods. It shows a diffraction pattern similar to that of PEG MME 5000, and the sharp crystalline peaks of NF were reduced, but it seemed that this reduction was less than PEG 6000 in the right-side region (cf. Figs. 9 and 10).

In general, x-ray diffraction of these solid dispersions revealed almost total loss of crystallinity of NF, and DSC thermograms supported the proposed crystalline changes.

Figure 11 shows the diffraction pattern of NF, SLS, and NF/SLS solid mixture. Both SLS and NF/SLS solid mixture showed crystalline regions. A semi-crystalline area could be detected in the solid mixture and new diffraction peaks appeared.

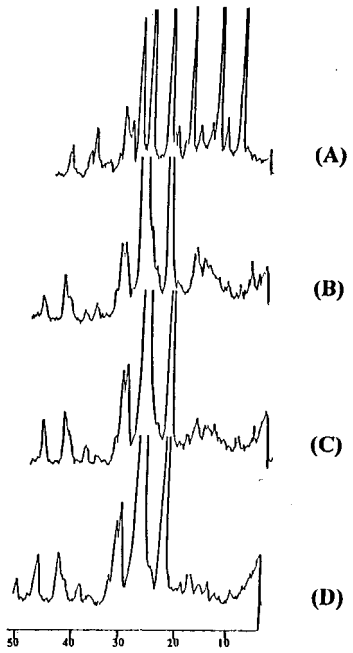

Figure 9. Powder x-ray diffraction pattern of (A) plain NF; (B) PEG 6000; (C) NF/95% PEG 6000 solid dispersion by fusion method; (D) NF/95% PEG 6000 by solvent method.

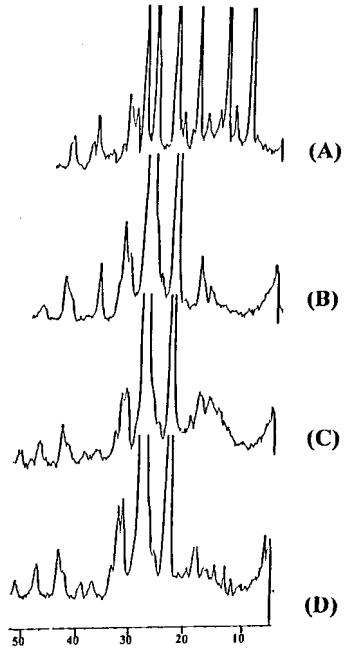

Figure 10. Powder x-ray diffraction pattern of (A) plain NF; (B) PEG MME 5000; (C) NF/95% PEG MME 5000 solid dispersion by fusion method; (D) NF/95% PEG MME 5000 by solvent method.

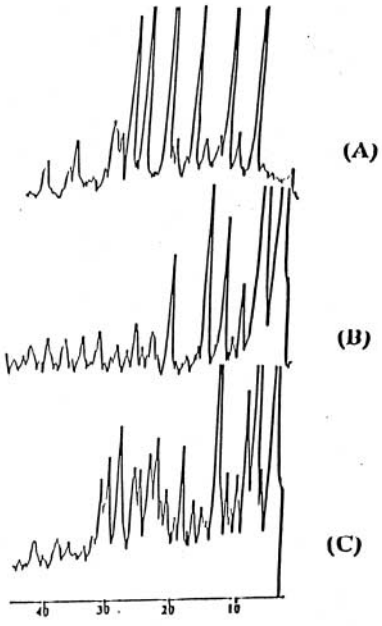

Figure 11. Powder x-ray diffraction pattern of (A) plain NF; (B) SLS; (C) NF/SLS solid mixture.

The x-ray diffraction pattern of NF-β-CyD kneaded complex is shown in Fig. 12. The x-ray pattern of the complex appeared to be different regarding the superposition of the NF and β-CyD patterns. These results confirm the formation of a new solid, from which we have the inclusion complex of NF inside the cyclodextrin cavity. On comparing the x-ray diffraction patterns of SLS and β-CyD, the obtained results might illustrate the lower dissolution rate of NF in SLS than that of NF-β-CyD complex.

Bioavailability Study

Certain preparations, which showed enhanced dissolution behavior and representing different preparation methods, were selected to enter the bioavailability study. These were NF solid dispersions in 95% PEG 6000 prepared by both the fusion and solvent methods, NF/SLS solid mixture, and NF-β-CyD kneaded complex. The plain NF was also considered for comparison.

The rabbit is an appropriate experimental animal for pharmacokinetic studies in certain situations.[27] While the elimination half-life of NF after administration of oral tablets has been found to vary between 6 and 11 hr in humans, it moves in a larger range, between 1.24 and 11.31 hr, in case of rabbits[28].

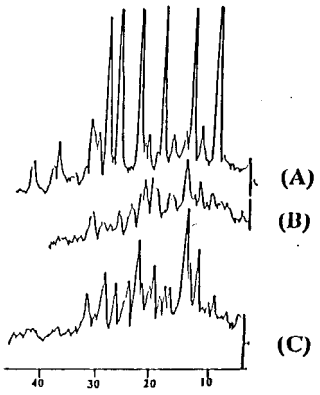

Figure 12. Powder x-ray diffraction pattern of (A) plain NF; (B) β-CyD; (C) NF-β-CyD kneaded complex.

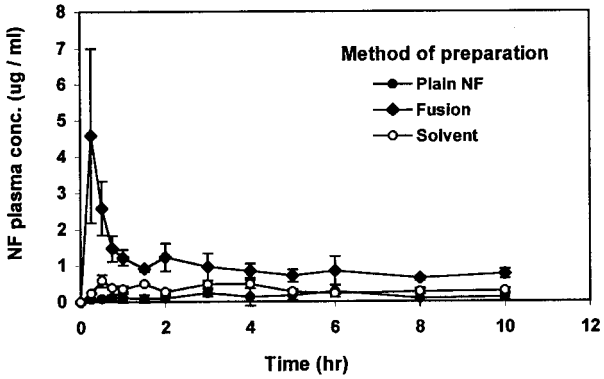

Figure 13. Mean NF plasma concentration following oral administration of NF/95% PEG 6000 (mean ± SE, $n = 4$).

Figure 13 shows the mean NF plasma concentration vs. time profiles following oral administration of plain NF and the selected solid dispersions. The results of pharmacokinetic parameters appear in Table 2. The data revealed that the mean C_{max} of plain NF was 0.39 μg/mL (range 0.19–0.59 μg/mL), which was lower than that of the prepared solid dispersions. There was an increase by 1.68- and 10.27-fold in C_{max} in case of NF/PEG 6000 prepared by solvent and fusion methods, respectively. The t_{max} was 3.33 hr (range 1–6 hr), compared to 0.58 hr (range 0.25–0.75 hr) and 1 hr (range 0.5–1.5 hr) for NF/PEG 6000 prepared by fusion

Table 2

Mean Pharmacokinetic Parameters of Nifedipine Following Oral Administration of Selected Preparations of Nifedipine

Preparation	Pharmacokinetic Parameters (Mean ± SD)[a]		
	CP_{max} (μg/mL)	t_{max} (hr)	$[AUC]_{0-10}$ (μg hr/mL)
Plain NF	0.39 ± 0.20 (0.19–0.59)	3.33 ± 2.52 (1.00–6.00)	1.51 ± 1.22 (0.17–2.58)
NF in 95% PEG 6000, prepared by fusion method	4.05 ± 3.79 (1.50–8.40)	0.58 ± 0.29 (0.25–0.75)	9.26 ± 2.89 (6.93–12.50)
NF in 95% PEG 6000, prepared by solvent method	0.67 ± 0.25 (0.49–0.84)	1.00 ± 0.71 (0.50–1.50)	3.14 ± 1.39 (2.16–4.12)
NF-β-CyD kneaded complex	0.90 ± 0.43 (0.44–1.43)	5.50 ± 5.21 (0.50–10.00)	3.86 ± 1.02 (2.52–4.97)
NF/SLS solid mixture	0.67 ± 0.20 (0.39–0.85)	4.38 ± 4.15 (1.00–10.00)	2.56 ± 1.36 (1.24–4.40)

[a]Ranges are shown in parentheses.

and solvent methods, respectively. The mean AUC_{0-10} of plain NF was found to be 1.5094 μg hr/mL (range 0.1696–2.576 μg hr/mL) and 9.26 μg hr/mL (range 6.9348–12.499 μg hr/mL) for NF/PEG 6000, prepared by fusion method, which was six times greater than the plain NF as shown in Table 2. On the other hand, in case of NF/PEG 6000 prepared by solvent method, the mean AUC_{0-10} was found to be only about twofold greater than the plain NF. This result (cf. Table 2) suggests that the gastrointestinal absorption of NF is enhanced due to the increased dissolution rate of NF from the prepared solid dispersions.

These in vivo results are contradictory to those expected according to the dissolution results (NF dissolution rate was higher from solid dispersions prepared by the solvent method than those obtained by fusion) (cf. Figs. 1 and 13). However, this is fortunate in view of the environmental and economic problems associated with the use of the solvent method.[29]

The study carried out by Watanabe et al.[9] indicated that administration of a matrix tablet containing a solid dispersion of NF in 83% PEG 6000 prepared by the fusion method resulted in an increase of the AUC_{0-10} by approximately four times over that of the powder form. This data agreed with the present bioavailability study, in which 95% PEG 6000 has been used.

Figure 14 shows the mean plasma concentration of NF after oral administration of a solid mixture of NF/SLS. The C_{max} was 0.6661 μg/mL (range 0.3853–0.8479 μg/mL), and the t_{max} was 4.3750 hr (1–10 hr) (cf. Table 2). Table 2 also shows that this mixture resulted in a 1.7-fold increase in the

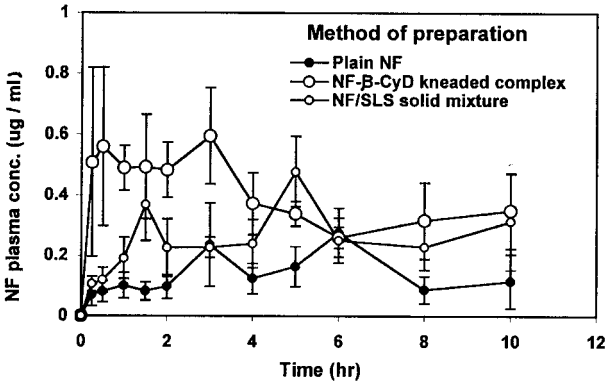

Figure 14. Mean NF plasma concentration following oral administration of NF-β-CyD kneaded complex and NF/SLS solid mixture (mean ± SE, $n = 4$).

AUC_{0-10}, with a mean value of 2.5618 μg hr/mL and ranging from 1.2378 to 4.3956 μg hr/mL.

Also, Fig. 14 shows the mean plasma concentrations following administration of NF-β-CyD kneaded complex. Table 2 shows that complexation of NF with β-CyD resulted in an increase of NF C_{max} compared to the plain drug by 2.3-fold. The C_{max} of the complex was found to be 0.9030 μg/mL (range 0.4395–1.4305 μg/mL), reached after 5.5 hr, t_{max} (range 0.5–10 hr). Table 2 also shows that the mean AUC_{0-10} of the complex was 2.6-fold higher than the plain drug, having a value of 3.8646 μg hr/mL (range 2.5228–4.9726 μg hr/mL).

A comparison of the mean AUC_{0-10} which is a measure of the overall bioavailability, indicates that the preparations could be arranged in the following

descending order: NF in 95% PEG 6000 fusion > NF-β-CyD kneaded complex > NF in 95% PEG 6000, solvent > NF/SLS solid mixture > NF plain powder (cf. Table 2).

In general, the results of NF bioavailability showed, in all cases, an intersubject variation which may be attributed, as in humans, to the variability in the rate of drug absorption.[30,31] Remunan et al.[28] also reported the existence of high intersubject variations during a bioequivalence study, in rabbits, of three sustained-release NF formulations compared to Adalat® Retard containing 20 mg NF. Kohri et al.[32] found that in the commercial fine granules containing 2 and 4 mg NF, the plasma levels varied noticeably among the rabbits and were reduced to undetectable levels at 8 hr in all cases.

CONCLUSION

Although a solid dispersion of NF/95% PEG 6000 prepared by the solvent method exhibited the highest in vitro dissolution enhancement, in vivo results revealed that a solid dispersion prepared by the fusion method gave a higher overall bioavailability than the solvent method preparation (cf. Figs. 1 and 13). However, it is known that the in vitro data and in vivo results are not always consistent; this might originate from the complexity of drug absorption or the weakness of the dissolution design.[33,34] Apart from this single exception, the in vivo data agreed well with the in vitro dissolution patterns and revealed that an increase of the absorption calculated as AUC_{0-10} was accompanied by an increase in the amount of NF dissolved. The NF-β-CyD complex possessed a higher enhancement capacity than the NF/SLS solid mixture, both in vitro and in vivo.

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