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DISSOLUTION AND ABSORPTION OF NIFEDIPINE FROM NIFEDIPINE-POLYVINYLPYRROLIDONE COPRECIPITATE

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ABSTRACT

Attempt was made to develop the solid dosage forms of nifedipine which showed good absorption rate and total bioavailability. Nifedipine is a poorly water-soluble drug, whose bioavailability is low when administered orally in the crystalline form. Solutions of nifedipine were well absorbed from the gastrointestinal tract. The dissolution rate of PVP-nifedipine copre-

cipitates exhibited rapid dissolution rate. X-ray diffraction data suggested the lack of crystallinity in the coprecipitate. The relation between the dissolution rates and average molecular weights of PVP was studied. Nifedipine in the coprecipitate was chemically stable to heat and humidity, but the dissolution rate of nifedipine from the coprecipitate stored at 21° and 75% R.H. markedly decreased. X-ray diffraction data revealed that it might be due to the transformation of amorphous form of nifedipine to crystalline form under higher relative humidity. The gastrointestinal absorption of nifedipine in beagle dogs after oral administration of the coprecipitate was increased than that after oral administration of the physical mixture.

INTRODUCTION

Dissolution studies have been receiving greater attention the last decade or two. Dissolution rates are shown to be a rate limiting step in the absorption of drugs from the gastrointestinal system by numerous workers (1). For drugs whose gastrointestinal absorption is rate limited by dissolution, a reducton of the particle size generally increases the rate of

absorption and its bioavailability. This commonly occurs for drugs with poor water-solubility.

Many methods to reduce the particle size have been reported. A unique approach of solid dispersion to reduce the particle size and increase the dissolution rates and absorptions of poorly water-soluble drugs was demonstrated (2). Coprecipitates of β-carotene and water-soluble polymers such as polyvinylpyrrolidone (PVP) prepared by evaporation from a $CHCl_3$ solution was exposed to water, then a colloidal dispersion of the drug was obtained (3). And now there are many studies concerning these solid dispersion and coprecipitate methods to reduce the particle size (4, 5, 6).

Nifedipine is a poorly water-soluble drug. Its solubility is about 11 mg/liter of water at 37°. In experiments on dogs (7), a ^{14}C-nifedipine gelatin capsule containing the crystalline compound was administered orally. The dogs excreted 15% of the administered activity. But in case of dogs which received gelatin capsule containing dissolved nifedipine, approximately 70% of the administered activity was eliminated via the kidney. From this result, it was found that the dosage form containing the crystal had poor bioavailability.

In this study the coprecipitate system was applied to develop the solid dosage forms of nifedipine which showed good absorption rate and total bioavailability.

MATERIALS AND METHODS

Materials

Nifedipine, mp 171-2°, on the market was used without further purification. Three grades of polyvinylpyrrolidone[1] were used as received. Other chemicals were used of J.P. IX or reagent grade.

Methods

All experiments were carried out in a dark room in view of the high sensitivity of nifedipine to light (8, 9).

Absorption in Rats

Nifedipine solutions (10 µg/ml) were prepared either in isotonic hydrochloride-citrate buffer at pH 2.0 for the stomach, or in isotonic phosphate buffer at pH 6.4 for small intestine.

Male Wister rats weighing 210-320 g were fasted for a whole night prior to experiments, but water was allowed freely. The rats were anesthetized with 20% urethan solution (0.6 ml/100 g body weight) and the small intestine was exposed by a mid-line abdominal incision. For experiments on the upper small intestine, the first 20 cm of the intestine beyond the ligament of Treiz was used. For experiments on the lower small intestine, a loop was prepared at the ileocecal junction and 20 cm proximally. Studies on gastric absorption were carried out by the in situ technique described by Schanker et al (10).

Three milliliters of a nifedipine buffer solution, previously warmed to 37°, were injected into the stomach or intestinal loops. After a given time, the solution was collected completely in a 100 ml measuring flask by washing with each buffer solution and allowed for assay. A half milliliter of blood was withdrawn at approximate time from the femoral artery using a polyethylene cannula. Nifedipine was assayed using a gas chromatograph equipped with an electron-capture detector (ECD-GC) according to a described procedure (11).

Preparation of Coprecipitates

All coprecipitate systems were prepared by the solvent method (4, 12). A suitable amount of the

nifedipine-carrier was weighed, dissolved in ethanol (minimum 99.5%) and then the solvent evaporated under reduced pressure below 50°. The residual solid was pulverized with a mortar and pestle, and various mesh fractions were collected. The coprecipitates were stored in an amber desiccator. The particle size of the nifedipine used as controls in the experimentation was 9.6 μm (determined by air permeatry). Physical mixtures were prepared by thoroughly mixing nifedipine (particle size: 9.6 μm) and PVP in the desired proportions.

Dissolution Study

A recycling and automatic recording system was used for the studies of the effect of particle size and of aging. The dissolution rate of nifedipine in different samples was run in 500 ml of water in a cylindrical beaker with a slightly concave bottom maintained at 37°. Sample powders containing the equivalent of 10 mg nifedipine were transferred directly into the dissolution medium and stirred at 150 rpm by means of four-bladed propeller. The solution was pumped at a rate of 50 ml/min through a glass filter stick (40-50 μm pore size) to a 0.5 cm flow cell and then back to the dissolution medium. The absorbance

of the solution was monitored by a recording spectrophotometer at 325 nm.

The dissolution method, which was used for the studies of the effect of nifedipine-PVP ratio and the effect of molecular weight of PVP, was the same beaker method as that reported by Sekikawa (4). An appropriate amount of the test sample containing 50 mg nifedipine equivalent was weighed and placed in 500 ml of water maintained at 25°. At various suitable time intervals, 5 ml of the sample solution were pipetted, and replaced by an equal volume of fresh water. The pipetted solution was then filtered (Millipore, 0.22 μm pore size) and it was assayed after appropriate dilution by means of a spectrophotometer at 340 nm.

Physical Measurements

To characterize nifedipine-PVP coprecipitates and physical mixture, solid samples were analyzed by X-ray powder diffractmetry[2] and differential thermal analysis.[3]

Chemical Stability

The coprecipitate and the physical mixture, both containing 1:3 ratio of nifedipine to PVP, were stored in air tight glass containers at 40°, or kept in a

desiccator under 75% relative humidity(R.H.) at 21°. The latter condition was attained by using a saturated solution of sodium chloride.

The chemical stability of nifedipine in each sample during storage was studied using thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). Silica gel TLC plates[4] were spotted with methanol solutions of standard nifedipine and each stored sample. These plates were placed in a chamber containing AcOEt:CCl_4:NH_4OH (80:60:1). After development, the plates were removed and dried, and then spots were visualized under ultraviolet light at 254 nm. Contents of nifedipine in the stored samples were determined by HPLC. Each sample was diluted with the methanol solution of m-dinitrobenzene (internal standard), and an aliquot of the sample solution was injected into HPLC system[5].

Plasma Level in Beagle Dog

Beagle dogs, 8-12 kg, were fasted for 24 hr prior to the experiment, but were allowed free access to water. Each dog was orally administered the test preparation containing 10 mg of nifedipine and 30 mg of PVP with 100 ml of water. Plasma samples were obtained at 20, 40, 60, 120 and 240 min after the

administration. The second administration were given 8 days after the initial administrations by the cross-over arrangement. Plasma samples were assayed for nifedipine by ECD-GC (11).

RESULTS AND DISCUSSION

Absorption of Dissolved Nifedipine from Rat Gastrointestinal Tract

It was reported that dosage forms containing crystalline nifedipine showed poor bioavailability (7). It may be due to its poor water-solubility. If this assumption is correct, the absorption of dissolved nifedipine from the gastrointestinal tract must be good. To confirm this assumption, the disappearance of nifedipine from the rat stomach, upper or lower small intestine were examined using the *in situ* ligatured loop method.

About 37% of the dose disappeared from the solution injected in the rat stomach in 40 min and approximately 92-94% of the dose disappeared from the upper or lower small intestine (Table 1). Generally, the disappeared amount may be equal to the absorbed amount. It was assured from these *in situ* experiments that dissolved nifedipine was well absorbed. Further,

TABLE 1

Absorption of Nifedipine from Different Sites of Rat Gastrointestinal Tract

Site	Residual % at 40 min	Blood level, ng/ml			
		5min	10min	20 min	40 min
Stomach	62.9 (6.7)	15.4 (2.8)	19.5 (3.1)	23.8 (4.7)	31.8 (2.1)
Upper small intestine	8.3 (1.0)	86.3 (13.2)	68.7 (11.6)	68.2 (17.0)	53.4 (15.8)
Lower small intestine	6.0 (1.4)	85.9 (7.3)	76.0 (8.7)	64.7 (7.9)	43.7 (1.3)

Average data for 3 to 4 experiments are represented and the standard errors of the mean are given in parentheses.

to confirm these results, the time courses of the blood levels of nifedipine were observed (Table 1).

These results indicate that dissolved nifedipine is well absorbed and its absorption rate is rapid. Therefore, it can be expected that bioavailability will be correlated with the dissolution of the dosage form.

Selection of Coprecipitate Carrier

Water-soluble polymers, such as PVP, hydroxypropylcellulose and polyethylene glycol, and organic acids such as citric and succinic acid have been proposed as possible dispersion matrices for water-

insoluble drugs (12). In the preliminary dissolution experiments on nifedipine dispersions prepared with these carriers, it was found that the PVP coprecipitate gave the fastest release rates.

X-ray Diffraction Pattern

X-ray diffraction patterns of pure nifedipine, PVP (K-30), their physical mixture and coprecipitates in different weight ratios are shown in Figure 1. Pure nifedipine and PVP proved to have crystalline and amorphous forms, respectively, by these patterns. A marked difference in X-ray diffraction patterns containing 1:3 and 1:9 weight ratios of nifedipine to PVP was observed. The data indicates the lack of crystallinity in these ratios. In the measurement with differential thermal analysis (DTA), endothermic peak accompanied by the melting of nifedipine (171°) disappeared in the coprecipitate containing 1:3 ratio of nifedipine to PVP. From this DTA result, it was reconfirmed that nifedipine is probably present in the amorphous form in PVP matrix. But in systems composed of nifedipine to PVP ratios of 1:0.5 and 1:1, a part of nifedipine might be present in the crystalline form. From these results, it was found that contents of PVP in the coprecipitate system should be more than 75 w/w%.

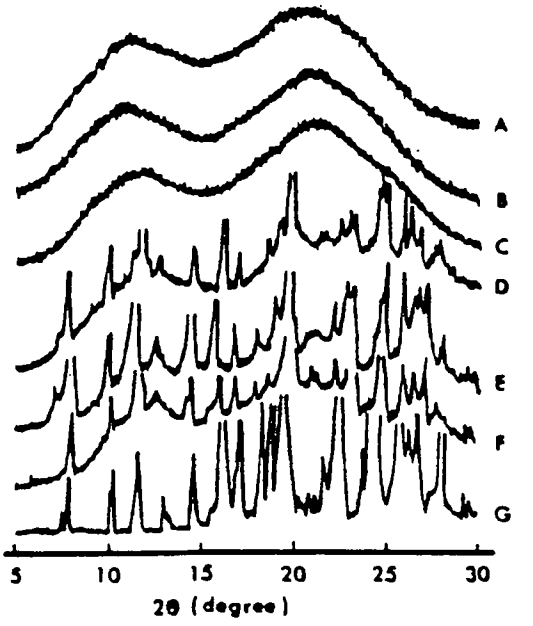

FIGURE 1

X-Ray Diffraction Patterns for (A) PVP alone, (B) 1:9 w/w Nifedipine-PVP Coprecipitate, (C) 1:3 w/w Coprecipitate, (D) 1:1 w/w Coprecipitate, (E) 1:0.5 w/w Coprecipitate, (F) 1:3 w/w Nifedipine-PVP Physical Mixture, (G) Nifedipine alone.

For this reason, 1:3 ratio of nifedipine to PVP was utilized for further studies in this report.

Dissolution Rate of Coprecipitate

All dissolution studies were based on three or four tests and were highly reproducible. So only average values are reported.

(1) Effect of particle size ——— It was reported that dissolution rates of griseofulvin from solid dispersions were found to be markedly affected by the particle size of solid dispersion, that is, the smaller the size, the faster the dissolution rate (13). So the dissolution characteristics of nifedipine-PVP (K-30) coprecipitates in various particle sizes, 12-16 mesh, 48-60 mesh, and less than 145 mesh size, were investigated.

As shown in Figure 2, all particle size samples were found to dissolve in a few minutes. However, the dissolution rate of the coprecipitate in 12-16 mesh size was slower than that in 48-60 mesh size. It would be due to the small surface area of the sample

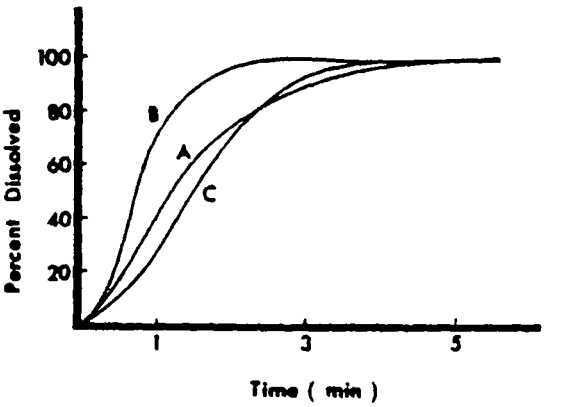

FIGURE 2

Dissolution Rates of 1:3 w/w Nifedipine-PVP Coprecipi-tates (amount equivalent to 10 mg of nifedipine) at 37°. Mesh Size: (A) 12-26 mesh, (B) 48-60 mesh, (C) less than 145 mesh.

in 12-16 mesh size. On the other hand, the dissolution rate of the particle less than 145 mesh size was nearly the same as that in 12-16 mesh size. It might be due to the fact that the small particle did not sink rapidly into the dissolution medium and drifted on the surface of the medium for a longer period.

In any case, as shown in Figure 2, it was found that particle size of the coprecipitate would have little effect on the dissolution rate of the drug. So the 60-100 mesh size fraction was used for further study in this paper.

(2) Effect of ratio of nifedipine to PVP ——— Figure 3 shows that the coprecipitates containing 1:3, 1:5 and 1:9 ratio of nifedipine to PVP (K-30) dissolved rapidly in the initial stage of dissolution, and yielded supersaturated concentrations. Then, the concentrations of nifedipine attained gradually to a constant value which were nearly equal to the solubility.

The binary system containing the 1:1 ratio, the physical mixture and pure nifedipine showed almost the identical behavior. The solubilities of nifedipine from these systems were much lower than that of the coprecipitate containing 1:3 ratio.

The solubility of nifedipine in water, in 0.5% and in 1% PVP aqueous solution at 37° was 11.5, 14.6 and 17.0 μg/ml respectively. These data showed that PVP

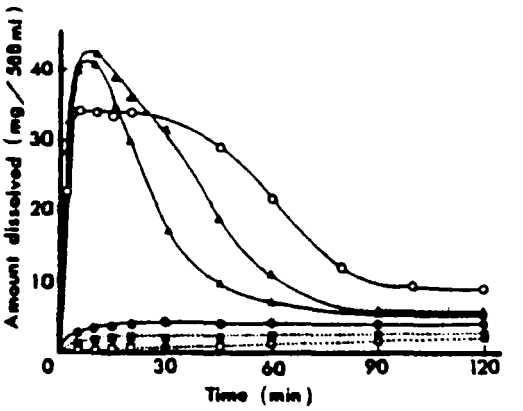

FIGURE 3

Dissolution Rates of Nifedipine from Test Preparations (amount equivalent to 50 mg of nifedipine) at 25°. Key: (△) 1:9 w/w nifedipine-PVP coprecipitate, (▲) 1:5 w/w coprecipitate, (○) 1:3 w/w coprecipitate, (●) 1:1 w/w coprecipitate, (■) 1:3 w/w physical mixture, (□) nifedipine alone (9.6 μm).

in the coprecipitate could hardly affect the solubility of nifedipine, because the amount of PVP in the medium (500 ml) after the dissolution of the 1:3 ratio coprecipitate was only 150 mg that was equivalent to 0.03% PVP aqueous solution. So it was confirmed that the rapid increase of solubility in Figure 3 was due to the coprecipitate formation.

(3) Effect of molecular weight of PVP ——— Since wide ranges of molecular weight of PVP are available, it was important to determine if any behavioral differences existed with a variation in molecular weight. To this end, coprecipitates containing 1:3 ratio of

nifedipine to PVP, using PVP of 10000, 40000 and 360000 average molecular weight, were prepared and their dissolution rates were determined (Figure 4). It was found that the molecular weight indeed significantly changed the dissolution rate of nifedipine, the 40000-molecular weight PVP being the most rapid of the three systems.

The inhibitory effect of PVP on the crystallization of several drugs to clarify the mechanism of coprecipitation of the drug with PVP was reported (5). The inhibitory effect decreased in the following molecular weight order; 40000>360000>10000. This order

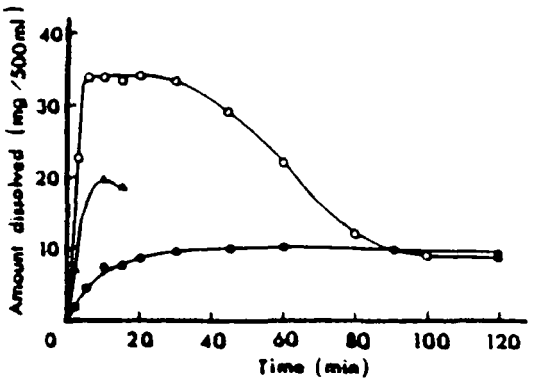

FIGURE 4

Dissolution Rates of 1:3 w/w Nifedipine-PVP Coprecipitates (amount equivalent to 50 mg of nifedipine) at 25° Average molecular weight of PVP: (●) 10000, (○) 40000, (△) 360000.

is consistent with the molecular weight order to affect the dissolution rate of nifedipine as shown in Figure 4.

These results indicate that the effect reaches maximum at a certain molecular weight, and decreases with further increases in molecular weight. For this reason the 40000-molecular weight PVP (K-30) was utilized for all studies reported in this paper.

Stability of Coprecipitate

These amorphous system may crystallize upon aging over a long period of time, especially under higher relative humidity. One would expect that a return to the crystalline state would result in a decrease in the dissolution rate of the system. The effect of aging or storage under various condition on the fast-release characteristics and chemical stability of the drug in the coprecipitate system had not previously been reported extensively.

Aging effects of corticosteroids utilizing sugar glass dispersion were reported (14). In their investigation it was revealed that the glass dispersion samples after storage for 30 days at 25° showed no decrease in the dissolution rate. But, this storage condition was very short-term. Further, it is neces-

sary to store the sample under high relative humidity to realize the transformation from the amorphous state to crystalline state.

In this section it was undertaken to evaluate the chemical stability of nifedipine in the coprecipitate and the effect of aging on the dissolution rate of the coprecipitate.

(1) Chemical stability ——— Any decomposition product in the coprecipitate containing 1:3 ratio of nifedipine to PVP after storage at 40°, or at 21° and 75% R.H. for 6 months respectively was not revealed by TLC. Further, the assay results of the coprecipitate by HPLC showed that nifedipine was stable under these conditions. Detail will be reported in the next report.

(2) Dissolution property ——— The coprecipitate samples containing 1:3 ratio of nifedipine to PVP were stored at 40°, and at 21° and 75% R.H. for 6 months. Forty milligram of each sample (equivalent to 10 mg of nifedipine) were dissolved in 500 ml of water. Storage at 40° for 6 months did not have any effects on the dissolution profiles of coprecipitates, so it was suggested that amorphous nifedipine in the coprecipitates was stable to heat. On the other hand, the storage at 21° and 75% R.H. resulted in marked changes of dissolution profiles as shown in Figure 5. It was

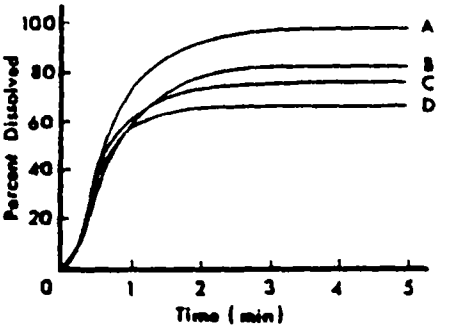

FIGURE 5

Dissolution Rates of 1:3 w/w Nifedipine-PVP Coprecipitates (amount equivalent to 10 mg of nifedipine) at 37°. The samples were stored at 21° and 75% R.H. for different durations. Key: (A) initial, (B) 0.5 month, (C) 1.5 months, (D) 4 months.

observed that the percent dissolved had decreased rapidly under storage condition.

This might be due to the formation of micro crystals of nifedipine, so X-ray diffraction patterns of the samples stored under humid condition were measured to examine the crystalline state of nifedipine. By the X-ray diffraction patterns of the stored samples, whose dissolution rates decreased markedly, many peaks which showed the presence of nifedipine crystals were observed.

The endotherm for nifedipine melting which is absent in the fresh coprecipitate would occur in the aged material. By measurement of its area we would be able to estimate the amount of crystalline material

present. So, we measured the DTA curves of the coprecipitate, its aged sample, and the physical mixture containing 1:3 ratio of nifedipine to PVP. But, we could not notice the endothermic peak accompanied by the nifedipine melting of the physical mixture. This result, that even the endothermic peak of the physical mixture dissapeared, shows that the crystalline form of nifedipine turns to the amorphous form during the sample heating with PVP. From these results, we could not estimate the amount of crystalline material present, and correlate with the data in Figure 5.

Plasma Level of Coprecipitate

In order to ascertain the effect of coprecipitate system on the bioavailability, the gastrointestinal absorption of nifedipine from the coprecipitate in beagle dogs was compared with that from the physical mixture. Mean plasma levels of nifedipine after oral administrations of 40 mg of the test samples (containing 1:3 ratio of nifedipine to PVP) to four dogs are shown in Figure 6 and Table 2. Three variables were examined in a bioavailability assesment using the plasma level data. These included: area under the time-plasma level curve (AUC) from 0 to 240min, maximum

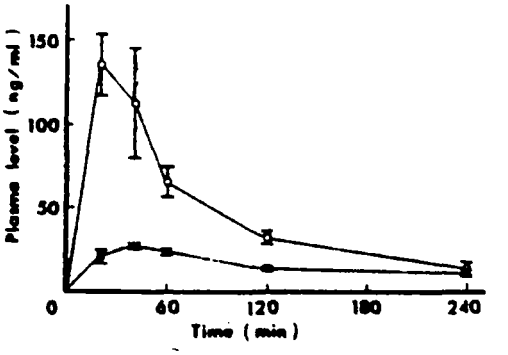

FIGURE 6

Plasma Levels of Nifedipine after Oral Administration of 40 mg of the Test Preparation in Dogs. Key: (○) 1:3 w/w nifedipine-PVP coprecipitate (60-100 mesh), (■) 1:3 w/w physical mixture. Each point indicates the mean of 4 experiments with the standard error of the mean.

TABLE 2

Statistical Comparison of Plasma Levels, Cmax, Tmax and AUC_{0-240} min.

	Plasma level, ng/ml		Significance
	Coprecipitate	Physical mixture	
20 min	135.1 (17.8)	20.3 (3.9)	P<0.001
40 min	111.9 (32.5)	27.5 (1.4)	P<0.05
60 min	64.9 (9.1)	24.0 (2.6)	P<0.01
120 min	31.7 (3.0)	14.0 (0.8)	P<0.005
240 min	13.4 (3.5)	11.7 (3.2)	N.S.[a]
Cmax, ng/ml	143.8 (25.0)	28.0 (1.5)	P<0.005
Tmax, minute	25.0 (5.0)	30.0 (5.8)	N.S.[a]
AUC_{0-240} min, μg·min/ml	11.1 (1.4)	3.8 (0.4)	P<0.005

Average data for 4 dogs are represented and the standard errors of the mean are given in parentheses.

[a] no significance

observed concentration (Cmax) and time to attain maximum ovserved level (Tmax).

Cmax and AUC of the coprecipitate increased about 5-fold and about 3-fold, respectively, more than those of the physical mixture. Statistical significant differences between the coprecipitate and the physical mixture were revealed in plasma levels beyond 120 min, Cmax and $AUC_{0-240\ min}$, although there was no significance in Tmax (Table 2). The reason for no significant difference in Tmax may be due to the fact that plasma levels of the physical mixture were so low that a sharp peak level could not be observed.

The results obtained in this absorption study are consistent with those of the dissolution study in Figure 3. From this absorption study, it was confirmed that the coprecipitate system was a good method to improve the bioavailability of nifedipine.

ACKNOWLEDGEMENT

The authors are grateful to Dr. I. Utsumi, Director of this Research Center, for his helpful advice and discussions throughout this work.

FOOTNOTES

1 PVP K-15, K-30 and K-90, average molecular weights are 10000, 40000 and 360000 respectively (Gokyo Traiding Co., Ltd., Osaka, Japan).

2 Rigaku Denki Geigerflex 2027 (Rigaku Denki Ltd., Tokyo, Japan) using Ni filtered Cu Kα radiation, Voltage 30 kV, Current 10 mA.

3 Shimadzu thermal analyzer DT 20B (Shimadzu Seisaku-sho Ltd., Kyoto, Japan).

4 Art. 5715, Dc-Fertigplatten Kieselgel 60 F_{254}, (E. Merck, Darmstadt).

5 Liquid chromatograph model 440 equipped with μBondapak C_{18} (Water Associates, Inc., Milford Massachusette). Detection, UV at 254 nm; eluent, MeOH:H_2O (60:40); flow rate, 1.1 ml/min.

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