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Inhibitory Effect of 2-Hydroxypropyl-β-cyclodextrin on Crystal-growth of Nifedipine During Storage: Superior Dissolution and Oral Bioavailability Compared with Polyvinylpyrrolidone K-30

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Abstract—To prevent the crystal-growth of nifedipine during storage, 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD) was employed as a hydrophilic drug carrier and compared with polyvinylpyrrolidone K-30 (PVP). Amorphous nifedipine powders were prepared by spray-drying with HP-β-CyD or PVP, and their crystal-growing behaviour at accelerated storage conditions were examined by X-ray diffraction analysis and microscopy. Although PVP initially retarded the crystallization of nifedipine, it failed to control the increase of crystal size after prolonged storage at 60°C, 75% r.h., resulting in a remarkable decrease in dissolution rate in water. In sharp contrast, a relatively fine and uniform size of nifedipine crystals was maintained in the HP-β-CyD system even after accelerated storage conditions. The enhanced dissolution observed for all the HP-β-CyD systems in a dissolution medium containing 0·1% non-ionic surfactant HCO-60 were clearly reflected in the in-vivo absorption of nifedipine following oral administration to dogs. These results suggest that HP-β-CyD is particularly useful in solving problems encountered on storage of amorphous nifedipine in solid dosage forms.

The bioavailability of nifedipine, a potent calcium antagonist, is significantly enhanced when administered orally in the amorphous form (Sugimoto et al 1982). However, amorphous nifedipine in a polyvinylpyrrolidone (PVP) matrix gradually crystallizes at high temperatures and high humidity, resulting in inferior dissolution and bioavailability due to this increase in crystallinity (Sugimoto et al 1981). Therefore, the prevention of crystal-growth during storage is particularly important to maintain the improved dissolution and bioavailability of poorly water-soluble drugs (Imaizumi et al 1983). We have recently reported that whisker-growth of isosorbide 5-mononitrate in solid form was markedly inhibited by binding to β-cyclodextrin (β-CyD) (Uekama et al 1985). β-CyD is a cyclic oligomer of seven glucose units which forms inclusion complexes with poorly water-soluble drugs and thus improves their water solubilities (Szejtli 1981). Widespread uses of β-CyD are, however, restricted by its low aqueous solubility (Uekama & Otagiri 1987; Duchêne 1987), and hydroxyalkylation of the hydroxy groups of β-CyD has been used to solve the problem of low solubility (Pitha 1988). Among the various hydroxyalkylated derivatives evaluated (Yoshida et al 1989), 2-hydroxypropyl-β-CyD (HP-β-CyD), which is an amorphous mixture of chemically related components, has gained some acceptance as a hydrophilic drug carrier in pharmaceutical applications, since its physicochemical properties and biological features have been well demonstrated (Brewster et al 1989; Uekama & Irie 1990). In the present study, an attempt was made to control the crystallization of amorphous nifedipine in spray-dried powders by HP-β-CyD during accelerated storage conditions.

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Materials and Methods

Nifedipine was donated by Bayer Yakuhin Ltd (Osaka, Japan), HP-β-CyD, with an average degree of substitution of 5·8, was purchased from Nippon Shokuhin Kako Co. (Tokyo, Japan), and PVP K-30 (average mol. wt 40 000) was from Nakarai Tesque, Inc. (Kyoto, Japan). Other materials and solvents were of analytical reagent grade. All experiments were carried out under light-protected conditions to prevent the photo-decomposition of nifedipine.

Preparation of amorphous nifedipine powders

Nifedipine powders were prepared by spray-drying (Lin & Kao 1989). Nifedipine and HP-β-CyD (1:4, w/w 1:1, m/m) or nifedipine and PVP (1:4, w/w), were dissolved in ethanol-dichloromethane (1:1, v/v) separately, and subjected to spray-drying, using a Pulvis GA32 Yamato spray-dryer (Tokyo, Japan). The drying conditions were as follows. Air flow rate: 0·45 m^3 min^{-1}, air pressure: 1·0 kg cm^{-2}, inlet and outlet temperatures: 85 and 55°C, respectively. Nifedipine in the spray-dried products thus obtained was confirmed to be amorphous by X-ray diffraction analysis. The spray-dried products of nifedipine with PVP K-60 and K-90 were very difficult to handle because of their viscous properties.

Ageing studies

The test powders (150 mg, < 100 mesh) were placed in glass containers in the desiccators at constant relative humidity (r.h.), and then stored in incubators at constant temperature. The accelerated storage conditions were 60°C and 75% r.h. At appropriate time intervals samples were withdrawn and used in the further studies. Samples were dried under reduced pressure at room temperature (25°C) for 24 h in phosphorous pentoxide desiccators before each experiment and water content of the powder thus determined by measuring weight change during the storage.

Measurements of crystal-growth behaviour

The X-ray diffraction profiles were measured using a Rigaku Denki Geiger Flex 2012 diffractometer (Tokyo, Japan) with Ni-filtered Cu-K α radiation. The degree of crystallinity of nifedipine in the physical mixture was taken as 100% and initial amorphous material was considered to have 0% crystallinity. The X-ray diffraction peaks of the internal standard sample (S_i) were measured and plots were made of the ratio of the peak height at $2\theta = 11{\cdot}8°$ due to the nifedipine crystal form and at $2\theta = 28{\cdot}4°$ due to S_i, the fractional crystallization (α) of the sample was obtained by the use of calibration plots. Microscopic observations on nifedipine crystals were performed using an Olympus BH-2 microscope (Tokyo, Japan) after removal of water-soluble ingredients by 3 min suspension of the sample in water; no appreciable change in crystal size was observed during this wash.

Dissolution studies

The dissolution test was essentially performed according to the paddle method in Japanese Pharmacopoeia XI, using an automatic dissolution testing apparatus described recently (Uekama et al 1990) at 37°C and with a stirring speed of 100 rev min^{-1}. Tablets (4 mm diameter) were prepared by compressing sample powder (5 mg nifedipine) under a pressure of 50 kg cm^{-2} in a hydraulic press, using a model R-303, Riken Seiki (Tokyo, Japan). A test tablet was placed in the bottom of the reservoir, and the dissolution medium (900 mL of water containing 0·1% non-ionic surfactant HCO-60, surface tension 50 dyne cm^{-1} (Takada & Watanabe 1987)) was continuously pumped through a glass filter stick (G3; $\phi = 20$–30 μm) at a flow rate of 3 mL min^{-1} to the flow cell in a UV detector and then back to the reservoir. The lag time from the reservoir to the UV monitor was 0·5 min. The

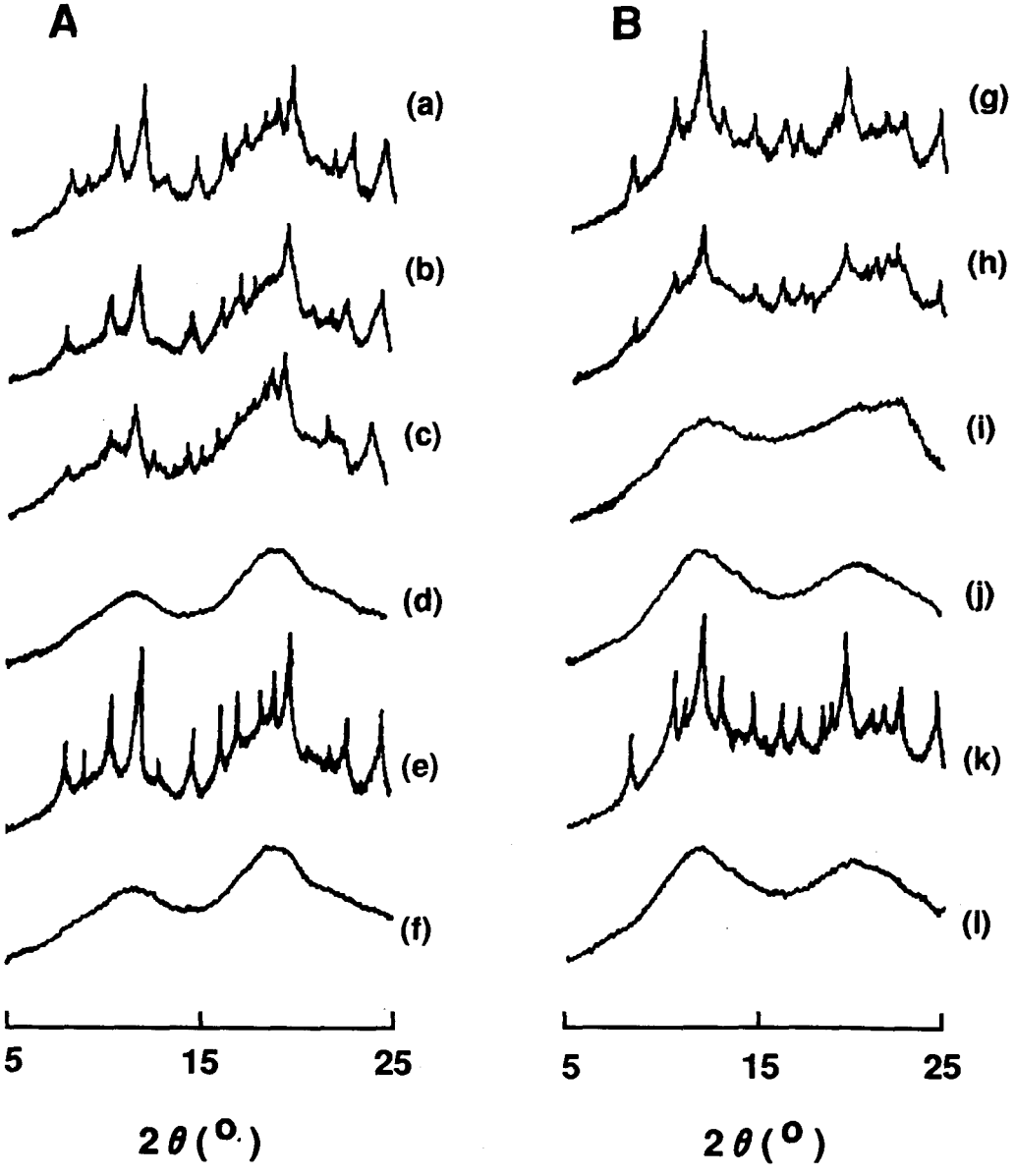

FIG. 1. Powder X-ray diffraction patterns of nifedipine-HP-β-CyD and nifedipine-PVP, stored at 60°C and 75% r.h. A, HP-β-CyD system. B, PVP system. Storage period, (a): 72 h. (b): 24 h. (c): 12 h. (d): initial. (e): physical mixture of nifedipine crystals and HP-β-CyD. (f): HP-β-CyD alone stored for 14 days. (g): 14 days. (h): 7 days. (i): 3 days. (j): initial. (k): physical mixture of nifedipine crystals and PVP. (l): PVP alone stored for 14 days.

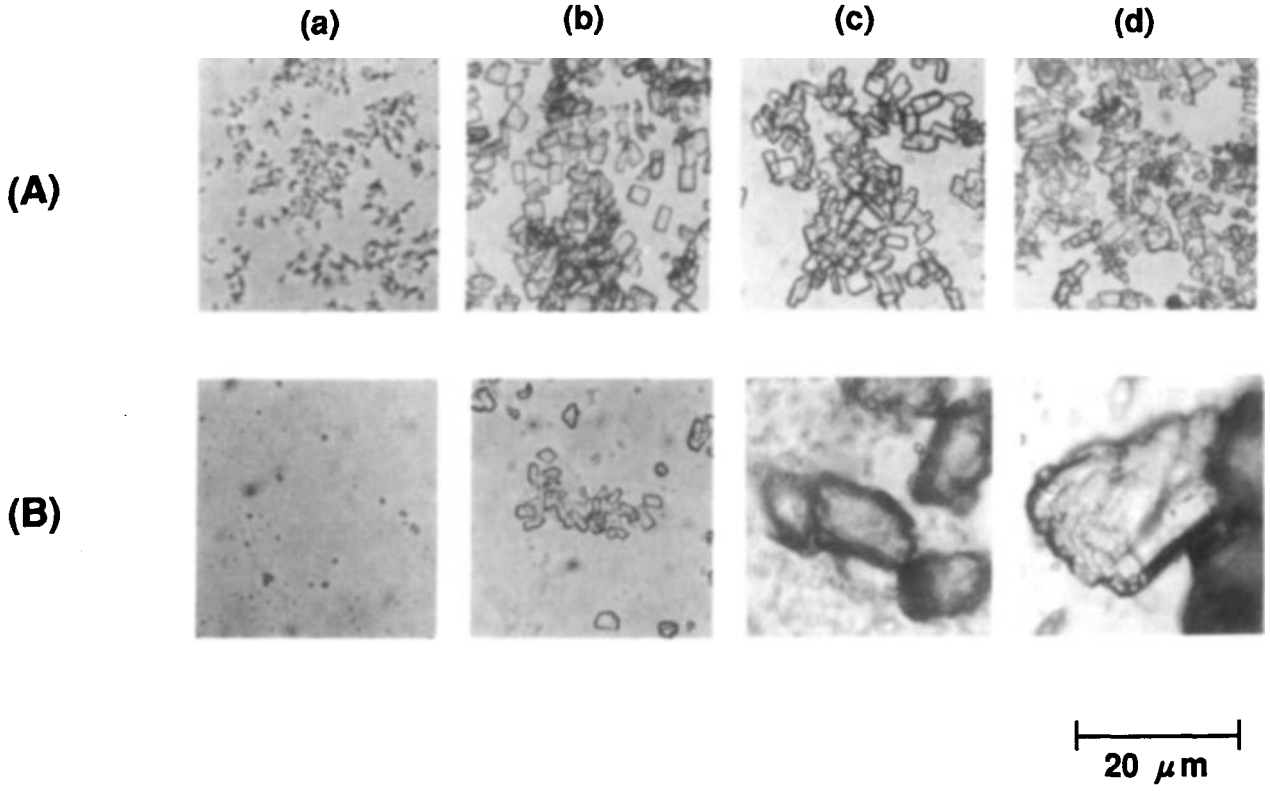

FIG. 2. Photomicrographs of nifedipine crystals in nifedipine-HP-β-CyD and nifedipine-PVP, stored at 60°C and 75% r.h. A, HP-β-CyD system. B, PVP system. Storage period, (a): initial. (b): 7 days. (c): 14 days. (d): 28 days.

amount of nifedipine dissolved in the medium was automatically measured by a UV monitor at a wavelength of 254 nm. The sink condition was maintained, since the amount of nifedipine dissolved in the medium was lower than its solubility.

In-vivo absorption studies

Male beagle dogs, 11–14 kg, fasted for 24 h, but allowed free access to water, received a test tablet (diameter: 7 mm), equivalent to 20 mg of nifedipine with 50 mL of water. The relatively high dose was needed to obtain blood levels appropriate for analytical detection and pharmacokinetic analysis. Plasma samples were collected at appropriate intervals (Fig. 5) after administration. Doses were administered by the crossover arrangement after a time interval of one week. Plasma samples were assayed for nifedipine by gas chromatography with electron-capture detection (Kondo et al 1980), using a Shimadzu GC-7A (Kyoto, Japan).

Results and Discussion

Crystal-growth of nifedipine during storage

As a typical example, the X-ray diffraction patterns of spray-dried products are shown in Fig. 1. The diffraction patterns of HP-β-CyD and PVP showed no sharp peak, while those of physical mixtures in which nifedipine was simply blended with HP-β-CyD or PVP, showed many sharp diffraction peaks due to nifedipine crystals. In the case of both spray-dried products before storage, no sharp peaks attributable to nifedipine were observed, indicating that nifedipine crystals were transformed to the amorphous state during the spray-drying process. However, sharp diffraction peaks gradually appeared with increasing storage time; rapid crystallization of nifedipine in the HP-β-CyD system was observed compared with that in the PVP system. Fig. 2 shows the photomicrograph of nifedipine crystals in both crystallization systems. Nifedipine crystals in the HP-β-CyD system could be observed after storage for 12 h. The average crystal size was estimated to be about 5·5 μm, and no obvious crystal growth was observed for further stored samples. On the other hand, an increase in crystal size was observed for the

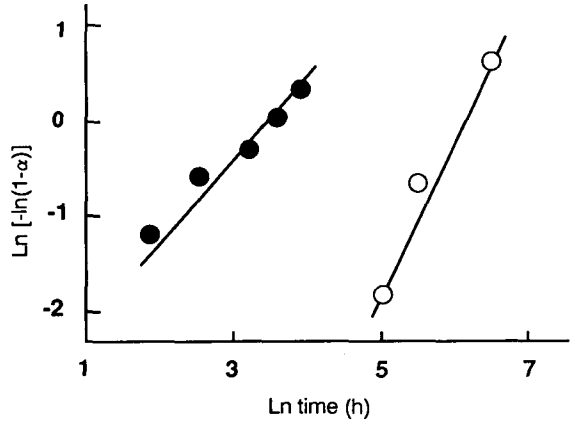

FIG. 3. Hancock-Sharp plots (eqn 1) for crystallization of nifedipine from HP-β-CyD and PVP systems, stored at 60°C and 75% r.h. ●, HP-β-CyD system. ○, PVP system.

PVP system with an induction period of about 3 days and the crystal size was much larger than that observed for the HP-β-CyD system. For example, the average crystal size of nifedipine in the PVP sample stored at 28 days was 55 μm in length, 12 times larger than that stored at 3 days (4.4 μm), and 10 times as large as that observed for the HP-β-CyD system. These results suggest that the crystal-growth mechanism of nifedipine in the HP-β-CyD system is different from that in the PVP system.

Kinetics of crystal growth

To gain preliminary insight into the crystal growth mechanism of nifedipine in the matrices, the changes in crystallinity during ageing were kinetically investigated according to the method of Hancock & Sharp (1972). In this method, plots of $-\ln[\ln(1-\alpha)]$ vs ln(time) (eqn 1) are used to distinguish crystal-growing mechanisms:

$$-\ln[\ln(1-\alpha)] = m \ln t + \ln B \quad (1)$$

where B is a constant which depends in part on the nucleation frequency and linear rate of grain growth, and m is a constant that can vary according to the geometry of the system. The time courses of the crystallinity changes of nifedipine in Fig. 1 were analysed according to equation 1. As shown in Fig. 3, linear plots with slopes (m) of 0·97 and 1·94 were obtained for the HP-β-CyD and PVP systems, respectively. Referring to the m values tabulated for a variety of nucleation- and growth-models (Hulbert 1969), it is proposed that the crystallization of nifedipine in an HP-β-CyD matrix follows the mechanism of one-dimensional growth of nuclei, while a two-dimensional mechanism operates in PVP. The crystallization of nifedipine seems to occur after the dissolution and dissociation of the complex to its components in the adsorbed water on the surface of matrices. Among the factors affecting the nucleation and crystal growth processes in super-saturated solutions, the molecular movement (diffusion process) of nifedipine for the access to crystal faces and the molecular arrangement (surface reaction process) of nifedipine for its incorporation into the crystal lattice may be of importance. Both processes can be affected by the capacity of nifedipine to interact with the matrix. During storage at 60°C and 75% r.h., it was found that the PVP system adsorbed almost twice as much water (about 20%) as the HP-β-CyD system (about 10%). Furthermore, the viscosity of the PVP system was found to be much higher than that of the HP-β-CyD system (4·9 and 1·2 cp, respectively, at 10% w/v). These results suggested that highly concentrated nifedipine in the hydrophilic PVP polymer matrix may allow isotropic contact of nifedipine with all crystal faces, forming three-dimensionally grown crystals (see Fig. 2), although the nucleation and growing rate would be slow due to the viscous nature of the PVP solution. In the case of the HP-β-CyD system, the nucleation and growing rate were faster than that of the PVP system, probably due to the lower viscosity of the solution. However, the nifedipine molecule can be effectively included in the hydrophobic cavity of HP-β-CyD to prevent the closer contact or suitable arrangement for attachment of the drug molecule to the crystal lattice, which may consequently inhibit the further growth of crystals, particularly in the higher-dimensions. We have recently reported the inclusion complex formation of HP-β-CyD with dihydropyridines such as nimodipine and nisoldipine in solution and solid state (Yoshida et al 1989, 1990). The detailed crystallization mechanism, together with the mode of inclusion of nifedipine within the HP-β-CyD cavity, will be reported elsewhere.

Dissolution behaviour

Because of the poor wettability and dissolution of nifedipine in water, 0·1% non-ionic surfactant HCO-60 was added to

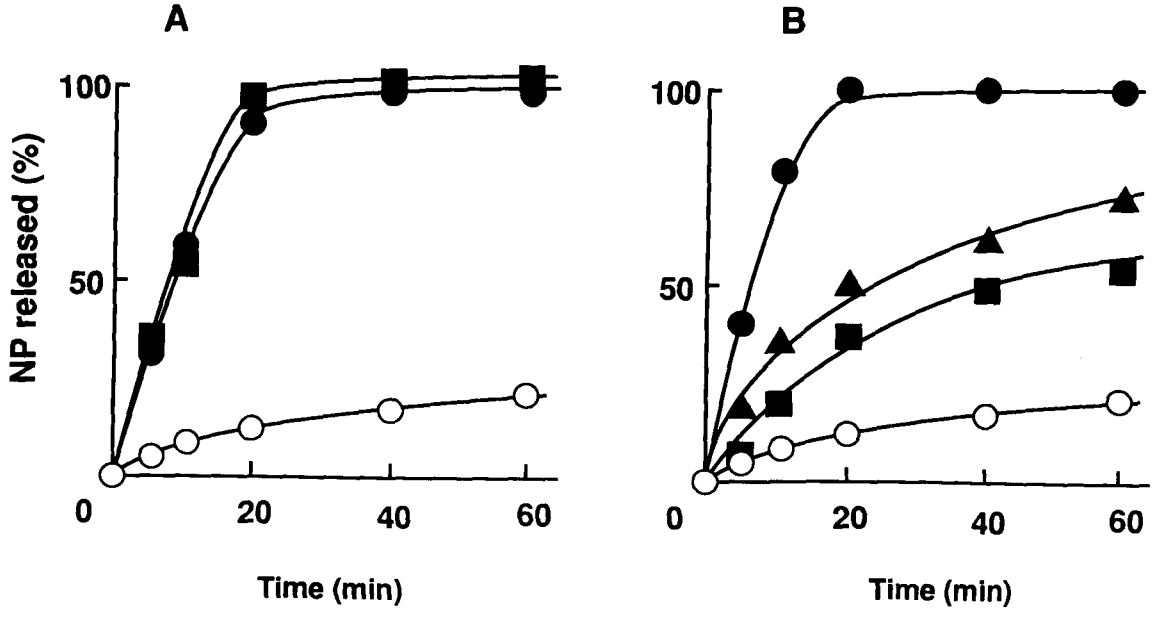

FIG. 4. Release profiles of nifedipine from tablets containing nifedipine, nifedipine-HP-β-CyD or nifedipine-PVP (equivalent to 5 mg nifedipine) in HCO-60 (0·1%) solution at 37°C, measured by the paddle method at 100 rev min^{-1}. A, HP-β-CyD system. B, PVP system. ○, Nifedipine (diluent, starch). ●, Initial. ▲, Stored for 7 days. ■, Stored for 14 days. Samples were stored at 60°C and 75% r.h.

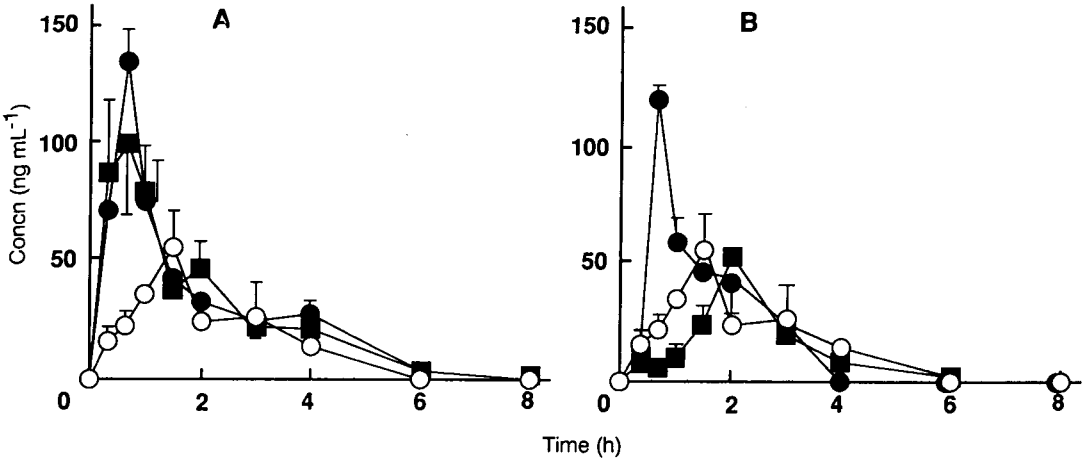

FIG. 5. Plasma levels of nifedipine following oral administration of tablets containing nifedipine, nifedipine-HP-β-CyD or nifedipine-PVP (equivalent to 20 mg nifedipine dosed) to dogs. A, HP-β-CyD system. B, PVP system. ○, Nifedipine (diluent, starch). ●, Initial. ■, Stored for 14 days. Samples were stored at 60°C and 75% r.h. Each value represents the mean ± s.e. of 4 dogs.

Table 1. Pharmacokinetic parameters of nifedipine following oral administration of tablets containing nifedipine, nifedipine-HP-β-CyD or nifedipine-PVP (equivalent to 20 mg nifedipine) to dogs.

System	C_{max}[a] (ng mL^{-1})	t_{max}[b] (h)	AUC[c] (h ng mL^{-1})
Nifedipine[d]	58·50 ± 15·17	1·63 ± 0·13	126·85 ± 17·24
Nifedipine-HP-β-CyD			
initial	134·36 ± 13·32**	0·67 ± 0·00**	213·22 ± 26·08*
14 days[e]	145·09 ± 4·80**	0·67 ± 0·14**	211·62 ± 23·59*
Nifedipine-PVP			
initial	120·36 ± 5·73*	0·67 ± 0·00**	148·39 ± 30·00
14 days[e]	54·45 ± 3·29	2·00 ± 0·00	103·20 ± 4·56

[a] The maximum plasma drug level; [b] the time required to reach the maximum plasma drug level; [c] the area under the plasma drug level-time curve up to 8 h post-administration; [d] diluent: starch; [e] after storage at 60°C, 75% r.h. Each parameter is expressed as the mean ± s.e. of 4 dogs. *,** Significantly different from nifedipine ($P < 0·05$ and $P < 0·01$, respectively, Student's t-test).

the dissolution medium to simulate the surface tension of the gastrointestinal fluids (Finholt & Solvang 1968). As shown in Fig. 4, the dissolution rate of the spray-dried products was faster than that of pure nifedipine, particularly for the HP-β-CyD system. The enhanced dissolution rate can be ascribed to the decrease in crystallinity and particle size of nifedipine together with the formation of a water-soluble complex during the spray-drying process (Lin & Kao 1989). According to the solubility method (Higuchi & Connors 1965), the stability constant of the inclusion complex of nifedipine with HP-β-CyD in water at 25°C was determined to be 370 M^{-1}. It is noteworthy that no appreciable ageing effect was observed for the dissolution rate of the HP-β-CyD system even after 14 days storage. In the case of the PVP system, however, the dissolution rate of nifedipine was significantly decreased with increasing storage time, indicating that the crystal-growth of nifedipine during storage was predominantly reflected in the dissolution behaviour.

In-vivo absorption

The effect of ageing on in-vivo absorption of nifedipine was examined for both spray-dried products in comparison with pure nifedipine. Fig. 5 shows the plasma levels of nifedipine following oral administration of the tablets to dogs, and their bioavailability parameters are listed in Table 1. The appearance of nifedipine in plasma was markedly enhanced by spray-drying with both HP-β-CyD and PVP; no ageing effect was observed for the HP-β-CyD system. On the other hand, the absorption pattern of the PVP system stored after 14 days at the accelerated storage conditions closely resembled that of intact nifedipine, as reflected by the bioavailability parameters in Table 1. Thus, the superior properties of HP-β-CyD as a hydrophilic drug carrier was further confirmed by these in-vivo experiments.

The results suggest that HP-β-CyD is much more effective than PVP in preventing the crystal growth of nifedipine owing to its ability to form inclusion complexes with dihydropyridine derivatives (Yoshida et al 1989, 1990), and may be useful in solving the bioavailability problems encountered by storage of amorphous nifedipine in solid dosage forms.

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