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Formulation development of an oral dosage form for an HIV protease inhibitor, AG1284

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Abstract

Several preformulation characteristics of a series of novel HIV protease inhibitors were examined as a prelude to expedient oral dosage form development. Initial studies indicated that these compounds were orally bioavailable in rats ($\leq 39\%$), but chemically unstable at low pH. AG1284 was selected as the lead compound from the series for further preclinical development based on its relatively high oral bioavailability and stability. The pH-rate profiles of AG1284 indicated a first-order degradative loss of a dimethylbenzyl group under acidic conditions. Concentrated solutions of an amorphous form prepared in various pharmaceutical solvents exhibited precipitation on standing. The precipitate was identified as crystalline AG1284 by X-ray powder diffraction (XRPD), differential scanning calorimetry (DSC) and polarized light/hot stage microscopy, and its solubility in water proved to be much lower than that of the amorphous form. Oral administration in dogs of a solid blend of AG1284 with polyethylene glycol 3350 (PEG 3350) in enteric-coated hard gelatin capsules did not yield any detectable AG1284 levels in plasma. When dosed in a propylene glycol/water (60/40) solution at 50 mg/kg to rats, oral bioavailability and C_{max} were 39% and 2.8 μg/ml, respectively. When delivered in a lyophilizable emulsion to rats at 100 mg/kg, oral bioavailability and C_{max} were 31% and 3.0 μg/ml, respectively. The lyophilized product could be reconstituted with WFI to regenerate an emulsion.

Keywords: HIV-protease inhibitor; Oral bioavailability; pH-rate study; o/w Emulsion; Lyophilization; Solid-state morphology

1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is known to be the etiologic agent in the pathogenesis of AIDS (Mitsuya et al., 1990). Although much effort is currently being directed toward discovering and developing novel anti-HIV agents, a number of factors will play a role in the overall success of any drug used to treat HIV. Among these are potency, rate of development of resistance, cumulative toxicity, and ability to reach the target site. Thus, it is unlikely that any single anti-HIV agent will be able to provide optimal therapy throughout the course of treatment, and various combination regimens will probably evolve

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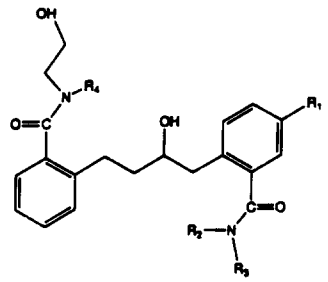
for management of this pathology (Ho, D.D., personal communication; Yarchoan et al., 1990). Furthermore, therapy will most likely be required for the lifetime of the patient, implying that effective oral formulations of such drugs will be critical to their success.

An important target in the viral replication cycle that has generated much attention with regard to therapeutic intervention is the enzyme HIV protease (Kempf et al., 1990; Yarchoan et al., 1993). Effective inhibitors of this enzyme have the potential to contribute significantly to the success of a combination regimen. AG1284 and its analogs, the structures of which are shown in Scheme 1, are examples of novel HIV protease inhibitors that were designed using protein structure-based techniques (Appelt, 1993; Reich et al., 1994). These compounds have demonstrated potent antiviral activity against HIV-1 infected CEM-SS and MT-2 cells. Relative stability and oral bioavailability in rats were used as criteria for selection of AG1284 as the lead compound from the series for further preclinical evaluation.

In this paper, the chemical stabilities of the compounds in this series and a pH-rate profile for AG1284 are presented. Solid-state morphology of AG1284 was investigated using XRPD, DSC and polarized light/hot stage microscopy (Qiu et al., 1988). Amorphous and crystalline forms were identified using these techniques, and the solubility of each form in various solvents of pharmaceutical interest was determined. A solid blend of AG1284 with PEG 3350 was prepared in an attempt to increase dissolution rate, and methods of protecting AG1284 from degradation in gastric fluids are presented. An oral, lyophilizable emulsion formulation similar to one previously reported in the literature (Vyas et al., 1992)



Chemical	Formula	MW	R_1	R_2	R_3	R_4
AG1157	$C_{30}H_{44}N_2O_5$	512.70	H-	2-hydroxyethyl	t-butyl	t-butyl
AG1274	$C_{41}H_{50}N_2O_5$	650.87	methyl	2-hydroxyethyl	dimethylbenzyl	dimethylbenzyl
AG1284	$C_{39}H_{52}N_2O_5$	628.86	methyl	2-hydroxyethyl	dimethylbenzyl	1-ethylcyclopentyl
AG1296	$C_{39}H_{52}N_2O_6$	644.86	methyl	2-hydroxyethyl	1-(4-hydroxyphenyl) -1-methylethyl	1-ethylcyclopentyl
AG1303	$C_{45}H_{57}N_3O_6$	735.97	methyl	2-hydroxyethyl	1-(4-(pyridin-3-yl methoxy)-phenyl) -1-methylethyl	1-ethylcyclopentyl
AG1313	$C_{41}H_{56}N_2O_6$	672.91	methyl	3-hydroxy-2-hydroxymethyl -propyl	dimethylbenzyl	1-ethylcyclopentyl
AG1314	$C_{41}H_{56}N_2O_6$	672.91	methyl	3,4-dihydroxy -butyl	dimethylbenzyl	1-ethylcyclopentyl
AG1318	$C_{38}H_{49}ClN_2O_5$	649.28	chloro	2-hydroxyethyl	dimethylbenzyl	1-ethylcyclopentyl
AG1323	$C_{30}H_{42}N_2O_5$	510.66	methyl	2-hydroxyethyl	H	1-ethylcyclopentyl

Scheme 1. Chemical structures of AG1284 and its analogs.

was developed in order to enhance GI stability and improve oral bioavailability. This lyophilized product could be regenerated to yield an o/w emulsion with oil droplets of less than 0.2 μm diameter. Results of bioavailability studies in rats with this o/w emulsion containing 2% AG1284 are presented.

2. Materials and methods

2.1. Materials

The compounds shown in Scheme 1 were synthesized at Agouron Pharmaceuticals, Inc., San Diego, California. Structures were confirmed by NMR spectroscopy and elemental analysis. Imwitor® (a mixture of mono-, di-, and triglycerides of caprylic/capric acids) and Miglyol® 812 (a triglyceride of caprylic/capric acids) were supplied from Huls America Inc., Edison, NJ. Tween 80, Span 80, propylene glycol (PG), PEG 3350, PEG 300, and PEG 400 were purchased from Fisher Scientific, Fairlawn, NJ. Cremophor® EL was obtained from BASF Corp., Parsippany, NJ. Soybean oil, oleyl alcohol, Crovol® M-40 and Crovol® PK-70 were from Croda Incorporated, Parsippany, NJ. Mannitol and sorbitol were purchased from Spectrum Chemical Manufacturing Corp., Gardena, CA. Ethanol was purchased from McCormick Distilling Co., Inc., MO. All ingredients were used as obtained. Both 0.2 and 0.45 μm nylon membrane syringe filters were purchased from Gelman Sciences, Ann Arbor, MI.

2.2. HPLC methods

A Hewlett-Packard 1090M HPLC system equipped with a diode-array detector and an autoinjector was used to quantitate each compound in the series. Separation of the compound of interest from its degradation products was achieved on a LiChrospher® 100 RP-8 reversed-phase column (5 μm, 125 × 4 mm). The mobile phase consisted of methanol and aqueous dibasic sodium phosphate (Na_2HPO_4, 0.01 M, pH 9.2) at a flow rate of 0.6 ml/min. The ratio of the two solvents was adjusted so that the retention time of the main peak fell within 4–6 min. The wavelength of detection was 220 nm. Fresh standards were prepared daily in methanol, and used for external calibration.

2.3. Solution stability studies

Preliminary stability studies were conducted initially to confirm that the HPLC assay was stability-indicating by comparing the results of inter- and intra-day sample assays. The stability of each compound in the series was determined at room temperature in solutions of pH 1–3. Drug solutions were prepared in appropriate buffers at a concentration of 20 μg/ml and stored at room temperature. At predetermined times, the solutions were assayed without further dilution using the stability-indicating HPLC method described above.

2.4. pH-rate study of AG1284

A pH-rate profile for AG1284 was generated using methods similar to those reported earlier (Chiang et al., 1994). Solutions of approx. 0.2 mg/ml of AG1284 in methanol were diluted by a factor of 12.5 in pH 1–8 buffers. Solution pH was recorded after dilution. Aliquots of buffered drug solution were placed in sealed ampoules and stored at 40, 50, and 80°C. Samples were removed at predetermined times and stored at −20°C prior to HPLC assay. Frozen samples were allowed to warm up to room temperature before assay.

2.5. Solubility measurements

The equilibrium solubility of AG1284 in various solvents was determined at room temperature by adding excess solid to the solvent in teflon-lined screw-capped tubes. Tubes were then sonicated for 30 min and rotated end-over-end overnight. Samples were removed the following day and filtered through a 0.45 μm nylon membrane syringe filter. It was determined in preliminary experiments that equilibrium was achieved in this time period. AG1284 concentration in the filtrate was assayed by HPLC after suitable dilution with methanol.

2.6. Microscopy

Small (< 1 mg) samples of AG1284 were examined by bright-field and polarized light microscopy (Optiphot-2, Nikon, Melville, NY) at 100 and 400 × magnification. The microscope was equipped with a Sony color video camera and printer for production of photomicrographs. Samples were also observed on a hot stage microscope (Thermovar, Reichert-Jung, Vienna, Austria) at 100 × while heating at ~ 5°C/min.

2.7. Differential scanning calorimetry (DSC)

DSC experiments were conducted for determination of the heat of fusion (ΔH_f), melting point (m.p.), and purity of AG1284 samples. The calorimeter (DSC-7 with a TAC 7/7 Instrument Controller, Perkin-Elmer Corp., Norwalk, CT) was operated at a heating rate of 5°C/min with a nitrogen purge maintained throughout each run. The system was calibrated with ultrapure indium to within ±0.1°C of its m.p. (156.6°C), and ±1% of its ΔH_f (28.45 J/g). Samples were placed in sealed aluminum pans and scanned over a temperature range of 25–350°C.

2.8. X-ray powder diffraction (XRPD)

Samples of AG1284 were prepared for XRPD by the powder pack method. Diffraction patterns were obtained using a Philips PW1710 automated powder diffractometer, with monochromatized CuK_α radiation ($K_{\alpha 1} = 1.54060$ Å; $K_{\alpha 2} = 1.54438$ Å). The instrument is equipped with a 2θ compensating slit and a curved graphite monochromator and was calibrated to within 0.02° (2θ) using the quartz peak at 26.66° (2θ). The minimum peak/background ratio was 0.75. Patterns were scanned from 2 to 62° (2θ) at a scanning speed of 6°/min.

2.9. Formulations

2.9.1. Capsules

Powder mixtures of AG1284 and PEG 3350 were prepared by dry-blending and manually filled into size 00 hard gelatin capsules. Capsules were dipped into a solution of 10% cellulose acetate phthalate (CAP) in acetone and dried at room temperature overnight. The amount of coating was determined by the difference of capsule weight before and after drying. These capsules were used to determine the oral bioavailability of AG1284 in dogs.

Disintegration studies were conducted to optimize the amount of coating in terms of its effect on disintegration. Capsules were filled with lactose as placebo. Disintegration of capsules was conducted in simulated gastric and intestinal fluids without enzymes (US Pharmacopeia, 1990) at 37°C using a USP basket apparatus (VanKel Industries, Inc., Edison, NJ). Capsules that remained intact in simulated gastric fluid for more than 3 h were removed, paper-towel dried and transferred into intestinal fluid to continue the study.

2.9.2. Emulsion

Several o/w emulsion formulations of AG1284 were prepared which were similar to one previously reported in the literature (Vyas et al., 1992). The oil phase consisted of either Imwitor®, Miglyol® 812, or soybean oil. The water phase consisted of an aqueous solution of Tween 80, Span 80, mannitol and sorbitol. AG1284 was first dissolved in the oil phase. A premix of Tween 80/Span 80 (1:4, by wt) was added to the oil phase, followed by 10 min of room temperature homogenization (Tissumizer® SDT-1810, Tekmar, Cincinnati, OH) with the water phase. These formulations were lyophilized on a tray lyophilizer (FTS Systems, Inc., Stone Ridge, NY) by weighing 2 g of each emulsion into 5-ml vials and operating a lyophilization cycle for approx. 35 h. The eutectic point was determined as −6°C.

2.10. Animal studies

The oral bioavailability of each compound in the series was evaluated by administering solutions by gavage to Sprague-Dawley rats and beagle dogs. Solutions in PG/water (60:40, w/w) at 10 mg/ml were prepared by dissolving drug in PG and then slowly adding water while stirring. Capsule and emulsion formulations of AG1284

were further evaluated in dogs and rats, respectively. A solution of AG1284 in PG/water (60:40) was used for intravenous administration in both species. Plasma levels of AG1284 were determined by HPLC as described below and oral bioavailability was calculated by comparing area under the plasma concentration-time curves after i.v. and oral dosing.

Analysis of plasma samples was conducted using Beckman HPLC Instruments (San Ramon, CA) comprising two solvent delivery pumps (Model 110B), auto-injector (Model 507), UV detector (Model 166 or 168) and Beckman System Gold data analysis system. Separation and detection was accomplished on a C_{18} column (Beckman, 4.6 × 150 mm, 5 μm) using a mobile phase consisting of water and acetonitrile (45:55) and a wavelength of 220 nm. The ratio of the two solvents was adjusted so that the retention time of the main peak fell within 5–8 min.

Calibration standards and samples of the drug in plasma were prepared prior to each analysis. Standard solutions of varying concentrations of drug in methanol were prepared fresh and appropriate volumes of the standard were transferred to 12 × 75 mm borosilicate culture tubes and the methanol evaporated at 40°C under a gentle stream of nitrogen. The residue was reconstituted with either 50 μl mouse plasma, or 100 μl rat plasma as appropriate. The final concentration of the calibration standards ranged from 0.5 to 100 μg of drug per ml of plasma. Plasma samples from the studies were thawed at room temperature and 100-μl plasma samples were transferred to 12 × 75 mm borosilicate culture tubes. Acetonitrile (1 ml) was added to each tube, vortexed (MultiTube Vortexer, Baxter, McGaw Park, IL) for 2 min and centrifuged (IECCentra, Damon/IEC Div., Needham Hts, MA) at 2000 rpm for 15 min. The supernatant was transferred to a new tube and evaporated at 40°C under nitrogen. Plasma from the corresponding calibration standards was treated in the same manner. Dried residues were then reconstituted in 250 μl mobile phase by vortexing for 2 min. The reconstituted samples were transferred to polypropylene auto-injector vials for drug analysis by reversed-phase HPLC.

3. Results and discussion

3.1. Solution stability studies

Buffer pH was not affected by the addition of any compound in the series, reflecting their neutral properties. All compounds exhibited first-order degradation profiles at pH 1.50, 2.16 and 3.33. The t_{50} values (time to reach 50% drug recovery) for all compounds at room temperature in solutions of pH 1.50, 2.16, and 3.33 are listed in Table 1. AG1157, the parent compound of the series, is the most stable but had relatively less potent antiviral activity than AG1284 against HIV-1 infected CEM-SS or MT-2 cells. AG1318 is also more stable than AG1284, but its oral bioavailability was lower than that of AG1284. In general, replacement of the *t*-butyl groups at positions R_3 and R_4 in AG1157 with larger groups, such as dimethylbenzyl (AG1274) or 1-ethylcyclopentyl (AG1284) decreases acid stability. Substitution of the methyl group at R_1 in AG1284 with a chloro group (AG1318) increases acid stability. The addition of one hydroxy group or a large pyridinylmethoxy group in the 4-benzyl position at R_3 in AG1284 reduces stability. A dihydroxy substitution at R_2 results in less stability than a monohydroxy substitution. Because of its relative stability and high oral bioavailability in rats (see the discusion below), AG1284 was chosen for further pH-rate studies at four tempera-

Table 1
t_{50} values for compounds tested in various buffer solutions

Compound	t_{50} (h)		
	1.50	2.16	3.33
AG1296	2	– [a]	10.46
AG1303	0.25	2.37	27.57
AG1313	0.29	1.96	45.82
AG1274	1.47	14.87	181.11
AG1314	2.37	19.22	207
AG1284	5.14	16.12	232.30
AG1318	8.65	85.21	stable [b]
AG1157	stable [b]	stable [b]	stable [b]

[a] Poor separation of AG1296 from its degradation products rendered the estimation of the t_{50} difficult.
[b] AG1318 and AG1157 were more stable than other compound. Their t_{50} values were not calculated.

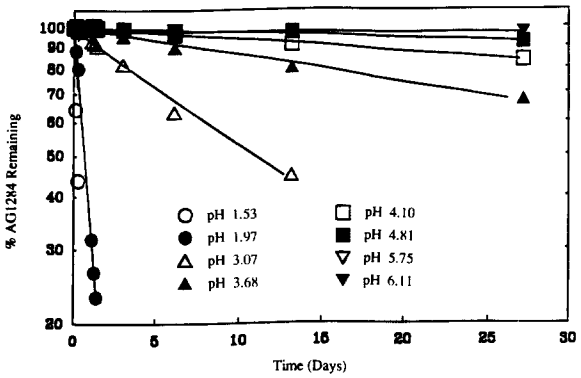


Fig. 1. First-order degradation profiles of AG1284 at room temperature.

tures over a wider pH range. In addition, more detailed preformulation and formulation studies were conducted on this compound before clinical evaluation commenced.

3.2. pH-rate study of AG1284

Degradation of AG1284 in buffer solutions was studied at four temperatures, i.e., room temperature, 40, 50, and 80°C. First-order degradation was observed in all cases, as shown in Fig. 1; plots of rate constant (k) vs pH are shown in Fig. 2. The pH-rate profiles show that AG1284 degrades faster at higher temperatures, and is most stable around pH 6 at all temperatures. However, the room temperature data did not appear to exhibit any distinct difference in rate around pH 4–5, probably due to experimental error.

A major degradation peak was observed in AG1284 stability samples. This degradation product was confirmed to be AG1323 by spiking with an authentic sample, suggesting that AG1284 loses its dimethylbenzyl group in acidic media (Scheme 2).

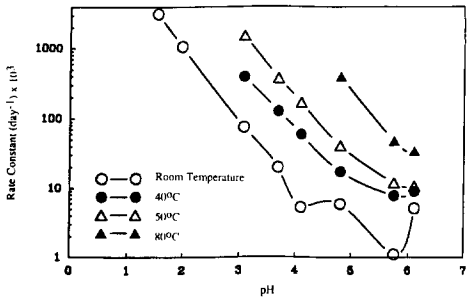
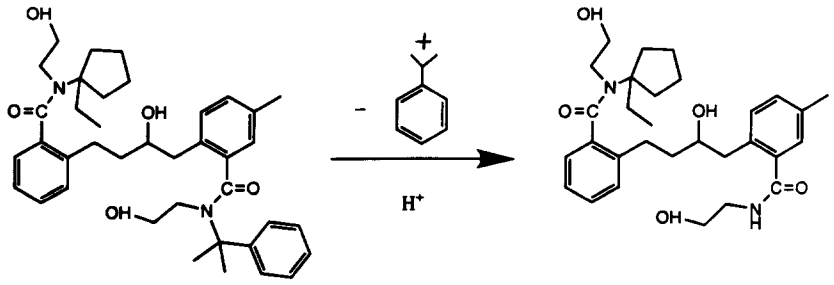


Fig. 2. pH-rate profiles of AG1284.

3.3. Solubility measurements

The apparent solubility of AG1284 in aqueous buffer solutions and several pharmaceutical solvents is summarized in Table 2. The data clearly show that the apparent aqueous solubility of AG1284 in various buffer solutions over the pH range 3.05–8.09 is about 0.07 mg/ml, irrespective of pH. At pH 1.08 and 2.00, however, AG1284 has lower solubility. This is probably due to a faster degradation rate at low pH relative to its dissolution rate into the vehicle. Table 2 also shows that the solubility of AG1284 in pharmaceutical solvents such as Cremophor® EL, PEG 300, propylene glycol, Novol®, Crovol® M-40, and Crovol® PK-70 is high. However, highly concentrated solutions of AG1284 prepared in the above vehicles exhibited precipitation upon standing.



Scheme 2. Degradation of AG1284 to AG1323.

The precipitate was collected by filtration and determined to be crystalline as demonstrated from the analysis to discussed in section 3.4. The solubility of crystalline AG1284 in deionized water is approx. 0.01 mg/ml (Table 2) which is 6–7 times lower than that of its amorphous form. In addition, the crystalline material is 3–4 times less soluble in PEG 400/ethanol than the amorphous form (Table 2).

3.4. Microscopy, DSC, and X-ray powder diffraction

Precipitates from the solubility studies were collected by filtration. Both the precipitate and the original bulk drug powder were examined by XRPD, DSC, and polarized light microscopy. The X-ray powder pattern for the precipitate exhibited distinct peaks, as compared to the diffuse

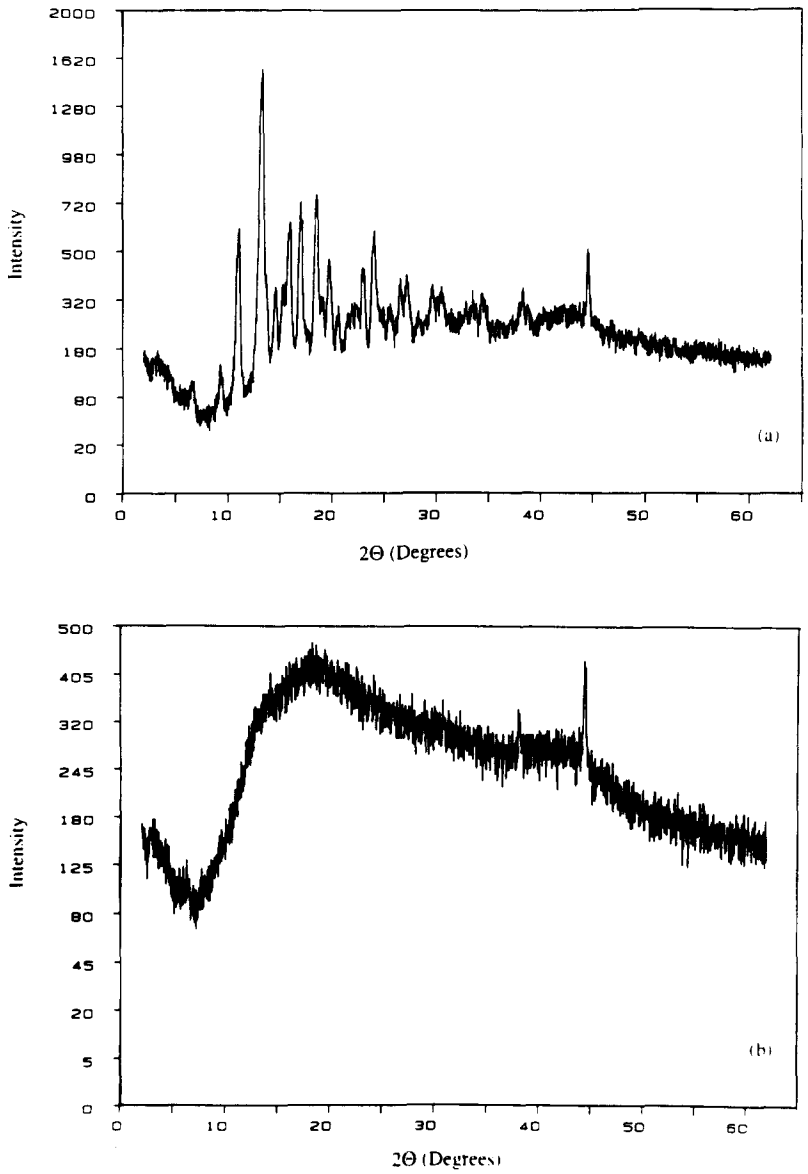


Fig. 3. X-ray powder diffraction of AG1284. (a) Crystal; (b) amorphous.

pattern observed with the bulk drug powder (Fig. 3a and b). By DSC, the precipitate exhibited a sharp endotherm at 150.7°C, indicating a distinct melting point (Fig. 4a). However, a sharp exotherm followed by a broad exotherm was seen with the bulk drug powder, indicating possible decomposition (Fig. 4b). On polarized light microscopy, the precipitate showed strong birefringence, but there was no light transmission for the bulk drug powder (Fig. 5a and b). These observations strongly suggested that the precipitate was a crystalline form and that the bulk drug powder was amorphous.

3.5. Disintegration of capsules with or without CAP coatings

Without any coating, capsules disintegrated in pH 1 and 7 solutions in 13 and 1 min, respectively. With CAP coatings of 12–32 mg, the capsules in pH 1 solution were still intact after 3 h. These capsules were then transferred into a pH 7

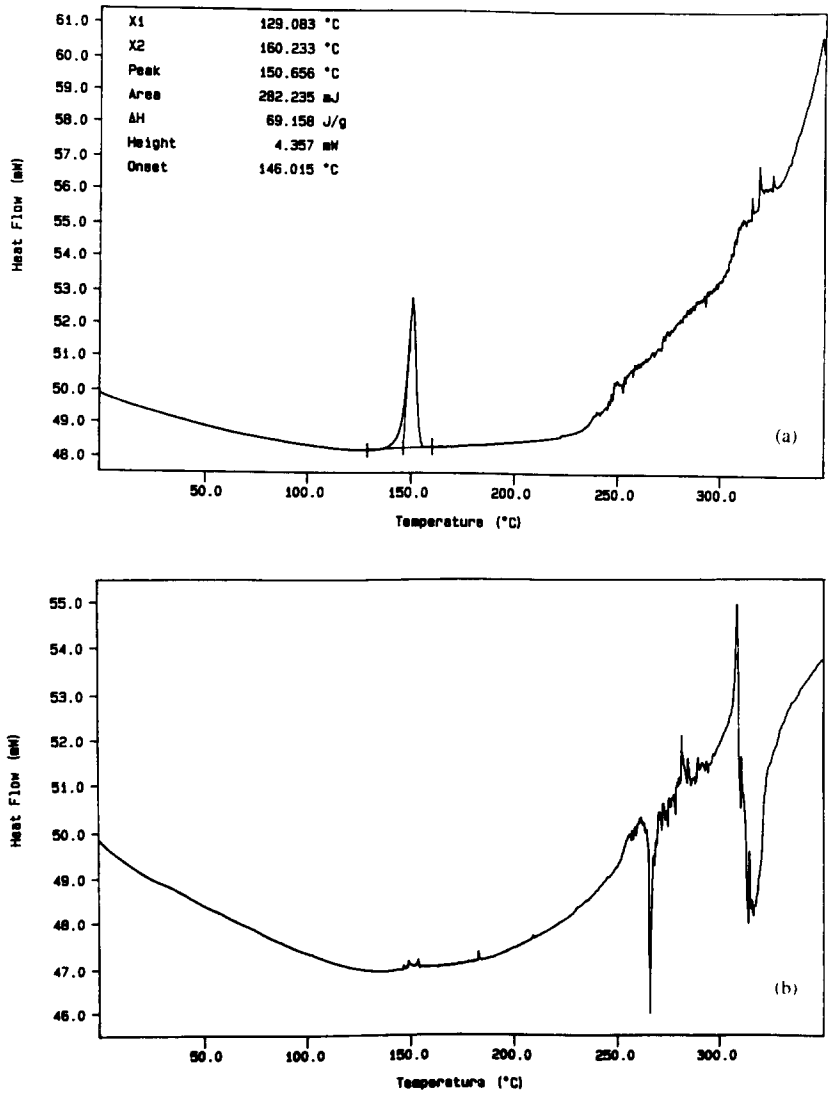


Fig. 4. DSC profiles of AG1284. (a) Crystals; (b) amorphous.

solution. All capsules disintegrated within 1–4 min. Results of the disintegration studies indicated that CAP coatings of 15–20 mg should protect the capsules from disintegration by gastric acid.

3.6. Emulsion formulations

The formulations were o/w emulsions as proved by examining the aqueous dilute of the emulsions under microscope. Imwitor® 742 (a

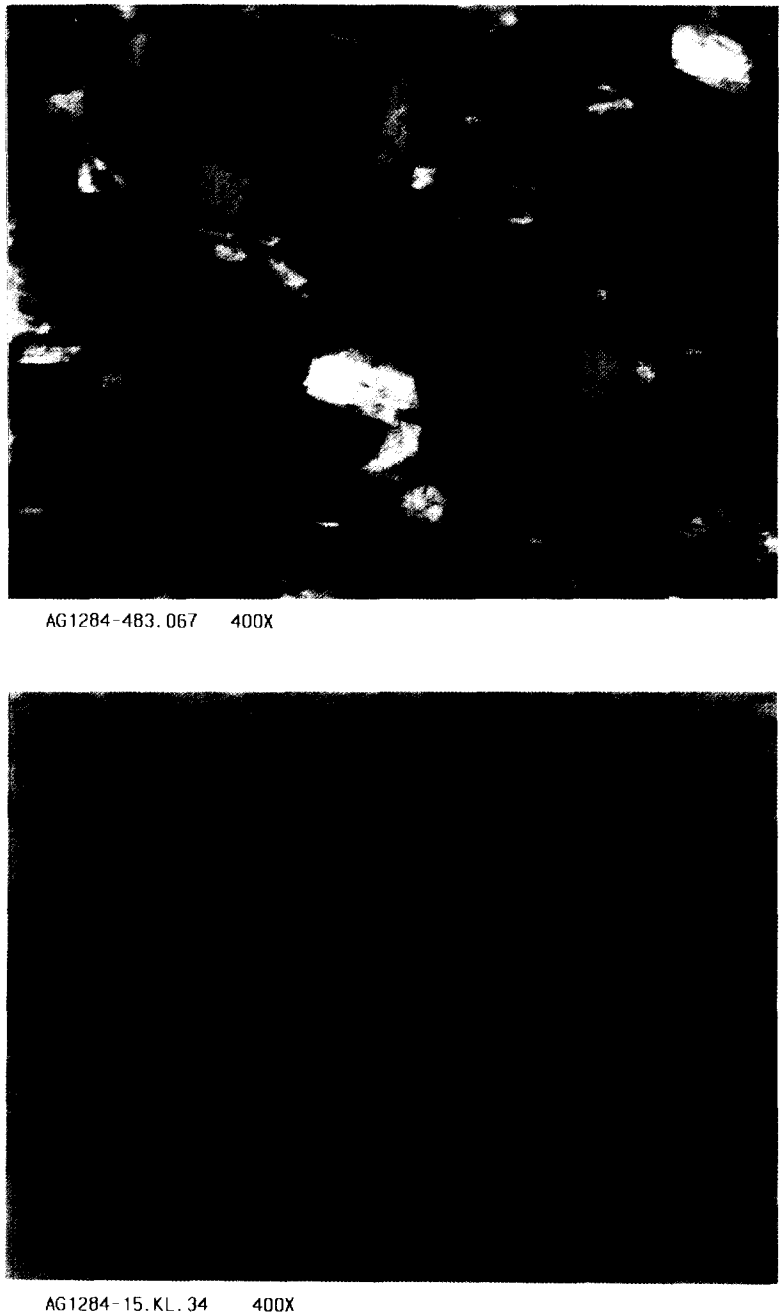


Fig. 5. Photomicrographs of AG1284 under polarizing light microscopy. (a) Crystalline; (b) amorphous.

Table 2
Apparent solubility of amorphous AG1284 in various pharmaceutical solvents and buffer systems

Vehicles	Solubility
0.40 M KCl/HCl (pH 1.08)	0.039 (mg/ml)
0.05 M HCl/KCl (pH 2.00)	0.067 (mg/ml)
0.01 M glycine/NaCl/HCl (pH 3.05)	0.069 (mg/ml)
0.01 M NaOAc/HOAc (pH 5.10)	0.073 (mg/ml)
0.01 M phosphate buffer (pH 6.41)	0.070 (mg/ml)
0.01 M phosphate buffer (pH 7.47)	0.067 (mg/ml)
0.01 M phosphate buffer (pH 8.09)	0.070 (mg/ml)
Deionized water	0.01 [a] (mg/ml)
Cremophor® EL	200 (mg/g)
PEG-300	300 (mg/g)
Propylene glycol	350 (mg/g)
Novol®	390 (mg/g)
Crovol® M-40	260 (mg/g)
Crovol® PK-70	225 (mg/g)
PEG-400/EtOH (92:8, w/w)	150–200 (mg/g)
PEG-400/EtOH (92:8, w/w)	52 [a] (mg/g)

[a] Solubility of crystalline AG1284.

mixture of medium chain glycerides), Miglyol 812 (triglyceride of caprylic/capric acids) or soybean oil was the oil phase. AG1284 was solubilized in oil phase. Sorbitol and mannitol were dissolved in water and were used as bulking agents. These two phases were emulsified with a mixture of Span 80 and Tween 80 (4:1). All emulsions underwent lyophilization and the resulting cakes were firm. After lyophilization, 2 ml of water were injected into each vial and the cake collapsed with gentle shaking. The resulting mixture appeared to be a homogeneous emulsion upon examination by light microscopy. Particle sizes of oil droplets before and after freeze-drying were found to be smaller than 0.22 μm. The concentration of Imwitor® 742 could be varied from 10 to 30% because of the high solubility of AG1284 in this oil.

A lead formulation, composed of 2% AG1284, 20% Imwitor® 742, 8% Span 80, 2% Tween 80, 20% mannitol and 27% water, was selected based on the cake formed after lyophilization and homogeneous distribution of oil droplets in emulsions after reconstitution. Oil droplet sizes in the emulsion are so small and evenly distributed that the total surface area available for AG1284 absorption is drastically enhanced. This formulation was used to determine oral bioavailability of AG1284 in the rat.

3.7. Animal studies

Capsules, containing a blend of AG1284/PEG 3350 (1:1) were coated with an average of 19.6 mg of CAP and administered to dogs. No blood levels were detected, probably due to the low dissolution rate of solid AG1284. AG1284 in PG/water (60:40) solution, when dosed into rats at 50 mg/kg, showed a C_{max} and oral bioavailability of 2.8 μg/ml and 39%, respectively. This solution, however, was not stable due to precipitation of AG1284 at room temperature after long-term storage. The o/w emulsion, when dosed to rats at 100 mg/kg showed a C_{max} and oral bioavailability of 3.0 μg/ml and 31%, respectively. The results of these animal studies indicate that the lyophilizable emulsion is a viable formulation for oral delivery of AG1284, and that it results in the attainment of comparable blood levels and bioavailability to the PG/water solution formulation.

4. Conclusion

A novel lyophilizable emulsion was developed for oral administration of AG1284. This emulsion was easily reconstituted with water. The regenerated emulsion is suitable for immediate administration, which would eliminate possible drug precipitation or crystallization during storage. This lyophilizable emulsion could also be used for other neutral compounds for which oral absorption is dissolution rate-limited. Such compounds usually show low water solubility and tend to precipitate from formulations when in gastric fluids. Different oils at various levels could be selected to optimize drug solubility and to form good cakes after lyophilization.

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