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Characterization of the Physicochemical Properties of KR-31378

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KR-31378 is a new drug candidate intended for the use in the prevention of ischemia-reperfusion damage. The objective of this preformulation study was to determine the physicochemical properties of KR-31378. The *n*-octanol to water partition coefficients of KR-31378 were 0.0504 at pH 3 and 0.8874 at pH 10. Accelerated stability of KR-31378 in solution and solid state was studied at 5, 40, 60°C. The stability testing indicated that the *t*90 for the drug in solid was estimated to be 2 years and 128.6 days at 25°C, while the that in aquesou solution was 68.6 days at 25°C. The KR-31378 was also found to be unstable under the relative humidity of 76%, probably because of the hygroscopic nature of the drug. In order to study compatibility of KR-31378 with typical excipients, potential change in differential scanning calorimetry spectrum was studied in 1:1 binary mixtures of KR-31378 and Aerosil, Avicel, Eudragit, lactose, PEG, talc, CMC, PVP, starch. As a result, CMC, PVP, and starch were found to be incompatible with KR-31378, indicating the addition of these excipients may complicate the manufacturing of the formulation for the drug. Particle size distribution of KR-31378 powder was in the size range of 9-93 μm with the mean particle size of 37.9 μm. The flowability of KR-31378 was apparently inadequate, indicating the granulation may be necessary for the processing of the drug to solid dosage forms. Crystallization of the drug with a number of organic solvents did not lead a crystalline polymorphism. In addition, dissolution of the drug from the powder was adequately rapid at 37°C in water.

Key words: KR-31378, Physico-chemical properties, Preformulation, Polymorphism, Solubility, Stability

INTRODUCTION

Both inhibition of lipid peroxidation induced oxygen species and stabilization of membrane have been proposed as neuroprotective strategies in stroke. In this context, a novel benzopyran derivative with *N*-cyanoguanidine group, KR-31378, (2S,3S,4R)-*N*''-cyano-*N*-(6-amino-3,4-dihydro-3-hydroxy-2-methyl-2-dimethoxymethyl-2*H*-benzopyran-4-yl)-*N*'-benzylquanidine (Fig. 1), was recently synthesized by Korea Research Institute of Chemical Technology as a new therapeutic agent for cardioprotective and neuroprotective (Hong *et al.*, 2002; Lee *et al.*, 2001; Yoo *et al.*, 2001) actions. The compound is currently under development as a new drug.

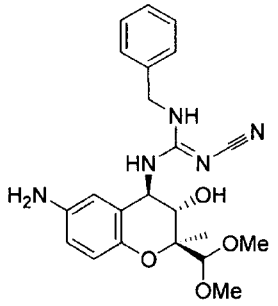

Fig. 1. Chemical structure of (2*S*,3*S*,4*R*)-*N*''-cyano-*N*-(6-amino-3,4-dihydro-3-hydroxy-2-methyl-2-dimethoxymethyl-2*H*-benzopyran-4-yl)-*N*'-benzylguanidine (KR-31378)

Prior to the development of major dosage forms with a new drug candidate, it is essential that certain fundamental physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined. This information will dictate many of the subsequent events and possible approaches in formulation development (Aulton, 2002; Lachman *et al.*, 1986). Typically, the preformulation studies focus on those physicochemical properties of the new compound that could affect drug performance and development of an efficacious dosage form. A through

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understanding of these properties may ultimately provide a rationale for formulation design, or support the need for molecular modification.

The objective of this study is to determine physicochemical properties of KR-31378 that are relevant in the formulation of the drug (e.g., stability, solubility and compatibility; Aulton, 2002; Lachman *et al.*, 1986).

MATERIALS AND METHODS

Materials and reagents

KR-31378 was supplied by Dongbu Hannong Chemical Company (Daejon, Korea). Other chemicals and solvents were of analytical reagent grade or HPLC grade and were used without further purification.

Apparatus

The powder X-ray crystallography diffraction (PXRD) patterns were measured with a Rigaku DMA S-IIIA (Rigaku, Japan) under the following conditions: Ni-filtered Cu-K radiation (1.542), a voltage of 30 Kv, a current of 20 mA. The differential scanning calorimetry (DSC) analysis was carried out using a Mettler DSC 12E thermal analyzer (Mettler, Swiss) and samples of approximately 2 mg were weighed in pierced aluminum pans with heating rate of 10 °C/min and temperature range from 35 to 200°C.

And UV/VIS spectrophotometry was measured with Beckman Du650 Spectrophotometer (Beckman, U.S.A). Flowability was estimated using a Flowability Tester (Erweka, Germany). Particle size distribution of KR-31378 powder was estimated using a laser particle size analyzer (Mastersizer 2000, Malvern, UK). The dissolution rate of KR-31378 was measured by paddle method of USP XXIII using dissolution tester (Duksan pure chemical Co., Korea).

Analytical method

Initially, the UV spectrum of KR-31378 in 0.1 mg/mL aqueous solution was studied in the range from 250 to 400 nm. The absorption maximum for the drug was obtained at 276 nm. To construct a calibration curve for KR-31378, known amounts of the prepared samples were dissolved in water (pH adjusted to 1.35 by the addition of 1 N HCl) and then the drug content was evaluated spectrophotomerically at 276 nm.

Six sets of aqueous standard solutions of KR-31378 were prepared twice a day in three-day period. The absorbance values of these solutions were measured at 276 nm. Analytical parameters such as linearity, precision and accuracy were, then, evaluated.

Apparent partition coefficients

N-Octanol (25 mL) was added to an equal volume of aqueous solution with different pH values (Hong *et al.*, 2002; Rivera et al., 2002). The mixture was shaken rigorously for 30 minutes in a separating funnel. After standing for 24 h, the phase was separated. Then, two phases were separately collected in 50 mL tube. This procedure was to saturate the aqueous and organic phase with other phases.

A 50 mg amount of KR-31378 was added to 5 mL of the aqueous phase. These systems were magnetically stirred for 3 h. They were centrifuged for 4 minutes at 4000 rpm and the solution was filtered through 0.45 μm membrane filter. After this solution was diluted, the concentration (C1) was determined spectrophotometrically. Aliquot (4.5 mL) of the aqueous phase was added to a fresh tube and an equal volume of *n*-octanol was added. The mixture was agitated by a magnetic stirrer, and centrifuged. The mixture was, then, stood for separation and the concentration of KR-31378 in aqueous solution was determined spectrophotometrically (C2).

The concentration of KR-31378 in the organic solution was estimated by the difference between the initial and the final concentration in aqueous solutions (viz, C1-C2). Apparent partition coefficients were determined from the ratio between the organic and aqueous concentration of the drug.

Determination of Hygroscopicity

Six glass desiccators, approximately 22 cm in diameter and 18 cm in height, were used to determine sorption isotherms by the traditional desiccator method. Saturated salt solutions at 25°C were prepared from analytical grade salts and purified water. The salts used were dibasic phosphate, ammonium sulfate, sodium acetate, sodium nitrite, zinc nitrate, and lithium chloride. Saturated salt solutions in a small beaker was placed alongside the exposed drug sample, and stored in a larger sealed container.

In this study, six desiccators with 95%, 81%, 76%, 66%, 42% and 15% of relative humidity were used. An amount of 200 mg of dehydrated KR-31378 was weighed and exposed to the desired relative humidity. The samples were kept in open plastic petri dishes at 24°C for 1 week at each of the humidity described, and the gain in weight of KR-31378 was then determined up to the saturation humidity.

Determination of particle size and flowability

Particle size distribution of KR-31378 powder was estimated using a laser particle size analyzer (Mastersizer 2000, Malvern, UK). In this study, triplicate measurement was carried out and expressed as mean of the measurements.

Flowability was estimated using a Flowability Tester (Erweka, Germany), by measuring the time of powder flowing through a hopper orifice.

Accelerated stability in solid and solution

In this study, the accelerated stability of KR-31378 in solution and solid was evaluated. For the assessment of stability of KR-31378 in solution, a sample solution (1 mg/6 mL) was prepared and an aliquot stored in vials with teflon-coated rubber stoppers. The sample solutions were stored at 40 or 60°C. The concentration of the stored solution was determined every day for 10 days by UV assay for KR-31378.

For the assessment of stability of the drug in solid, 2 mg of KR-31378 powder was placed in brown vials and stored at 5, 40 and 60°C for 1 to 7-month period. When it was necessary to determine the remaining content, an aliquot of the sample was dissolved in water (pH adjusted to 1.35 by 1 N HCl) and the concentration determined spectrophotometrically. Then, the content remaining was calculated from the concentration.

Compatibility with excipients

Thermal analysis was used to study compatibility of KR-31378 with typical excipients (Botha *et al.*, 1989; Holgado *et al.*, 1995; Sohn *et al.*, 1999). Thus, 1:1 binary mixture of KR-31378 with Aerosil, Avicel, Eudragit, lactose, PEG, talc, CMC, PVP or starch was prepared and the differential scanning calorimetry spectrum in the mixtures assessed.

Polymorphism

Recrystallization was performed to study the potential crystalline polymorphism for KR-31378. In this study, methanol, ethanol, acetonitrile, acetone, chloroform, dichloromethane, dichloroethane, dimethylformamide, isopropylalcohol, isoamylalcohol, ethylacetate, buthanol, dioxane, heptane, tetrahydrofuran, trichloroethane, tetrachloroethane, and tetrachlorocarbon were used as re-crystallization solvents. Each solvent was saturated with KR-31378 powder, then the saturated solvent was standed at -72°C, -30°C, -10°C, 5°C, and room temperature.

In order to study whether the alteration in the crystal morphology lead to a polymorphic transition, X-ray powder diffraction analysis was used (Haleblian *et al.*, 1969; Sohn *et al.*, 2000).

Dissolution

The dissolution rate of KR-31378 was measured by paddle method of USP XXIII using dissolution tester (Duksan pure chemical Co., Korea). The dissolution vessels contained 900 mL of filtered distilled water at 37°C and a paddle rotation speed of 100 rpm was used throughout the study.

At pre-determined time intervals, an aliquot (1mL) was withdrawn and immediately filtered through a 1.45 μm syringe filter, 1 mL of water at the same temperature was replaced in the vessel. The concentration of the drug in the sample was determined by the UV assay for KR-31378.

RESULTS AND DISCUSSION

Determination of KR-31378 concentration

The concentration KR-31378 was readily determined in the concentration range of 0.04 to 0.15 mg/mL. The equation of the fitted model was: Absorbance=0.01341+5.5969 · concentration.

The calibration curve was used throughout the study to determine the concentration of KR-31378.

Apparent partition coefficient of KR-31378

N-Octanol to water apparent partition coefficient for KR-31378 was 0.0504 at pH 3 and 0.8874 at pH 10. Since the drug contained primary and secondary amines, KR-31378 is a weak base. In this study, the partition coefficient was dramatically increased with the pH, consistent with the basic nature of the drug.

Hygroscopicity of KR-31378

Fig. 2 depicts the percentage of saturation humidity as a function of the relative humidity. The results indicated that KR-31378 is physically stable in an atmosphere up to 76% of relative humidity at 25°C. Therefore, KR-31378 should be stored below 76% relative humidity to prevent the adsorption of water vapor, which may lead to a potential instability.

Particle size and the distribution of KR-31378 powder

Mean particle size and the distribution were studied for KR-31378 powder. The distribution of frequencies was found to be close to the normal distribution (Fig. 3) with the mean particle size of 37.942 μm and the size range of 9-93 μm.

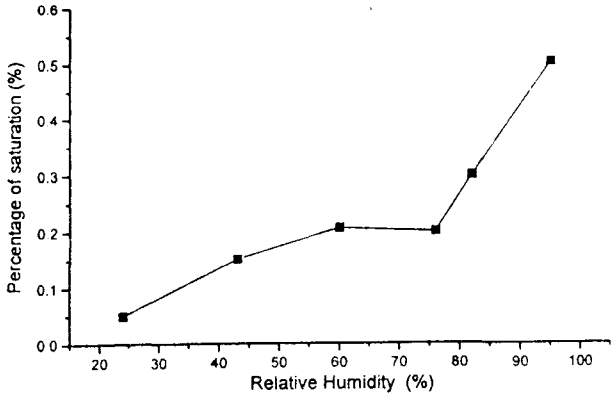

Fig. 2. Plot of percentage of saturation humidity versus relative humidity

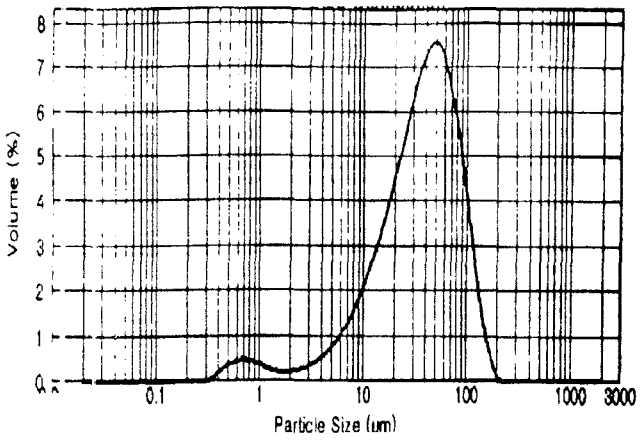

Fig. 3. Particle size distribution of KR-31378

Flow property of KR-31378 powder

Flow property of KR-31378 was also assessed. Therefore, 10 g of powder flowed for 9.35 seconds through a hopper orifice. And flow angle of KR-31378 was 72 deg 46 min. Free flowing particle is likely to have a high flow rate and low flow angle. Thus, flow angle above 50 deg would indicate that the powder flows only with great difficulty, if at all. Since the observed flow angle of KR-31378 is over 70 degree, the flow characteristics is clearly inadequate. During the manufacture of solid dosage forms, flowability is an important factor since content uniformity may determine by the physicochemical property. Therefore, granulation of KR-31378 may be necessary to improve the flowability for a successful formulation of KR-31378 into solid dosage forms (e.g., tablet and/or hard-gelatin capsule).

Accelerated stability testing for KR-31378 in solution and solid

The method of accelerated testing of pharmaceutical products based on the Arrhenius equation. According to this technique, elapsed time to 90% remaining at elevated temperatures (40, 60°C) were obtained and plotted against the reciprocals of the absolute temperature. Using the relationship, the expected stability (i.e., 90% remaining) at room temperature may be extrapolated to 25°C.

In this study, stability in elevated temperature was studied in aqueous solution (Fig. 4A) and solid (Fig. 4B). The time elapsed to 90% remaining from the original strength was evaluated by the linear regression and calculated for elevated temperatures in solution and solid. The time to 90% of the original potency was then plotted against 1/temperature in solution (Fig. 4A, inset) and solid (Fig. 4B, inset). The decomposition data revealed that a t_{90} value (elapsed time to 90% of the original potency) at 25 was 68.6 and 858.6days (i.e., 2 years and 128.6 days) for solution and solid, respectively.

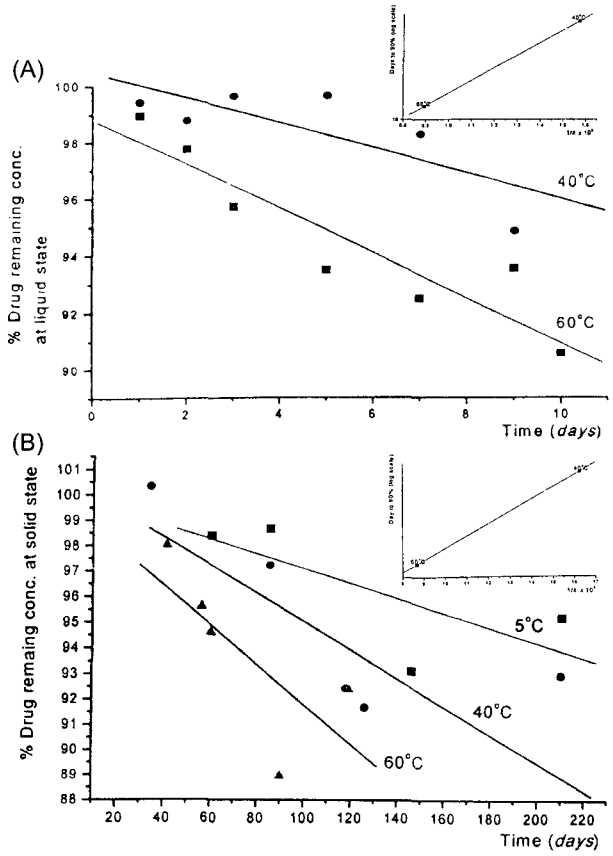

Fig. 4. Accelerated stability assessment for KR-31378 in aqueous solution and solid. Pane A: Temporal profiles of percent remaining KR-31378 from the original potency in aqueous solution. Inset, Panel A: Time elapsed to 90% remaining KR-31378 from the original potency in aqueous solution vs. the reciprocal of the temperature. Panel B: Temporal profiles of percent remaining KR-31378 from the original potency in solid. Inset, Panel B: Time elapsed to 90% remaining KR-31378 from the original potency in solid vs. the reciprocal of the temperature.

Compatibility with excipients

In this study, compatibility of KR-31378 with typical excipients was studied. The selected excipients represent components that are generally added during the formulation process. The typical thermogram of KR-31378 is shown in Fig. 5A with the estimated melting point of 179 °C. No additional endotherm was observed from this thermogram, and the peak was a very sharp, indicating that KR-31378 has a well defined crystalline phase. Sum of DSC curve for the drug and Aerosil was almost superimposable for that (Fig. 5B) obtained from 1:1 binary mixture of the drug and Aerosil, indicating that there was no appreciable interaction between the drug and Aerosil. Similar DSC characteristics were obtained from 1:1 binary mixture of the drug and Avicel (Fig. 5C), Eudragit (Fig. 5D), lactose (Fig. 5E), PEG (Fig. 5F) or talc (Fig. 5G). These observations indicated that Aerosil, Avicel, Eudragit,

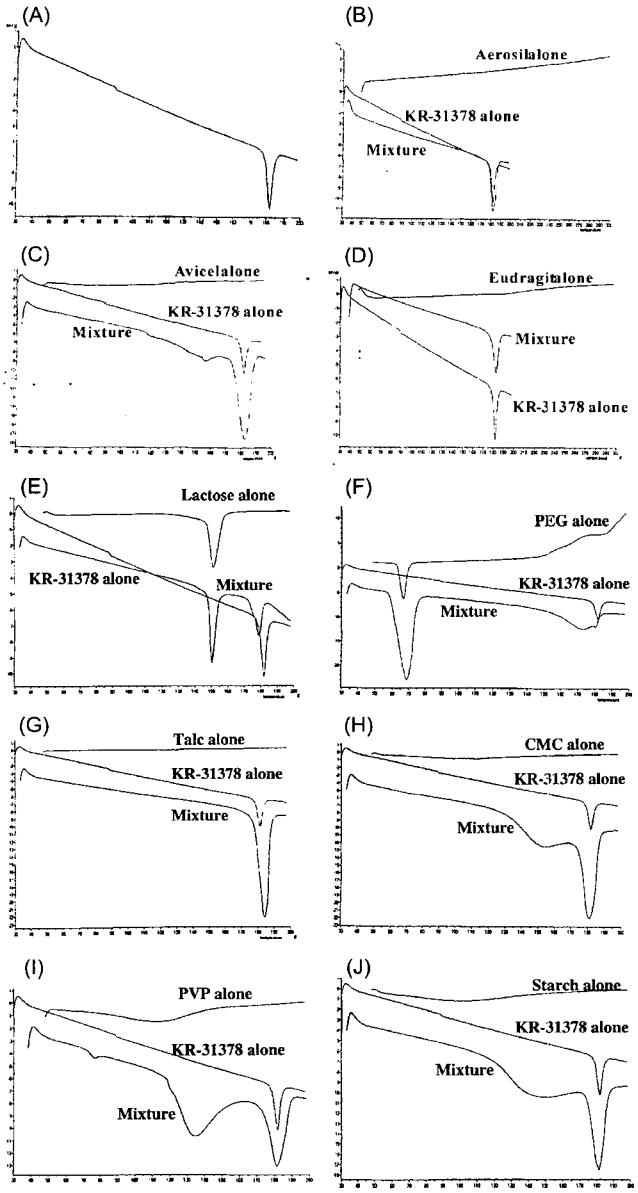

Fig. 5. DSC thermogram of KR-31378, typical excipients and 1:1 binary mixture of the drug and excipeints. Panel A: DSC curve of KR-31378 alone. From panel B to I: DSC curves of KR-31378, the excipients, and the mixture with the drug and the corresponding excipients. Panel B: Aerosil. Panel C: Avicel. Panel D: Eudragit. Panel E: lactose. Panal F: PEG. Panel G: talc. Panel H: CMC. Panel I: PVP. Panel J: starch.

lactose, PEG and talc are compatible with KR-31378 and, thus, the excipients may be added in the formulation of the drug.

In contrast, there appeared to be a significant interaction between KR-31378 and excipients such as CMC (Fig. 5H), PVP (Fig. 5I) and starch (Fig. 5J). In general, DSC thermogram of KR-31378 in the presence of the excipients indicates an observable change in the peak shape and area. The peak was broaden at the point of 179°C (i.e., the melting point of the drug). Furthermore, additional

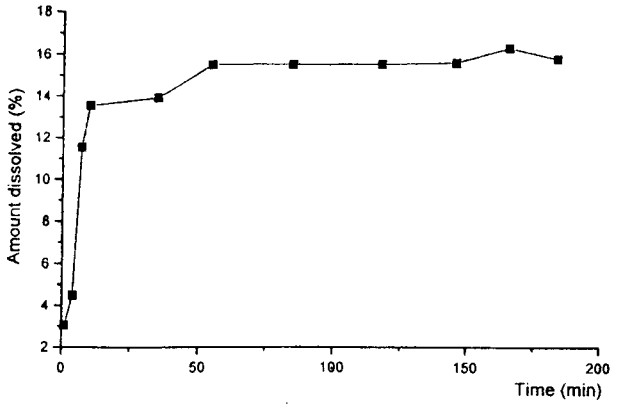

Fig. 6. Dissolution pattern of KR-31378 in distilled water at 37°C

endothermic peaks were detected at 150°C (Fig. 5H) for CMC, 130°C (Fig. 5I) for PVP and 140°C (Fig. 5J) for starch. Therefore, a strong interaction of KR-31378 with CMC, PVP and starch may occur and, thus, the excipients may not be added during the formulation of the drug.

Polymorphism

Under the re-crystallization study with various organic solvents, different polymorphic crystalline form was not resulted. Therefore, this observation indicates that KR-31378 does not have crystalline polymorphism.

Dissolution

Fig. 6 depicts pattern of dissolution for KR-31378 solid in water at 37°C. The pattern indicated that the dissolution of the drug is rapid for the first 10 minutes and the dissolution rate was in equilibrium after 55 minutes. These observation indicated that solubilization of KR-31378 to fully dissolve the drug from the formulation.

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