Research Article

Improvement of solubility and bioavailability of aceclofenac using cocrystallization

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ABSTRACT

Aim: The aim of the present investigation was to carry out the *in vitro* and *in vivo* studies of the cocrystals of a poorly soluble BCS Class II drug aceclofenac with two selected cocrystal formers, i.e., gallic acid and nicotinamide and hence to improve its solubility and bioavailability. **Materials and Methods:** The *in vitro* dissolution studies of the cocrystals which were formed by solvent evaporation method in the stoichiometric ratio of 1:1 after screening were carried out in pH 1.2 buffer solution using Shimadzu 1201 UV-Visible spectrophotometer. The *in vivo* bioavailability studies were carried out on adult male New Zealand albino rabbits following a parallel design. The blood samples were analyzed using high-performance liquid chromatography. The various pharmacokinetic parameters such as Cmax, Tmax, and area under the curve for the two cocrystals were calculated. **Conclusion:** Both the cocrystals showed a better dissolution profile than the pure drug and the physical mixture of the pure drug and the cocrystals former in the *in vitro* dissolution studies. The oral bioavailability also showed a significant increase of 1.77 and 1.37 times when compared to that of pure aceclofenac ($P < 0.05$).

KEY WORDS: Aceclofenac, Bioavailability studies, Cocrystals, Dissolution studies

INTRODUCTION

The rate of dissolution of a poorly water-soluble active pharmaceutical ingredient controls its rate of absorption and, hence, bioavailability.[1] Many techniques have been reported in the literature so far for enhancing the dissolution characteristics of poorly water-soluble drugs. These include a reduction in particle size, solubilization, use of prodrugs, formation of water-soluble complexes, and manipulation of the solid state of drug substance, i.e., by decreasing crystallinity of drug substance. Recently, crystal engineering approaches, which can potentially be applied to a wide range of crystalline materials, have offered an alternative and potentially fruitful method for improving the solubility, dissolution rate, and subsequently the bioavailability of poorly soluble drugs.[2] Through the application of crystal engineering of cocrystals physicochemical properties of the application programming interface (API) properties can be modified while maintaining the intrinsic activity of the drug molecule. A cocrystal may be defined as a crystalline material that consists of two or more molecular electrically neutral species held together by non-covalent forces.[4] Of the two, one is an active pharmaceutical ingredient and other is a pharmaceutically acceptable, non-toxic cocrystallizing agent(s) called the cocrystal former which is chosen so as to result in a pharmaceutically acceptable product. This limits the cocrystallizing agent to be safe for human consumption be classified as generally recognized as safe .[3] Coformer selection for an API is the most important aspect for designing and screening of cocrystals. For selection of suitable coformers and screening of cocrystals, researchers have used some different knowledge-based approaches which include hydrogen-bonding propensity, synthonic engineering, supramolecular compatibility by Cambridge Structural Database, pKa-based models, lattice energy calculation, the Conductor-like Screening Model for Real Solvents, Hansen solubility parameter, virtual cocrystal screening, thermal analysis, measuring saturation temperature, Kofler contact method, and synthon matching. Traditional methods based on the solution and grinding methods have been used for the preparation of cocrystals. Different types of

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Website: jprsolutions.info | ISSN: 0975-7619

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Received on: 17-08-2018; Revised on: 24-09-2018; Accepted on: 11-10-2018

solution methods are solvent evaporation, solution crystallization technique, anti-solvent addition, slurry conversion method, and reaction crystallization method, whereas grinding methods include two types: Neat grinding and solvent drop grinding. Some newly emerging methods used for the formation of cocrystals are ultrasound-assisted solution method supercritical fluid atomization technique, spray drying technique, and hot melt extrusion technique.[4]

MATERIALS AND METHODS

Materials

The drug aceclofenac was gifted by Suraksha Pharma, Hyderabad, India. Nicotinamide was supplied by Sigma-Aldrich, India. Gallic acid was supplied by CDH Chemicals, New Delhi. Methanol and water used were of the high-performance liquid chromatography (HPLC) grade.

Cocrystal Screening

The screening of the cocrystal formers was done by calculating the solubility parameters using Hoftyzer and Van Krevelen solubility parameters and slurry crystallization technique using seven cocrystal formers.[5,6]

Cocrystal synthesis

After cocrystal screening, cocrystals of aceclofenac were prepared with nicotinamide and gallic acid in the stoichiometric ratio of 1:1 by solvent evaporation technique.

Solid-State Characterization

The prepared cocrystals were characterized by Fourier-transform infrared spectroscopy, differential scanning calorimetry, X-ray diffraction, and scanning electron microscopy techniques.

In vitro Studies

Standard plot for aceclofenac in acid buffer (pH 1.2)

Accurately weighed 100 mg of aceclofenac was dissolved in 10 ml of methanol in a 100 ml of volumetric flask and make up the volume with pH 1.2 buffer solutions. 10 ml of this solution was taken in a 100 ml of volumetric flask and make up the volume with pH 1.2 buffer solutions to get working stock solution having concentration of 100 µg/ml. From this stock solution, aliquots 1, 2, 3, 4, and 5 ml were pipetted out into a series of 50 ml volumetric flasks and make up to mark with pH 1.2 buffer solution to get concentration within the Beer's range from 2 to 14 µg/ml. The absorbance of the resulting solution was then measured at 275 nm using ultraviolet (UV) spectrophotometer against respective parent solvent as a blank. The standard curve was obtained by plotting absorbance versus concentration in µg/ml.[7]

Dissolution Study

The *in vitro* dissolution studies of the pure drug Aceclofenac (ACE), the physical mixture of the drug and the cocrystal formers (PM(ACE-GA) and PM(ACE-NIC)), and the prepared cocrystals ACE-GA and ACE-NIC were carried out using 8 Station USP type-1 dissolution apparatus (Electrolab, Mumbai, India). *In vitro* release profile was examined in 900 ml of pH 1.2 buffer from 0 to 1 h in the basket and rotated at a constant speed of 100 rpm. The medium was maintained at 37°C ± 0.5°C. Aliquots of samples were withdrawn after every 10 min, and the same volume of fresh medium was added immediately to the test medium. The concentration of the drug release at different time intervals was then determined by measuring the absorbance at 275 nm spectrophotometrically using Shimadzu 1201UV-visible spectrophotometer. Corresponding concentrations in the sample were calculated from standard plot and cumulative percentage of drug release from each formulation was calculated, and a graph is plotted for the same.[8]

Pharmacokinetic Study

Protocol of the study

Pharmacokinetics of different aceclofenac cocrystals, i.e., aceclofenac gallic (AG) and aceclofenac nicotinamide (AN) were studied on adult male New Zealand albino rabbits, weighing 1.75–2.00 kg in accordance with an approved protocol by the Institutional Animal house Ethics Committee (IAEC) registration no. IAEC/NIET/2016/011. The rabbits underwent a washout period of 14 days. They were housed in individual cages throughout the study period. The rabbits were provided with water *ad libitum* during fasting and throughout experiment. Before the experiment, they were kept on fasted for overnight.

Drug administration

The study design was a parallel design, in which each animal received one sample at a time. Rabbits were divided into three groups: The first group (3 rabbits) was fed with pure aceclofenac (standard) at a dose of 3 mg/kg body weight; the second group (3 rabbits) was administered with prepared aceclofenac cocrystals of formulation code AG, at an equivalent dose of 3 mg aceclofenac/kg body weight; and the third group (three rabbits) was kept as control. The cocrystals were dissolved in 10 ml of water and were administered orally by a catheter.

Sampling Procedure

The blood samples were collected through the marginal ear vein of the rabbit in heparinized glass centrifuge tubes with the aid of sterilized disposable plastic syringes 0, 0.5, 1, 2, 4, and 6 h after drug administration. Blood samples were centrifuged immediately at 2500 rpm for 5 min in

Centrifuge Apparatus (Remi Motors, India). Then, supernatant was collected and acetonitrile was added to precipitate the proteins. The precipitated proteins were settled by centrifugation at 1800 rpm for 15 min. The supernatant was collected and stored in −20°C until assayed.

Drug Analysis

The drug concentration was determined by HPLC analysis. HPLC analysis was carried out using a UV-detector (L-7400) and WinChrom software. The column used for the separation of aceclofenac was a Li Chro CART 250-4 LiChrosorb RP-18 (4.6 mm i.d.) column. The mobile phase contained a mixture of methanol and water (70:30, v/v). The flow rate of mobile phase was 1.5 ml/min, and the wavelength was 278 nm. All reagents used for the preparation of mobile phase were of HPLC grade. Similarly, the same protocol was carried out for AN.[9]

RESULTS AND DISCUSSION

Cocrystal Screening

The cocrystal formers were screened using Hoftyzer and Van Krevelen solubility parameters and slurry crystallization technique. Of the seven cocrystal formers, all showed good miscibility except for tartaric acid.

Cocrystal Synthesis

The cocrystals were prepared by the traditional solvent evaporation method with the selected cocrystal formers such as nicotinamide and gallic acid in the stoichiometric ratio of 1:1 using ethanol as the solvent.

Cocrystal Characterization

All the characterization techniques confirmed the formation of cocrystals.

In vitro Studies

Standard calibration curve of aceclofenac by UV spectrophotometry

A standard curve of the pure drug aceclofenac was obtained from the stock solution in the concentration range of 2–14 µg/ml using pH 1.2 (acid buffer) at an absorbance of 275 nm. The standard plot of aceclofenac is shown in Figure 1. The regression equation of absorbance on concentration is given by "Absorbance = 0.013, Concentration - 0.000" with $R^2 = 0.995$.

Dissolution Studies

Dissolution studies were carried out for the pure drug ACE, the physical mixture of the pure drug and the cocrystal formers PM(ACE-NIC) and PM(ACE-GA), and the prepared cocrystals ACE-GA and ACE-NIC. Since two cocrystal formers are used for the preparation of cocrystals, we have two

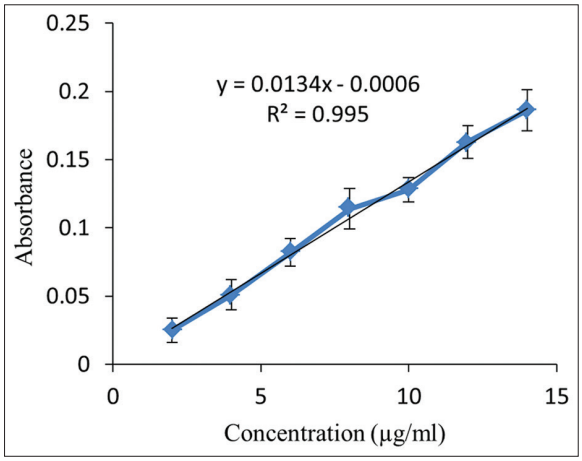

Figure 1: Standard curve of aceclofenac in pH 1.2

different dissolution profiles. The first one shows the percentage cumulative drug release profile of the pure drug aceclofenac, the physical mixture of drug aceclofenac and the cocrystal former nicotinamide, and the cocrystals of drug aceclofenac and the cocrystal former nicotinamide in the stoichiometric ratio of 1:1 which is shown graphically in Figure 2. It can be clearly seen that the percentage cumulative drug release is better in case of the cocrystals as compared to the pure drug or the physical mixture of the drug and the cocrystal former. About 75% of the drug is released in 30 min.

The second dissolution profile shows the percentage cumulative drug release profile of the pure drug aceclofenac, the physical mixture of drug aceclofenac and the cocrystal former gallic acid, and the cocrystals of drug aceclofenac and the cocrystal former gallic acid in the stoichiometric ratio of 1:1 as shown graphically in Figure 3. Here, also we find that the release profile of the cocrystal is better than the plain drug and the physical mixture. Nearly 75% of the drug is released in 20 min. Further, when the release profiles of both the cocrystals are compared, it is found that the release profile of the gallic acid crystals is better than the nicotinamide cocrystals.[10,11]

Pharmacokinetic Studies

The pharmacokinetic parameters such as the peak plasma concentration (Cmax), time to reach peak plasma concentration (Tmax), and area under the curve (AUC) were calculated from the plasma concentration-time curves which are shown in Figure 4 and Table 1. A significant ($P < 0.05$) 1.60-fold increase in the peak plasma concentration (Cmax) was observed in case of AG acid cocrystals as compared to the pure aceclofenac. The Cmax of AN cocrystals also increased significantly ($P < 0.05$) when compared with free drug by 1.41 times. The AUC of AG acid cocrystals and AN cocrystals also showed about 1.77 and 1.37 times significant increase in the oral

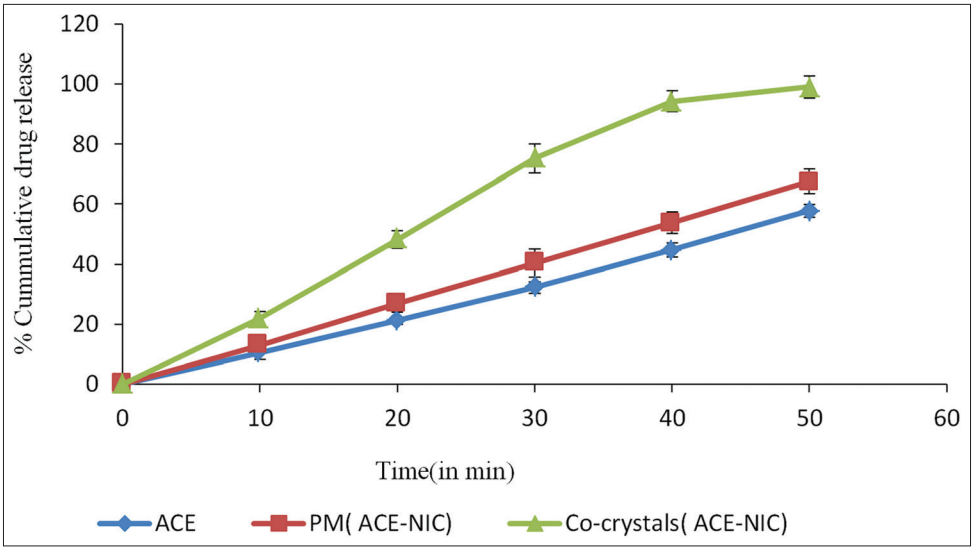

Figure 2: Percentage cumulative drug release profile of ACE, PM (ACE-NIC), and cocrystals (ACE-NIC)

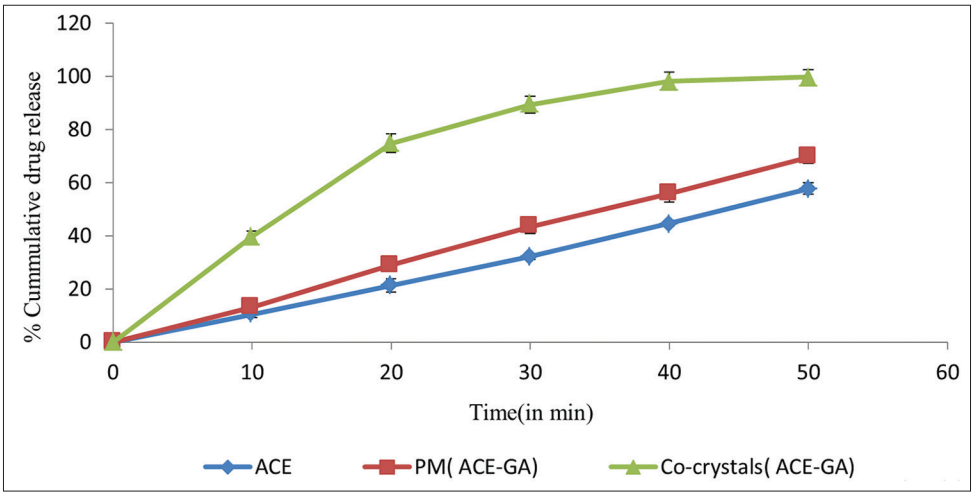

Figure 3: Percentage cumulative drug release profile of ACE, PM (ACE-GA), and cocrystals (ACE-GA)

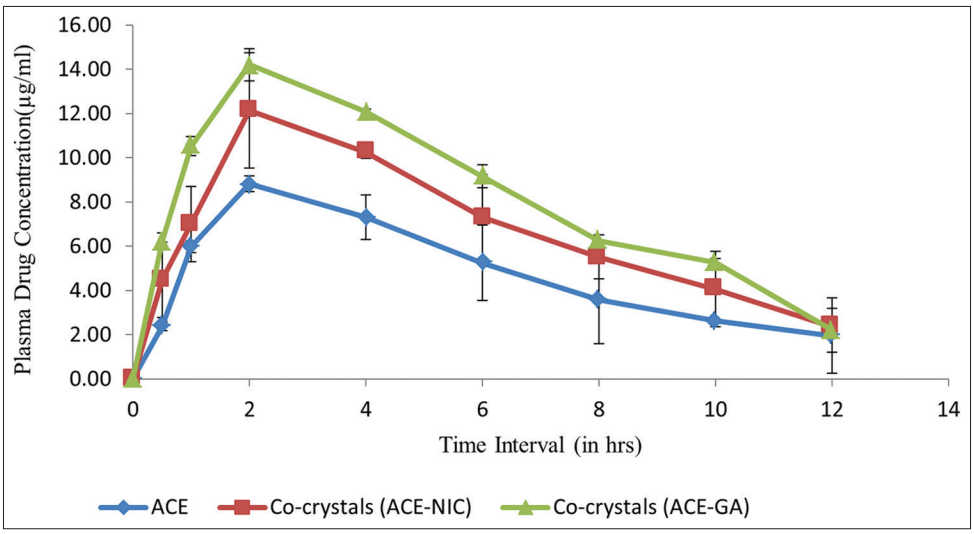

Figure 4: Comparative plasma drug concentration profile of ACE, cocrystals (ACE-GA), and cocrystals (ACE-NIC)

bioavailability, respectively, when compared to that of pure aceclofenac ($P < 0.05$). This level of bioavailability enhancement is comparable or superior to that obtained with other methods.[12]

Table 1: Mean pharmacokinetic parameters

Sample	Cmax (µg/ml)	Tmax (h)	AUC µg/h/ml
Pure ACE	8.83±0.36	2.0	27.89±0.35
Cocrystals (ACE-GA)	14.21±0.4	2.0	49.64±2.0
Cocrystals (ACE-NIC)	12.14±0.62	2.0	38.28±0.99

AUC: Area under the curve

CONCLUSION

In this study, prepared aceclofenac cocrystals of aceclofenac with nicotinamide and aceclofenac and gallic acid exhibited excellent dissolution rate and bioavailability profiles as compared to the pure drug. If this process can be scaled-up to manufacturing level, the crystals can definitely prove to be a great tool to increase the solubility and, hence, biopharmaceutical performance of the poorly soluble drug aceclofenac. However, stability, toxicity, and clinical pharmacokinetic studies are required to be done before commercialization.

ACKNOWLEDGMENT

The authors thank the management of Noida Institute of Engineering and Technology, Pharmacy Institute, Greater Noida, from the bottom of their heart for providing the necessary facilities for carrying out this research work. Our heartfelt is also due to Diya Labs, Mumbai, for carrying out the HPLC analysis of the blood samples.

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Source of support: Nil; Conflict of interest: None Declared