Preparation, characterization and evaluation of aspirin: benzoic acid cocrystals with enhanced pharmaceutical properties

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Abstract

Background: The cocrystallization process in pharmaceuticals has gained widespread attention as a recent method of modifying physicochemical properties without altering the pharmacological characteristics of drugs. Cocrystallization provides a couple of benefits like it can be employed for a large number of APIs (acidic, basic, ionizable, or non-ionizable), and secondly, the availability of a large number of potential coformers increases the possibility of the cocrystals (CCs) that can be synthesized for an API. The main objective of this study was to investigate the effects of cocrystallization on drugs having poor aqueous solubility.

Results: Aspirin (AN) and benzoic acid (BZ) were cocrystallized by using the solvent evaporation technique. CSD (Cambridge Structure Database) software and ΔpKa value method were used for the selection of the drug and coformer and for prediction of CC formation. The analysis of CCs was performed using DSC (differential scanning calorimetry), FT-IR (Fourier transformation infra-red spectroscopy) and XRD (X-ray diffraction) techniques. In vivo anti-inflammatory studies were conducted on 24 Wistar rats divided into four groups.

Conclusions: Here, in this study, in vitro dissolution studies revealed an improved solubility profile of CCs compared to pure drug and marketed formulation viz. 87%, 31% and 60% respectively. The in vivo anti-inflammatory studies exhibited improved anti-inflammatory activity compared to pure drug. So, on the basis of outcomes of this study, we concluded that cocrystallization process have a direct impact on the improvement of physicochemical characteristics of APIs having issues like solubility or stability without any modification and alteration of their pharmacological actions.

Keywords: Cocrystallization, Aspirin, Homosynthon, Anti-inflammatory studies, Solubility

Background

It is a challenging task for pharmaceutical researchers and industry to develop suitable formulation with higher physicochemical properties. The process of cocrystallization is long known; however, in the recent times, this approach has gained enormous importance in pharmaceuticals as a relatively new method for enhancement of solubility, bioavailability, stability, thermal properties, permeability, tabletability and other related physicochemical properties [1]. Cocrystals (CCs) are multicomponent systems in which two components, an active pharmaceutical ingredient and a coformer, are present in stoichiometric ratio and bonded together with non-covalent interactions in the crystal lattice [2]. Cocrystallization offers better

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optimization of not even physicochemical properties but also therapeutic response and pharmacological properties of APIs. The design of a cocrystallization experiment is based on robustness, hydrogen bonding rules and potential intermolecular interactions [3]. Aspirin (AN) is one of the most broadly utilized medications in the world. It was discovered as an anti-inflammatory agent in 1874 [4]. Since then, many other benefits of this drug like antipyretic, anti-platelets, and anticancer have been demonstrated in animals and humans by various researchers from time to time [5]. AN is poorly dissolvable in water and causes gastrointestinal (GI) disturbance. Orally administered AN requires high and successive dosing in light of the fact that it experiences broad presystemic metabolism [6]. Likewise, long term and chronic oral AN is related with genuine gastrointestinal symptoms. AN has been cocrystallized in numerous previous studies like drug: drug CCs of meloxicam: AN [7], AN: nicotinamide CCs [8], and pentoxifylline: AN CCs [9] showing enhanced dissolution profile, but in most of earlier studies, either AN was used as a coformer with other drugs or complete in vitro and in vivo analysis of its CCs were not performed. Here, in this study, we cocrystallized AN with BZ through the solvent evaporation method. The primary objective of this study was to evaluate the effect of cocrystallization on the solubility profile of poorly water-soluble drug. The techniques like CSD software [10] and ΔpKa value [11] method were used to select the drug and coformers along with prediction of CC formation. The cocrystallization was completed in a fixed stoichiometric ratio of 1:1 for both drug and coformer respectively. The purity analysis of drug and characterization of drug, coformer, their physical mixture (PM) and CCs were performed using DSC, FT-IR and XRD techniques. In vitro dissolution and in vivo anti-inflammatory studies were conducted to analyse the drug release profile of drug, CCs and marketed formulation. In most of previous studies, Wistar rats animal species were used for in vivo anti-inflammatory studies. The outcomes of this study revealed an enhanced dissolution profile and a better anti-inflammatory response of CCs compared to the pure drug.

Methods
AN was purchased from Loba Chemie Pvt Ltd. (Mumbai, India). BZ was obtained from Sigma-Aldrich Chemical Limited, carrageenan from Central Drug House (P) Ltd. New Delhi, sodium hydroxide from Loba Chemie Pvt. Ltd., Mumbai, and all other reagents and chemicals used were of analytical grade. The solvent evaporation method was used to prepare CCs. In vivo studies were performed as per the IAEC guidelines and approval of IAEC Committee. Twenty-four Wistar rats of average body weight 180 g were selected from the institutional animal house. The study was approved by Institutional Animals Ethics committee [1767/GO/Re/S/14/CPCSEA; dated: August 31, 2017]. Authors have obtained written informed consent to use the animals in the study from the institute.

In vivo studies
The animal experiments complied with the ARRIVE guidelines. The ARRIVE checklist has been attached as an additional file.

Selection of drug and coformer
The cocrystallization process basically depends upon synthon formation between two molecules. The synthon formation process between two similar functional groups like carboxylic acid functional groups of two molecules is known as homosynthon formation while the interaction between two different functional groups like carboxylic acid and amide functional group is called heterosynthon [11]. Here, in this study, ΔpKa value method was utilized for prediction of CC formation, where difference between pKa value of acidic component (AN pKa 3.5) was subtracted from pKa value of less acidic or comparatively basic component (BZ pKa 4.2) and the ΔpKa value obtained (0.7) between 0 to 1, which is the most favourable range for CC formation [12]. CSD software was used to check the most prone functional group for synthon formation (Fig. 1). AN structure was analysed by using CSD code ‘ACSA LA’. AN consists of centrosymmetric carboxylic acid dimer moieties (O–O) that are, in turn, linked via centro-symmetric methyl C–H–O(C–O) contacts of acetyl groups, thereby forming 1D chains (Fig. 1a, b). The carboxylic acid functional group of AN was found to be the most favourable functional group on CSD analysis, while in case of BZ, only carboxylic acid is present, so it was hypothesized that homosynthon formation could take place between COOH functional of both components via hydrogen bonding.

Analytical method development for the determination of AN
Determination of absorption maxima
For the determination of absorption maxima (λ_max), UV spectrophotometric method was selected for the present work. Two hundred fifty milligrams of pure drug was dissolved in 1000 ml
of 0.05 M, 4.5 pH sodium acetate buffer solution to prepare stock solution. 7.5 mcg/ml solution was scanned over between 200 and 400 nm using UV spectrophotometer (UV-1800, Shimadzu Corp., Japan) [13].

![Plotting the calibration curve](image)

The following concentrations were prepared by using stock solution. The various concentration of stock solution (Table 1) was diluted with 100 ml of 0.05 M, 4.5 pH sodium acetate buffer solution.

![Table 1](image)

**Table 1** Concentrations of samples for plotting calibration curve

| Amount of stock solution added in 100 ml of buffer solution | Concentration (mcg/ml) |
|-------------------------------------------------------------|------------------------|
| 1 ml                                                         | 2.5                    |
| 2 ml                                                         | 5                      |
| 3 ml                                                         | 7.5                    |
| 4 ml                                                         | 10                     |
| 5 ml                                                         | 12.5                   |
| 6 ml                                                         | 15                     |

Fig. 1 Packing of AN molecules. a One-dimensional chains sustained by alternating carboxylic acid and acetyl group centrosymmetric dimers. Acid dimers are connected via catemeric methyl C–H–O and phenyl C–H–O (not shown) hydrogen bonds.

**Table 2** In vivo anti-inflammatory study

| S. no. | Group* | Treatment | Dose    | Route of administration |
|--------|--------|-----------|---------|-------------------------|
| 1.     | I (control) | Carrageenan | 0.05 ml of 1% sol. Injected in plantar side of the left hind paw |
| 2.     | II     | AN        | 100 mg/kg | Oral route              |
| 3.     | III    | AN: BZ CCs | 50 mg/kg | Oral route              |
| 4.     | IV     | AN: BZ CCs | 100 mg/kg | Oral route              |

*Total number of rats = 24 (six animals in each group)
The samples taken were analysed by UV spectrophotometer (UV-1800, Shimadzu Corp., Japan) at 267 nm [13].

**Preparation of AN:BZ CCs**

On the basis of intense literature survey as well as concerned feasibility and practicability, the solvent evaporation method was selected for preparing the CCs [14]. AN and BZ were used as drug and coformer in equimolar amount in the fixed stoichiometric ratio respectively. AN (180 mg) and BZ (244 mg) were taken in the fixed stoichiometric ratio (1:2) and dissolved in ethanol. Sufficient amount of ethanol was used as a solvent for dissolving both components. The solvent was evaporated at room temperature, and after complete evaporation of the solvent, CCs were procured for further experiments [15].

**Characterization of AN, BZ, their PM, and CCs**

For analytical purposes, AN, BZ, their PM and CCs were used. The PM of AN (100 mg) and BZ (100 mg) was prepared by mixing equal amount of both components respectively. No solvent was added in this PM.

### Table 3  Concentration vs absorbance data

| Concentration (mcg/ml) | Absorbance |
|------------------------|------------|
| 2.5                    | 0.124      |
| 5                      | 0.237      |
| 7.5                    | 0.368      |
| 10                     | 0.479      |
| 12.5                   | 0.596      |
| 15                     | 0.713      |

DSC analysis

DSC analysis of AN, BZ, their PM and CCs was performed by using DSC Q10 V9.9 Build 303, US instrument. Two milligrams of the sample taken in a closed aluminium pan was heated from 20 to 160 °C in an atmosphere of nitrogen gas pursing at a flow of 60 ml/min. An empty aluminium pan was taken as the reference pan.

FT-IR analysis

FT-IR study of AN, BZ, their PM and CCs was performed by employing KBr disc technique. The spectrum was recorded over the range of 4000–400 cm⁻¹. The graph obtained was interpreted for the peaks obtained. The study was performed by using FT-IR Alpha Bruker 1206 0280, Germany instrument.

XRD analysis

XRD analysis of AN, BZ, their PM and CCs was performed by using the XRD model ‘XPERT PRO’ instrument with continuous scanning type at 2θ angle position.

**In vitro drug release study**

In vitro drug release was studied using USP type II dissolution apparatus (Lab India DS-8000) [13]. One hundred milligrams of drug was taken for dissolution studies [16]. The dissolution profile of equivalent amounts of CCs and marketed formulation was compared taking 0.05 M, 4.5 pH sodium acetate buffer solution (500 ml) as the dissolution medium. USP type II dissolution apparatus was used for dissolution studies at paddle speed of 50 rpm at 37 ± 0.5 °C temperature for 90 min. Five millilitres of samples was withdrawn at an interval of 15 min.

![Fig. 2 Calibration curve of AN in 0.05 M, 4.5 pH sodium acetate buffer](image-url)
which was replaced simultaneously by an equivalent amount of dissolution medium. The samples were filtered through 0.45-μm syringe filters and suitably diluted for spectrophotometric analysis. The samples taken were analysed by UV spectrophotometer (UV-1800, Shimadzu Corp., Japan) at 267 nm to calculate the amount of drug release [13].

In vivo anti-inflammatory study

Carrageenan-induced paw edema

In vivo studies were performed in the laboratory of the institution under prescribed conditions as per the IAEC guidelines and approval of the IAEC Committee. Animals were kept in air-conditioned housing facilities. Four transparent polycarbonate unbreakable
cages with corn bedding were used for storing the animals. Six animals in each cage were stored. They were treated with hygienic food and fresh water twice daily. The institute provided air-conditioned transportation facilities. For in vivo studies, AN pure drug was used as standard drug (100 mg/kg) while equivalent dose (100 mg/kg) and half dose (50 mg/kg) of CCs were used to evaluate the anti-inflammatory activity (Table 2). Half dose of CCs (50 mg/kg) was used because of improved dissolution profile of drug.
after cocrystallization. Wistar rats were starved overnight. To ensure uniform hydration, the rats received 5 ml of water by stomach tube. After 30 min, the rats were challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the planter side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically after injection (of subcutaneous 0.05 ml of 1% solution of carrageenan), at an interval of 1, 2, 3, 4 and 5 h after challenge [17, 18]. Anaesthetic agents were not used since the procedures were not associated with routine blood withdrawal.
Result

Determination of absorption maxima

7.5 mcg/ml solution was scanned over between 400 and 200 nm using UV spectrophotometer (UV-1800, Shimadzu Corp., Japan). The absorption maxima ($\lambda_{\text{max}}$) was obtained at 267 nm [13].

Establishment of calibration curve

Various concentrations of AN in 0.05 M, 4.5 pH sodium acetate buffer were taken (Table 3). Absorbance of each concentration was determined at $\lambda_{\text{max}}$ 267.40 nm. The graph was plotted between absorbance and concentrations (Fig. 2). $R^2$ value was calculated on the basis of calibration curve, and it was found to be 0.9996. $R^2$ is a statistical measure of fit that indicates how much variation of a dependent variable is explained by the independent variable(s) in a regression model.

Characterization of AN, BZ, their PM and CCs

DSC analysis

DSC analysis was performed by using DSC Q10 V9.9 Build 303, US instrument. The DSC thermogram of AN (Fig. 3) shows a sharp endothermic peak representing its melting point at 141.08 °C. The DSC thermogram of BZ (Fig. 4) shows a sharp endothermic peak representing its melting point at 126.44 °C. No other peak appeared in both thermograms which shows that the samples procured were pure. In the thermogram of PM (Fig. 5), the corresponding peaks of AN and BZ are absent; instead, a sharp peak at 106.67 °C shows the physicochemical interaction of AN with BZ. The DSC thermogram of CCs (Fig. 6) shows a sharp endothermic peak at 106.67 °C. No other peak appeared in this thermogram which shows that the drug has been completely cocrystallized.

| Functional group | AN           | BZ           | PM            | CCs          |
|------------------|--------------|--------------|---------------|--------------|
| C=O stretch      | 1176–1214    | 1175–1279    | 1177–1294     | 1067 (alcohol) |
| C–H bending      | 1366–1451    | –            | –             | 1386–1450 (alkane) |
| C=O stretch      | 1684 (carbonyl) | 1649–1743 (carbonyl) | 1624 (carbonyl) |
| O–H stretch      | –            | 3609–3794 (alcohol (non-bonded)) | 3461–3797 (alcohol (non-bonded)) | 3405 (alcohol (bonded)) |
| C=C stretch      | 1600–1748 (carbonyl) | –          | –             | –            |
Fig. 10 FT-IR spectra of CCs

Fig. 11 XRD spectrum of AN
FT-IR analysis

The characteristic peaks of AN (Fig. 7), BZ (Fig. 8), their PM (Fig. 9) and CCs are explained in (Table 4) respectively. The characteristic peaks present in FT-IR spectra of AN and BZ, viz. C=C stretch 1600–1748 and 1684 for carbonyl functional group and C-O stretch

| Pos. [°2θ] | FWHM total [°2θ] | d-spacing [Å] | Rel. int. [%] | Area [cts*°2θ] |
|------------|------------------|---------------|--------------|----------------|
| **AN**     |                  |               |              |                |
| 7.7701     | 0.0912           | 11.36892      | 77.66        | 2228.16        |
| 15.5695    | 0.1450           | 5.68690       | 100.00       | 4056.83        |
| 16.7211    | 0.1610           | 5.29772       | 3.37         | 167.37         |
| 20.5856    | 0.1995           | 4.31109       | 8.13         | 464.94         |
| 22.5687    | 0.1776           | 3.93656       | 28.63        | 1584.68        |
| 23.1687    | 0.1197           | 3.83595       | 17.60        | 838.48         |
| 23.4501    | 0.0738           | 3.79055       | 4.68         | 88.28          |
| 26.8909    | 0.2891           | 3.31283       | 22.35        | 2179.81        |
| **BZ**     |                  |               |              |                |
| 7.9682     | 0.1546           | 11.08670      | 72.62        | 712.10         |
| 8.1026     | 0.1439           | 10.90312      | 85.34        | 924.48         |
| 16.1232    | 0.2723           | 5.49282       | 62.71        | 1029.17        |
| 17.0640    | 0.1480           | 5.19203       | 61.59        | 691.46         |
| 17.1394    | 0.0789           | 5.16936       | 100.00       | 685.06         |
| 18.8945    | 0.3026           | 4.69296       | 8.42         | 145.97         |
| 21.0225    | 0.0892           | 4.22247       | 8.87         | 71.29          |
| **AN to BZ PM (1:1)** | | | | |
| 7.8562     | 0.0795           | 11.24444      | 19.74        | 2005.94        |
| 8.1908     | 0.1036           | 10.78594      | 100.00       | 10,521.72      |
| 15.6594    | 0.1318           | 5.65445       | 26.34        | 3504.45        |
| 16.3305    | 0.1194           | 5.42355       | 39.16        | 4689.20        |
| 16.8154    | 0.1291           | 5.26821       | 0.82         | 137.65         |
| 17.2844    | 0.0837           | 5.12631       | 8.02         | 798.11         |
| 17.7801    | 0.1798           | 4.98450       | 0.18         | 28.84          |
| 18.1978    | 0.1678           | 4.87101       | 0.25         | 56.54          |
| **AN to BZ CCs** | | | | |
| 5.1023     | 0.1283           | 14.47183      | 66.48        | 707.89         |
| 6.4690     | 1.3361           | 9.74326       | 5.67         | 250.19         |
| 7.6130     | 0.1444           | 11.30652      | 33.70        | 1273.85        |
| 9.5991     | 0.1417           | 10.82663      | 15.49        | 1556.17        |
| 11.4826    | 0.1138           | 7.83616       | 34.12        | 127.16         |
| 12.8963    | 0.1239           | 10.90924      | 96.28        | 3824.37        |
| 14.4608    | 0.5332           | 6.38386       | 29.54        | 79.94          |
| 15.2950    | 0.1800           | 5.67762       | 24.22        | 1446.88        |
| 16.2736    | 0.1760           | 5.44237       | 20.24        | 1267.30        |
| 17.1773    | 0.1288           | 5.15803       | 0.49         | 2877.58        |
| 19.2767    | 1.0006           | 5.12857       | 0.63         | 402.56         |
| 19.9027    | 0.1189           | 5.12821       | 100.00       | 7890.53        |
| 20.6179    | 0.2173           | 4.30441       | 1.17         | 225.30         |
| 21.0057    | 0.1905           | 4.22581       | 0.53         | 64.39          |
1176–1214 and 1175–1279 for carboxylic acid functional group respectively, are also present in their PM (Fig. 9) with minor peak shifting due to some weak Van der Waals forces [19]. This conformed that without addition of solvent in the mixture of drug and coformer, they did not interact chemically with each other. After addition of ethanol in the mixture of AN and BZ in the stoichiometric ratio of 1:2, homosynthon formation occurred due to possible hydrogen bonding between the COOH group present in both drug and coformer, which was evident through the corresponding FT-IR spectra (Fig. 10) of CCs.

**XRD analysis**

The XRD spectrum of AN (Fig. 11) showed major characteristic peaks on 2θ angle position at 7.7701, 15.5695, 22.5687 and 26.8909 with relative intensity (in percentage) of 77.66, 100.00, 28.63 and 22.35 respectively (Table 5). The XRD spectrum of BZ (Fig. 12) showed major characteristic peaks on 2θ angle position at 7.9682, 8.1026, 16.1232 and 17.1394 with relative intensity (in percentage) of 72.62, 85.34, 62.71 and 100.00 respectively (Table 5). The XRD spectrum of PM (Fig. 13) of AN and BZ showed major characteristic peaks on 2θ angle position at 8.1908, 15.6594 and 16.3305 with relative intensity (in percentage) of 100.00, 26.34 and 39.16 respectively (Table 5). The relative intensities of peaks revealed the purity of drug and coformer samples. The XRD spectrum of CCs (Fig. 14) showed major characteristic peaks on 2θ angle position at 5.1023, 7.6130, 11.4826, 12.8963 and 19.9027 with relative intensity (in percentage) of 66.48, 33.70, 34.12, 96.28 and 100.00 respectively (Table 5).

**In vitro drug release study**

The in vitro drug release of AN, marketed formulation and CCs in 0.05 M, 4.5 pH sodium acetate buffer solution (500 ml) are presented in Fig. 15. The pure drug exhibited approx. 31% drug solubility over a 90-min period while marketed formulation and CCs exhibited 60% and 87% drug release respectively (Fig. 16) (Table 6).

**In vivo anti-inflammatory study**

The anti-inflammatory effects of CCs on carrageenan-induced edema in rat’s hind paws are presented in Fig. 17. The dose of CCs in group III was kept half compared to the standard drug AN, and in group IV, equivalent dose of CCs to the standard drug was used (Table 2). There was no reduction in inflammation found in case of group I or control group rats treated with saline. The results showed that CCs significantly reduced the inflammation in 100 mg/kg dose followed by standard anti-inflammatory drug, AN 100 mg/kg. The values of reduction in paw volume in each group, 0.99 ± 0.03, 0.78 ± 0.04, 0.81 ± 0.009 and 0.63 ± 0.01, were found respectively at 5 h after carrageenan administration (Table 7).

**Discussion**

The cocrystallization process basically depends upon synthon formation between two molecules. For analysis of synthon formation, there is a need to study different functional groups in molecules prone to make synthons with each other. There are numerous techniques to detect these functional groups like CSD and COSMOS-RS.
techniques. Another method used for prediction of cocrystal formation is ΔpKa value method [10]. Here, in this study, we used CSD and ΔpKa value method to select the API and coformer. In the DSC study, different sharp peaks were obtained at 141.08 °C, 126.44 °C and 104.82 °C in the thermogram of AN (Fig. 3), BZ (Fig. 4) and PM (Fig. 5) respectively. In the thermogram of the CCs (Fig. 6) and PM of AN and BZ, the corresponding peaks of AN and BZ are absent, instead of a sharp peak at 106.67 °C and 104.82 °C respectively, which shows that the drug has been cocrystallized with coformer when the temperature of their PM in the DSC pans was increased. In the FT-IR study, the characteristic peaks in FT-IR spectra of AN and BZ, viz. C=C stretch 1600–1748 and 1684 for carbonyl functional group and C–O stretch 1176–1214 and 1175–1279 for carboxylic acid
Injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw.

Inflammation in rat paw after carrageenan injection.

Establishment of plethysmometer assembly.

Measurement of inflammation by using plethysmometer assembly.
### Table 6 Drug release study data

| Drug abs Conc (mcg/ml) | Dilution factor | Actual drug conc in 1 ml (mcg/ml) | Drug in 5 ml (mcg/ml) | Total drug (mcg/ml) | Total cumulative drug release | Drug in mg | % cumulative drug release |
|-----------------------|-----------------|-----------------------------------|-----------------------|---------------------|-----------------------------|------------|-----------------------------|
| AN                    |                 |                                   |                       |                     |                             |            |                             |
| 0.369                 | 7.685440678     | 20                                | 153.7288136           | 768.640678          | 76,864.0678                 | 768.640678 | 25.62146893                |
| 0.378                 | 7.87718644      | 20                                | 157.5423729           | 787.7118644         | 79,539.8305                 | 79.5398305 | 26.51327684                |
| 0.382                 | 7.961864407     | 20                                | 159.2372881           | 796.1864407         | 80,406.3559                 | 80.4063559 | 26.80211864                |
| 0.401                 | 8.36440678      | 20                                | 167.2881356           | 836.440678          | 84,440.254244               | 84.44025424 | 28.14675141                |
| 0.409                 | 8.533898305     | 20                                | 170.6779661           | 853.3898305         | 86,175.42373                | 86.1754237 | 28.72514124                |
| 0.444                 | 9.275423729     | 20                                | 185.5084746           | 927.5423729         | 93,607.62712                | 93.60762712 | 31.20254237                |
| CCs                   |                 |                                   |                       |                     |                             |            |                             |
| 0.529                 | 11.07627119     | 25                                | 276.9067797           | 1384.533898         | 138,453.3898                | 138.4533898 | 46.15112994                |
| 0.768                 | 16.13983051     | 25                                | 403.4957627           | 2017.478814         | 203,132.4153                | 203.1324153 | 67.71080508                |
| 0.836                 | 17.58050847     | 25                                | 439.5127119           | 2197.563559         | 221,773.8347                | 221.7738347 | 73.92461158                |
| 0.886                 | 18.63983051     | 25                                | 465.9957627           | 2329.978814         | 235,195.4449                | 235.1954449 | 78.30848164                |
| 0.942                 | 19.82627119     | 25                                | 495.6567797           | 2478.283898         | 250,158.3686                | 250.1583686 | 83.38612288                |
| 0.992                 | 20.88559322     | 25                                | 522.1398305           | 2610.699153         | 263,548.1992                | 263.5481992 | 87.84939972                |
| Marketed formulation  |                 |                                   |                       |                     |                             |            |                             |
| 0.465                 | 9.720338983     | 25                                | 243.0084746           | 1215.042373         | 122,719.2797                | 122.7192797 | 40.90642655                |
| 0.598                 | 12.53813559     | 25                                | 313.4533898           | 1567.266949         | 157,941.7373                | 157.9417373 | 52.64724576                |
| 0.645                 | 13.53389831     | 25                                | 338.3474576           | 1691.737288         | 170,740.9958                | 170.7409958 | 56.91366525                |
| 0.678                 | 14.23305085     | 25                                | 355.8262712           | 1779.131356         | 179,604.8729                | 179.6048729 | 59.88829096                |
| 0.683                 | 14.33898305     | 25                                | 358.4745763           | 1792.372881         | 181,016.4195                | 181.0164195 | 60.3388065                 |
| 0.686                 | 14.40254237     | 25                                | 360.0635593           | 1800.317797         | 181,824.1525                | 181.8241525 | 60.60805085                |

### Carrageenan induced paw volume data

Fig. 17 Change in paw volume with time
functional group, respectively, are also present in their PM (Fig. 9) with minor peak shifting due to some weak Van der Waals forces [19]. The hydrogen bonding was conform by shifting of characteristic peak of non-bonded hydrogen of BZ from 3609–3794 to 3405. The C=O stretch of carbonyl group for AN at 1677 and for BZ at 1684 was shifted to 1624 due to homosynthonic interaction between the drug and coformer [20]. The characteristic peak present in the XRD spectra of AN (Fig. 11) and BZ (Fig. 12) is absent in the XRD spectrum of CCs. There are numerous newer peaks are present which also differ from characteristic peaks present in PM (Fig. 13) of drug and coformer [21]. The homosynthon formation between OH–O and O–OH present at carboxylic acid of both drug and coformer. Due to hydrogen bonding between carboxylic acids of drug and coformer, newer crystal structure has been generated with different crystalline properties. There are minor changes in relative intensity of characteristic peaks in PM, which may be the resultant of some minor interaction between both components during the mixing process to make PM. The outcomes of the in vitro drug release study revealed an increased dissolution profile of CCs compared to AN and marketed formulation (Fig. 15). As AN is a BCS class II drug, so on the basis of dissolution study, we conclude that cocrystallization of BCS class II drugs could improve their dissolution profile. The in vivo anti-inflammatory activities of CCs were found to have an effect in a dose-dependent manner (Table 7). There was a gradual increase in edema paw volume of rats in the control group. However, in the AN group (100 mg/kg), half dose of CCs (50 mg/kg) and full dose of the CC group (100 mg/kg) showed a significant reduction in the edema paw volume (Fig. 17).

Conclusion

Herein, AN and BZ CCs have been synthesized successfully in a fixed stochiometric ratio (1:2) by using the solvent evaporation technique. The interaction of drug and coformer was found to be via hydrogen bonding between the carboxylic acid of both components. The CCs exhibited an improved dissolution profile and enhanced in vivo carrageenan-induced paw edema model based on anti-inflammatory activity compared to parent pure drug. Thus, the cocrystallization of AN provides another approach to the development of products having better physicochemical properties and higher pharmaceutical characteristics compared to parent molecules. Further deep investigations of in vitro and in vivo correlation studies are required for better understanding of this approach.

Table 7 Carrageenan-induced paw volume data

| Time   | Group 1* | Group 2a | Group 3a | Group 4a |
|--------|----------|----------|----------|----------|
|        | Control group | AN (100 mg/kg) | ANBZ CCs (50 mg/kg) | ANBZ CCs (100 mg/kg) |
| 1 h    | 0.556 ± 0.03 | 0.423 ± 0.01 | 0.506 ± 0.01 | 0.368 ± 0.02 |
| 2 h    | 0.748 ± 0.03 | 0.65 ± 0.02 | 0.665 ± 0.01 | 0.538 ± 0.03 |
| 3 h    | 0.865 ± 0.03 | 0.753 ± 0.02 | 0.746 ± 0.02 | 0.636 ± 0.01 |
| 4 h    | 0.946 ± 0.02 | 0.805 ± 0.03 | 0.803 ± 0.01 | 0.651 ± 0.006 |
| 5 h    | 0.998 ± 0.03 | 0.788 ± 0.04 | 0.813 ± 0.009 | 0.63 ± 0.01 |

*Values are mean of 6 ± S.D.
Competing interests
The authors declare that there is no competing interest.

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