DESIGN, FORMULATION, AND CHARACTERIZATION OF APREMILAST-SACCHARIN COCRystals LOADED WITH TOPICAL GEL

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ABSTRACT

Objective: Most of the drugs are relevant to BSC class II and class IV having solubility problems. Cocryrstallization of drug with conformer is an immense approach used to explore the physicochemical properties of drug. The objective of the present work was to design formulate and evaluate the drug cocryystals of poorly soluble drug apremilast (APR) with saccharin.

Methods: Cocryystals of APR were prepared using the solvent evaporation technique. The saturated solubility study and in vitro dissolution study of cocryystals were carried out. The prepared cocryystals were characterized by differential scanning calorimetry (DSC), X-ray diffraction (XRD), and Fourier-transform infrared (FTIR) spectroscopy. The topical gel of APR cocryystals was formulated optimized and evaluated using three-level factorial design.

Results: The cocryystals of APR were prepared in 1:1 molar ratio with saccharin. APR cocryystals showed the improvement in solubility and dissolution as compared to pure APR. The formation of cocryystals was confirmed from change in endothermic peak of DSC and from shifting of FTIR spectra of cocryystals. Crystalinity of cocryystal was confirmed from XRD pattern and noteworthy change in 2θ values of the intense peak. The topical gel of APR cocryystals was formulated and optimized using three-level factorial design using Carbopol-940 and hydroxypropyl methylcellulose (HPMC) as a gelling agent.

Conclusion: The cocryystals with altered physicochemical properties of APR were prepared with saccharin and formulated as a topical gel to overcome the problems related to oral administration.

Keywords: Apremilast, Saccharin, Cocryystal, Solvent Evaporation, Solubility, Factorial Design, Topical gel.

INTRODUCTION

The solubility of drug is one of the major factors taken into consideration during the development of different forms of dosage forms in the pharmaceutical industry [1]. The therapeutic activity of drug mainly depends on its bioavailability and ultimately depends on the solubility of the drug. Solubility is the most important parameter to get the desired concentration of drug in systemic circulation for achieving appropriate pharmacologic effect [2]. Solubility can be defined into two types both qualitatively and quantitatively. Qualitatively, it can be defined as the solute concentration in a saturated solution at a particular temperature, whereas qualitatively, it can be stated as spontaneous interaction of two substances to produce homogenous molecular dispersion. In past decades, various techniques have been used to improve the solubility of the drug. Some of these techniques include the particle size reduction, solid dispersion, cosolvency, and inclusion of cyclodextrin complex [3].

In pharmaceutical field more than 60 % are synthesized drug. Overall synthesized drug upto 40 % have bioavailability problem due to its poor solubility [4]. Pharmaceutical cocryystal is a building tool to improve the solubility, dissolution of the drug, and physicochemical properties of the drug. Cocryystalization of drug with conformer is a promising approach to enhance the solubility of drug without altering its pharmacological effect [5]. Cocryystals can be defined as “a stoichiometric multicomponent system which connected by non-covalent interaction and present in solid form under ambient condition.” Pharmaceutical cocryystal consists of two components one is an active pharmaceutical ingredient and other components called as conformer [6]. Pharmaceutical cocryystals have achieved more interest from pharmaceutical industries because they offer multiple opportunities for modification of the physicochemical characteristics of drug substance without making or breaking any covalent bonds [7].

Cocryystals of apremilast (APR) were prepared using the solvent evaporation method. APR is a novel, orally administered small molecule used in the treatment of psoriasis or psoriatic arthritis. (PSA) [8] Psoriasis or PSA is a chronic inflammatory skin disease affecting approximately 125 million people worldwide. The disease is characterized by scaly, erythematosus skin plaques, or along with joint pain in PSA [9]. APR acts by inhibiting the enzyme phosphodiesterase four which leads to the spontaneous inhibition of tumor necrosis factor-alpha from the human rheumatoid synovial cell [10]. The objective of the present investigation was to design, formulate, and evaluate the APR cocryystal loaded with topical gel. Since the APR has two major problems when administered orally; first, it has low solubility and irritant effect in GIT can cause ulcerative colitis with bleeding [11,12].

MATERIALS AND METHODS

Materials
APR was purchased from a suitable commercial source. Saccharin and methanol and all other chemicals were obtained from Analab Fine Chemicals Mumbai. Double distilled water and analytical grade quality solvents were used throughout the research work.

Formulation of cocryystal
APR cocryystals were prepared using the solvent evaporation method. Accurately weighed APR and conformer saccharin in 1:1 molar ratio were dissolved in an appropriate quantity of methanol as a solvent. The prepared mixture was heated on a hot plate till clear solutions were collected, dried, and stored in an airtight container until further use [13].
Characterization of cocrystal

Physical appearance

Prepared APR cocrystals were visually characterized to study its color, odor, and texture.

Differential scanning calorimetry (DSC)

DSC was performed using DSC 60A calorimeter to study the thermal behavior of drug alone and prepared APR cocrystals. The samples were heated in hermetically sealed aluminum pans under nitrogen flow (30 ml/min) at the scanning rate of 100 °C/min from 500 °C to 3000 °C [14].

X-ray diffraction (XRD)

The silicon sample holders were utilized to get diffraction patterns of pure APR and cocrystal (Shimadzu XRD7000). The instrument was equipped with a fine focus X-ray tube and each sample was placed on to goniometer head that was motorized to permit the spinning of the sample during data acquisition. The sample was scanned at 6 deg/min and voltage 40 kV [15,16].

Fourier-transform infrared (FTIR) spectroscopy study

The APR and APR cocrystals FTIR spectra were obtained using FTIR (Shimadzu IR affinity 1s Japan) spectrophotometer. The samples were mixed with potassium bromide in 1:1 molar ratio and compressed into a disc before scanning between 4000 and 400 cm⁻¹ with a resolution of 4 cm⁻¹ the IR spectroscopy was used to determine the interaction between drug and conformer [17].

Drug content

Weighed quantity (10 mg) of prepared APR cocrystals was taken and dissolved in 100 ml methanol. Then, the solution was ultrasonicated for 15 min to get a uniform solution. After that, the absorbance of the obtained solution was measured using an ultraviolet (UV)-visible spectrophotometer at 230 nm [18].

Saturated solubility study

Accurately weighed dried APR cocrystal equivalent to APR 100 mg of reconstituted with 50 ml of double-distilled water in a conical flask plugged with cotton. It was shaken for 48 h using orbital shaker (Labline Sr. No. 213004), the concentration of dissolved APR was determined using validated UV-visible spectrophotometry. The same procedure was repeated for pure APR and physical mixture of APR:saccharin (1:1) [19].

In vitro dissolution study of cocrystal

The dissolution studies (%) were carried out in pH 6.8 phosphate buffer solution (900 ml) for 60 min at 37 ± 0.5 and 50 rpm using United States Pharmacopeia (USP) type II dissolution test apparatus (Electro-lab, Mumbai, India). The pure drug APR and APR cocrystal equivalent to 20 mg of drug were used for the study. Five milliliters of samples were withdrawn after a specified time interval and immediately replaced with an equal volume of fresh dissolution medium. The sample was filtered using Whatman filter paper. Later suitable dilutions of the sample were done and analyzed using a UV-visible spectrophotometer at 230 nm [20].

Formulation of APR cocrystals loaded topical gel

APR cocrystals loaded to pical gel was formulated and optimized by two factors and two levels by a three-level factorial design using Design-Expert software (version 12). Two independent variables (Table 1) were (a) concentration of Carbopol-940 and (b) concentration of HPMC (Table 2). All formulations were evaluated for in vitro drug release (Y₁) and spreadability (Y₂) as dependent variables [21].

Preparation of APR cocrystal loaded gel

Accurately weighed quantity of Carbopol 940 and HPMC was dissolved in 10 ml of distilled water (70°C) in beaker A. In another beaker B, 100 mg of APR cocrystal was dissolved in 8 ml of propylene glycol. Then, 2 ml of triethanolamine and sufficient quantity of methyl paraben was added to a mixture containing APR cocrystal. Finally, beaker B containing solution was added into the beaker A. Properly mixed the above mixture and stirred well using mechanical stirrer to get a homogeneous mixture.

Characterization of gel loaded with APR

Physical appearance

The formulated gels were inspected visually for its color, consistency, and appearance.

Homogeneity

The formulated gels were checked for its homogeneity by visual inspection after filled into a suitable container. The gels were observed for their appearance and presence of any particulate matter.

pH determination

pH of the formulated gels was determined using digital pH meter and observed readings were noted.

Spreadability

The spreadability (cm) of the gel formulations was determined by placing accurately weighed 1 g of gel between two horizontal glass plates and 1 kg of weight applied over the plate for 1 min. Later, the spreadability was determined by measuring the diameter of gel spread over the plate in 1 min [22].

Viscosity

The viscosity (cps) of the prepared gels was determined using Brookfield Viscometer. The spindle was rotated at 10 r/min and the sample was allowed to settle for 30 min at temperature 25°C before the readings were taken [23].

In vitro drug release study

In vitro drug release study (%) was carried out using vertical Franz diffusion cell apparatus. The cellophane membrane was used for this study. An accurate amount of gel (0.5 g) was applied on cellophane membrane sandwiched between donor and receptor compartment of Franz diffusion cell. Entire surface of the membrane was in contact with a receptor compartment filled with 20 ml phosphate buffer of pH 6.8 as a diffusion media. The whole assembly was placed on a magnetic stirrer to get a uniform solution. The sample was allowed to settle for 30 min at temperature 25°C before the readings were taken [23].

Table 1: Independent variables and their levels

| Independent variables | Levels |
|-----------------------|--------|
|                        | −1     | 0     | 1     |
| Concentration of Carbopol-940 (%w/w) | X₁ | 0.2   | 1.25  | 5   |
| Concentration of HPMC (%w/w)    | X₂ | 0.2   | 1.25  | 5   |

Table 2: Central composite design for the formulation of APR cocrystal loaded gel

| S. No. | Concentration of Carbopol-940 (%) | Concentration of HPMC (%) |
|--------|----------------------------------|---------------------------|
| 1.     | 1.25                             | 1.25                      |
| 2.     | 0.5                              | 0.5                       |
| 3.     | 2.31066                          | 1.25                      |
| 4.     | 1.25                             | 1.25                      |
| 5.     | 2                                | 2                         |
| 6.     | 1.25                             | 1.25                      |
| 7.     | 1.25                             | 1.25                      |
| 8.     | 1.25                             | 1.25                      |
| 9.     | 0.5                              | 2                         |
| 10.    | 0.18934                          | 1.25                      |
| 11.    | 1.25                             | 0.18934                   |
| 12.    | 2                                | 10.5                      |
| 15.    | 1.25                             | 2.31066                   |

APR: Apremilast
stirrer and the solution was stirred continuously at 200 rpm with the temperature maintained at 37±1°C. The sample (1 ml) was withdrawn at a specific time interval and replaced with the same volume of fresh phosphate buffer to maintain sink condition. Further suitable dilution of the sample was made and analyzed using a UV-visible spectrophotometer at 230 nm [24,25].

**Stability studies**

Prepared APR cocrystal loaded formulations were filled in a suitable container and subjected to stability study as per ICH guidelines. Formulations were kept at 40°C/75% RH, 25°C/60% RH, and room temperature for 3 months. Samples were withdrawn at different time interval (1 month, 2 months, and 3 months) and evaluated for pH, physical appearance, viscosity, spreadability, and drug release [26,27].

**RESULTS AND DISCUSSION**

The cocrystals of APR with saccharin were prepared successfully using the solvent evaporation method. Prepared cocrystals were subjected to different physicochemical evaluations and the formulation of the topical gel of the cocrystals.

**Preformulation study**

**Physicochemical parameter of APR cocrystal**

Prepared APR cocrystals were found to be white in color.

**Determination of drug content**

The drug content of the APR cocrystals was found to be 88.2%. The obtained drug content was quite sufficient to the formulation of cocrystals in a suitable dosage form.

**DSC**

DSC analysis was used to evaluate the phase transformation during the formation of cocrystals. DSC thermogram of APR in (Fig. 1) showed an endothermic pic at 160° corresponding to its reported melting point and the reported melting point of saccharin was 228.8°. There was a shift in the thermogram observed in the case of APR cocrystal (Fig. 2) and the peak was showed at 136°. The non-covalent interaction between the drug and conformer is an indication of the formation of cocrystals. This non-covalent interaction between drug and conformer is occurred due to the formation of a hydrogen bond between the polar functional group. This interaction resulted into the change in the molecular structure of the cocrystals formed which gives a new crystalline form of drug with altered physical properties such as solubility and melting point [28].

**XRD**

XRD analysis was carried to evaluate the solid or crystalline form of the drug. XRD study was stated that the alteration in the crystalline nature of a drug and conformer consequent to cocrystallization. Pure APR and APR cocrystals diffractogram were shown in Figs. 3 and 4. XRD pattern of pure APR showed characteristic peaks crystallinity at different 2θ values such as 10.21, 12.37, 16.05, 18.25, and 19.64. In contrast to pure APR, some additional sharp peaks were observed in APR cocrystals diffractogram at 2θ of 26.99, 13.53, and 24.74 which was an indication of the formation of a new crystalline phase, which can be attributed to crystal formation [29].

**FTIR spectroscopy study**

FTIR is an important medium used for the conformation of cocrystals formation and it showed the formation of hydrogen bond between
pure drug and conformer. FTIR peak for pure APR and APR cocrystals was recorded and shown in Figs. 5 and 6. The principle bands were identified and significant changes were recorded. The pure APR spectra of IR showed the characteristic peak which was recorded at 2944 cm$^{-1}$ -NH stretching, 1763 cm$^{-1}$ aromatic -C=O ketone stretching, 1617 cm$^{-1}$ -C=O amide stretching, and 2944 cm$^{-1}$ C-H stretching. The IR spectra of the APR cocrystals were showed the peak at 2578 cm$^{-1}$, 1704 cm$^{-1}$, 1519 cm$^{-1}$, and 3363 cm$^{-1}$ for C-H, -NH, C=O ketone, and C=O amide, respectively. The change in peak shape, peak intensities, and peak broadening was observed which indicates the formation and confirmation of the APR cocrystal with a new crystalline phase [9].

Saturated solubility study
Saturated solubility (ug/ml) of pure APR and APR cocrystals was performed successfully. The solubility of pure APR was found to be 6.89 ug/ml in distilled water 148.93 u/ml. It clearly stated that the solubility of APR was increased in the cocrystal form of drug. The solubility of cocrystals was increased due to molecular interaction of non-covalent bonds and hydrogen bond formation between drug APR and conformer saccharin.

In vitro dissolution study
In vitro dissolution (%) study of pure APR and APR cocrystals were carried out successfully. The dissolution curve of pure APR and APR cocrystals in 6.8 pH phosphate buffer is shown in Fig. 7; it was an evident that the cocrystals of APR with saccharin clearly showed the improvement in dissolution rate as compared to pure drug.

Evaluation of APR cocrystals loaded gel
Physical appearance
The formulated APR cocrystals loaded gels were inspected visually. The gel was found to be white in color and smooth appearance.
Viscosity and pH
The viscosity and pH of all formulations were determined successfully. The obtained data were given in Table 3.

Spreadability (cm)
From the p values less than presented that the quadratic model was found to be significant for spreadability with model f value of 133.17

Fig. 6: Fourier-transform infrared spectra of apremilast cocrystal

Fig. 7: Drug release for apremilast (APR) cocrystal and APR

Fig. 8: Three dimensional graph for spreadability
Equation reveals that both factors (X1 and X2) affect the spreadability characteristics of gel significantly. Three dimensional graph showed that as the concentration of Carbopol increases, spreadability also increases. Increases in HPMC concentration also goes on increases. The spreadability of all formulations was found in between a range of 5.4 and 6.5 (Figs. 8 and 9).

In vitro drug release (%)
From the p value presented in the quadratic model was found to be significant for in vitro drug release of model f value of 51.98 (p<0.0500). The quadratic equation generated by the software is as follows:

\[
\text{Spreadability} = 25.13817 + 22.26910 \text{ Conc. of Carbopol} + 14.51493 \text{ Conc. of HPMC} - 4.44444 \text{ Conc. of Carbopol} \times \text{ Conc. of HPMC} - 6.76556 \text{ Conc. of Carbopol}^2 - 3.37444 \text{ Conc. of HPMC}^2.
\]
Drug release = + 42.17436 + 38.60250 + Conc. of Carbopol + 56.18546 Conc. of HPMC + 1.60889 Conc. of Carbopol * Conc. of HPMC – 19.21444 Conc. of Carbopol 2 – 21.69000 Conc. of HPMC 2.

From the graph, it was showed that the Carbopol in the concentration range of 1.1–1.75%, the drug release was maximum. The effect of HPMC on drug release showed that maximum drug release was found between the concentration ranges of 1.1 and 1.7% (Figs. 10 and 11).

Optimization of study
From the evaluation of all the prepared formulations (F1 to F13 batches), F1 batch was found to be optimized batch having spreadability and drug release 5.85 cm and 99.26%, respectively.

Stability study
During the storage of APR cocrystals loaded gel, there may be chances of changes in the physicochemical parameters such as spreadability and drug release of the formulation. Hence, the prepared formulations were subjected for the stability study at room temperature and accelerated condition for a period of 3 months to define the stability. It was found that the APR loaded gel was stable at both conditions. The obtained data were given in Table 4.

CONCLUSION
In the present work, cocrystals of APR and saccharin were prepared using the solvent evaporation technique. The prepared APR-crystals exhibit good physicochemical properties such as solubility and dissolution. Solid state characterization of drug and cocrystals was carried out using DSC, XRD, and FTIR studies. APR cocrystal formation was confirmed. The altered thermal changes, IR bands along with intensities and change in 2θ values in DSC, FTIR, and XRD, respectively, give the evidence of the formation of a new crystalline phase. The prepared APR-cocrystals were formulated into a topical gel using three-level factorial design. Carbopol-940 and HPMC were used as a gelling agent as independent variables. F1 formulation was found to be optimized batch and selected variables show a significant effect on the responses that are drug release and spreadability. From the overall conducted study, we can conclude that the newly developed crystalline form of APR with saccharine showed increased solubility and dissolution rate and it was given in topical formulation to overcome problems related to oral administration of drug.

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AUTHORS’ CONTRIBUTIONS
All the authors have contributed equally.
CONFLICTS OF INTEREST
There are no conflicts of interest.

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