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Studies On Cocrystallization Of Pitavastatin: Solid State Characterization

Baskar Bangar[1], Subhranshu Panda[1], Adhikrao Yadav[2]

[1] School of Pharmaceutical Sciences, Jaipur National University, Jaipur, Rajasthan, India.
[2] KCT's, Krishna College of Pharmacy, Karad, Maharashtra, India.

***Corresponding Author:** Baskar Bangar
Email:bhaskarbangar44@gmail.com

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Abstract

The poor solubility and dissolution rate is major constrain in oral delivery of most of drugs. The most of drug synthesized nowadays are BCS class II and IV drugs. Many scientific investigators have utilized various approaches for dissolution rate enhancement of drug. The formulation of cocrystals is recently explored way for solubility enhancement of drug. Pitavstatin is HMG CoA reductase inhibitor used in management of increased cholesterol level. The poor solubility is major limitation associated with oral delivery of pitavastatin. Thus, present study has planned to formulate pitavastatin cocrystals.
The cocrystals of pitavastatin were prepared using various coformer by solvent evaporation method. The formulated cocrystals showed enhanced saturation solubility than pure drug and more drug release than pure drug. Thus, cocrystals could be promising alternative for delivery of drug.

Keywords: Pitavastatin, Solubility enhancement, Cocrystals, Solvent evaporation

Introduction

The poor aqueous solubility and dissolution rate of API is one of the main challenges in pharmaceutical development and is becoming more common among new drug candidates over the past two decades due to the use of high throughput and combinatorial screening tools during the drug discovery and selection phase. The lead molecules discovered utilizing these screens is increasingly larger and more lipophilic. The improvement of solubility and dissolution profiles of these lipophilic drug molecules without altering the molecular structure is a particular challenge for the successful development of pharmaceutical products. According to the Biopharmaceutics Classification System (BCS), a drug compound is poorly soluble if the highest dose strength is not soluble in 250 ml aqueous media over the pH ranges at 37 Degree Celsius. These compounds mostly belong to Class II, which are poorly soluble and highly permeable according to the pH of the gastrointestinal fluid and tend to present dissolution-limited absorption. Despite their high permeability, these drugs often have low oral bioavailability because of their slow and limited release of drug in gastrointestinal fluid. Therefore, one of the major challenges of the pharmaceutical industry is to apply strategies that improve the dissolution and/or apparent solubility of poorly soluble drugs to develop such problematic compounds into orally bioavailable and therapeutic effective drugs [1].
Recently cocrystals have gained much importance owing to their amenability to design and their ability to tailor physicochemical properties [2]. The development of cocrystals was resulted by designing crystals with a purpose, as the properties of a compound depend on the arrangement of the atoms in the crystal structure which helps in modifying its properties. Cocrystals are "long known but little studied" group of compounds and was popularized by Etter. The first cocrystals to be synthesized was Quinhydrone, a 1:1 cocrystal between benzoquinone and hydroquinone and was synthesized by Wohler in 1844. In 1963 Hoogsten synthesized a complex between 1-methyl thiamine and 1- methyl adenine as seen in DNA base pairing and used the term "cocrystal" for the first time [3].
Generally, chemists and engineers in pharmaceutical industry seek to deliver crystalline forms of their active compounds due to inherent stability of crystalline materials. Active pharmaceutical ingredients (API's) can exist in different solid forms, including polymorphs, solvates, hydrates, salts, cocrystals and amorphous solids [4]. Each solid form displays unique physicochemical properties that can influence bioavailability, purification, stability, manufacturability and other performance characteristics of the drug.
Thus, present study has started with an aim to formulate and evaluate cocrystals of pitavastatin using various coformers.

Materials and methods

Pitavastatin was purchased from Zenvito Healthcare, India. Benzoic acid, acetone, citric acid and itaconic acid were purchased from SD Fine Chemicals Ltd., Mumbai, India. All other reagents and solvents were analytical grade and purchased locally.

Methods

Preparation of pitavastatin cocrystals

The cocrystals of pitavastatin with saccharin (SAC), maleic acid (MA), itaconic acid (IA) and benzoic acid (BA) were prepared in 1:1 stoichiometric ratio by solvent evaporation (SE) technique [6].

To prepare pitavastatin-SAC cocrystal by this method, 323.24 mg of pitavastatin and 183.19 mg of SAC were dissolved in 10 ml of acetone by stirring. Stirring was continued till a clear solution was obtained. The system was covered with an aluminium foil and about 5- 6 fine holes were pierced in the foil. Solvent from the clear solution was allowed to evaporate at a slow constant rate at room temperature with constant stirring. The process was continued till solid cocrystals were obtained. The obtained cocrystals were dried at room temperature and evaluated.

Characterization of pitavastatin cocrystals prepared by solvent evaporation and melt fusion method

Visual morphology

The photographic images of plain pitavastatin, benzoic acid, itaconic acid and developed cocrystals were taken using Canon Powershot A480 Digital camera and organoleptic characteristics were studied and compared.

Melting point

Melting point of coformers (SAC, IA and BA) and obtained cocrystals were determined by using Expo Hi-Tech Melting point apparatus (India).

In vitro drug release

The *in vitro* release profiles of cocrystals was studied using USP Dissolution Apparatus II (Paddle Type) and dissolution experiments were performed in triplicates [7-9]. 80 mg of pitavastatin and an amount of pitavastatin cocrystals equivalent to 80 mg of pitavastatin were weighed and placed in hard gelatin capsules size 1. The hard gelatin capsules were clamped using sinkers and placed in 900 ml of phosphate buffer pH 7.4. Dissolution was performed at 100 rpm at 37±0.5^{o}C. Aliquots of 10 ml were taken at 5, 10, 15, 30, 45 and 60 minutes and filtered through Whatman filter paper. 1 ml of the resulting solution was diluted 10 times with phosphate buffer pH 7.4. The absorbance of resultant solutions was measured using UV-Visible spectrophotometer (Jasco 530V, Japan). Sink conditions were maintained by replacing 10 ml of the aliquot sample with phosphate buffer pH 7.4. The standard equation obtained by plotting a standard plot of pitavastatin in phosphate buffer pH 7.4 was used for calculating the drug release. All the measurements were performed in triplicates.

Saturation solubility studies

Saturation solubility studies of pitavastatin and cocrystals were performed in phosphate buffer pH 7.4 [10-12]. Briefly, an excess amount of pitavastatin was taken in the eppendorf to which 1 ml of phosphate buffer pH 7.4 was added. Thereafter, the eppendorf were sealed and shaken for 72 hrs at 37^{o}C in water bath shaker (Remi, CM 101, Mumbai, India). Subsequently, the solutions were filtered through 0.22 µ filter and the supernatant was suitably diluted and pitavastatin content was quantified on a Jasco-V-530 UV spectrophotometer, using a validated UV spectrophotometric technique. To eliminate the solvent effect on absorbance, phosphate buffer pH 7.4 was used as blank. Saturation solubility studies of cocrystals were performed in a similar way. All the experiments were performed in triplicates.

Determination of drug content

Drug content was determined by dissolving cocrystals quantity equivalent to 80 mg of pitavastatin in 50 ml of methanol and the volume was adjusted to 100 ml with methanol AR [13-15]. The solution was filtered through Whatman filter paper no 41 and 1 ml of the resulting solution was diluted 100 times with methanol AR. Absorbance of the resultant solution was measured using UV spectrophotometer (Jasco 530V, Japan).

Stability studies of Cocrystals

Introduction

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light; and to establish a retest period for the drug substance or a shelf life for the drug product and recommended storage conditions. Stability studies include exposing the drug product to normal storage conditions (long term storage condition) for a period of time sufficient to cover the proposed retest period, also the drug product is subjected to accelerated conditions of temperature and humidity so as to determine the stability of product under accelerated conditions. The studies designed to increase the rate of chemical degradation or physical change of a drug substance or drug product by using exaggerated storage conditions are known as accelerated stability studies. The product is evaluated at different time points for various parameters, such as physicochemical characters, drug content and the data obtained from the studies carried out is used

to determine the shelf life of a product at a particular storage condition. Shelf life is a period till which not more than 10% of the drug is degraded.

Stability testing as per ICH Guidelines

International conference on harmonization (ICH) has established a guideline for "Stability testing of new drug substances and products Q1A (R2). The guideline recommends the following storage conditions for drug products intended to be stored at room temperature:

Table 1 Storage conditions for stability studies

Study	**Storage condition**
Long term	25^0C/60% RH ± 5% RH
Accelerated	40^0C/75% RH ± 5% RH

Results and Discussion

Preparation of pitavastatin cocrystals by solvent evaporation (SE)

Pitavastatin cocrystals were successfully formulated by solvent evapouration technique using various cocrystals. The difference in organoleptic properties of cocrystals and plain drug were observed which indicated conversion of drug into different crystalline form. The organoleptic properties of various cocrystals are represented in table 2. The colour of pure pitavastatin was white, whereas cocrystals were found to be pale yellow to brown. The cocrystals prepared using maleic acid coformer where found to be semisolid, which were difficult to process further. Thus, maleic acid cocrystals were not used in further study.

Table 2 Organoleptic characteristics of cocrystals obtained by solvent evaporation

Cocrystals	**Appearance**
Pitavastatin-SAC	Pale yellow coloured flaky powder
Pitavastatin -IA	Pale yellow coloured flaky powder
Pitavastatin -BA	Yellow-brown coloured flaky powder
Pitavastatin-MA	Semisolid, orange coloured compound

Characterization of pitavastatin cocrystals

Melting point

Melting point is the temperature at which the solid phase is at equilibrium with the liquid phase. It is an important property considered during drug development and is a fundamental physical property. Much research work has been done to investigate if melting point of a cocrystal changes with respect to individual components. In the present study melting point of cocrystals of pitavastatin prepared by solvent evaporation were analysed. All the formed pitavastatin cocrystals had considerable difference in their melting points when compared to pitavastatin and coformers used.

Table 3 Melting point of drug and cocrystals

S. No.	**Sample details**	**Melting point (^{0}C)**
1	Pitavastatin	219-230
2	SAC	227-228
3	IA	162-164
4	BA	121-122
5	SAC Cocrystals (Solvent Evaporation)	122-125
6	IA Cocrystals (Solvent Evaporation)	82-85
7	BA Cocrystals (Solvent Evaporation)	80-81

***In vitro* drug release**

Dissolution is an official test used by official compendia for drug release from solid and semisolid dosage forms and it is used in Quality Control (QC) and Research and Development (R&D). The purpose of in vitro drug release studies in QC is to confirm batch to batch consistency and detection of manufacturing deviation while in R&D the focus is to provide some predictive estimate of drug release in respect to the in vivo performance of a drug product.

The dissolution curves of pure drug alone and cocrystals in phosphate buffer pH 7.4 are shown in figure 1. From the graphs it is evident that the cocrystals have significantly improved the dissolution rate of drug compared to the pure drug.

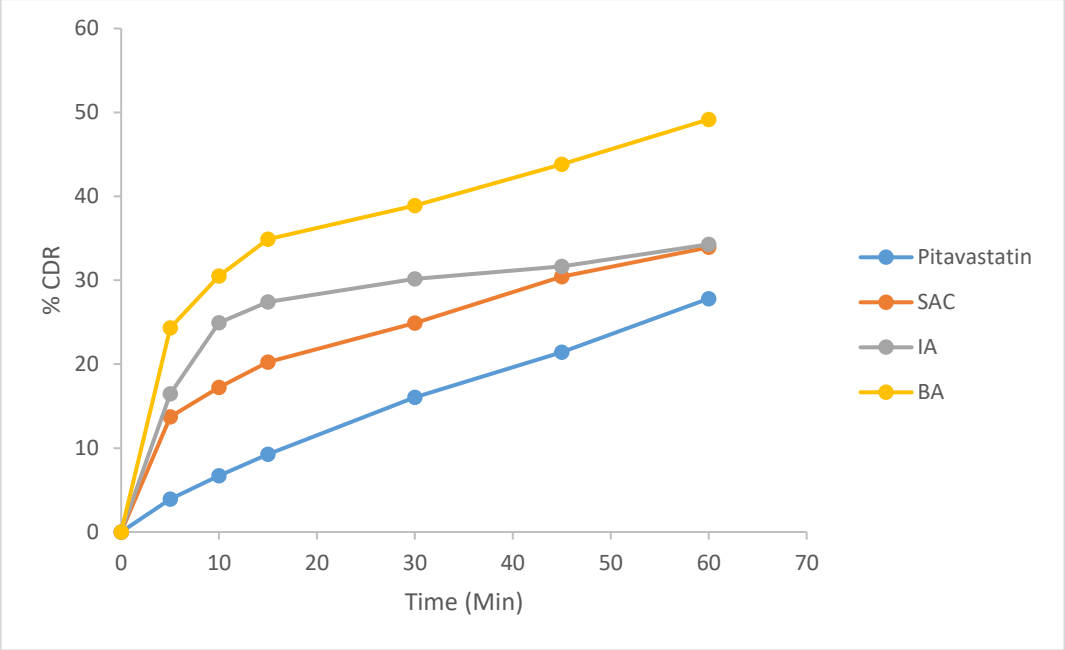

Figure 1 *In vitro* release profile of pitavastatin and pitavastatin cocrystals prepared by SE

The dissolution profile of pitavastatin cocrystals prepared by SE depicted significant increase in the dissolution rate of pitavastatin as compared to plain pitavastatin. Almost 3.52 fold increase in dissolution rate of pitavastatin was observed from pitavastatin -SAC cocrystals prepared by SE whereas it was found to be 4.22 fold and 6.23 fold greater from pitavastatin -IA and pitavastatin -BA cocrystals prepared by SE respectively within 5 minutes. Percent cumulative release of 33.92%, 34.28% and 49.17% were obtained from pitavastatin-SAC, pitavastatin-IA and pitavastatin-BA cocrystals prepared by SE respectively within 60 minutes. Whereas the percent cumulative release of pure drug within the same time was observed to 27.81%.

Saturation solubility studies

Solubility is the concentration of the solute in a solution when equilibrium exits between pure phase and the solution phase. Knowledge of the solubility of a drug is a critical parameter in formulating products, developing analytical method and evaluating drug transport or distribution problems. Saturation solubility of the drug and cocrystals in phosphate buffer pH 7.4 was examined. Saturation solubility results of pitavastatin cocrystals with BA and IA demonstrated that as the ratio of coformer was increased, saturation solubility of cocrystals also increased.

The saturation solubility of 1:1, 1:2 and 1:3 stoichiometric ratios of pitavastatin-BA and pitavastatin-IA cocrystals prepared by solvent evaporation were found to be 5.78, 7.38, 8.32, 6.30, 6.50 and 5.83 mg/ml respectively. There was about 4-6 fold increase observed in the solubility of cocrystals prepared by solvent evaporation compared to pure pitavastatin.

Cocrystals prepared by solvent evaporation technique exhibited better enhancement in solubility than those prepared by melt fusion method in saturation solubility studies. This might be because by solvent evaporation technique of cocrystallization the drug and coformer interact at a molecular level is higher by this technique.

Further BA was found to be better coformer than IA in improving the solubility. This could be due to existence of correlation between melting point of cocrystal and its solubility. Similar results were reported by Bak et al in the study of AMG 517 reported that cocrystals with high melting points results in lower solubility (Bak et al., 2008). Less improvement in dissolution of IA coformer could be due to low proportion of complex with higher negative energy. However the ratio of this proportion needs to be confirmed using advanced analytical methods like Raman spectroscopy and X-ray crystallography. Physical mixtures of drug and coformers did not show any enhancement in saturation solubility indicating absence of intermolecular interaction between drug and coformer.

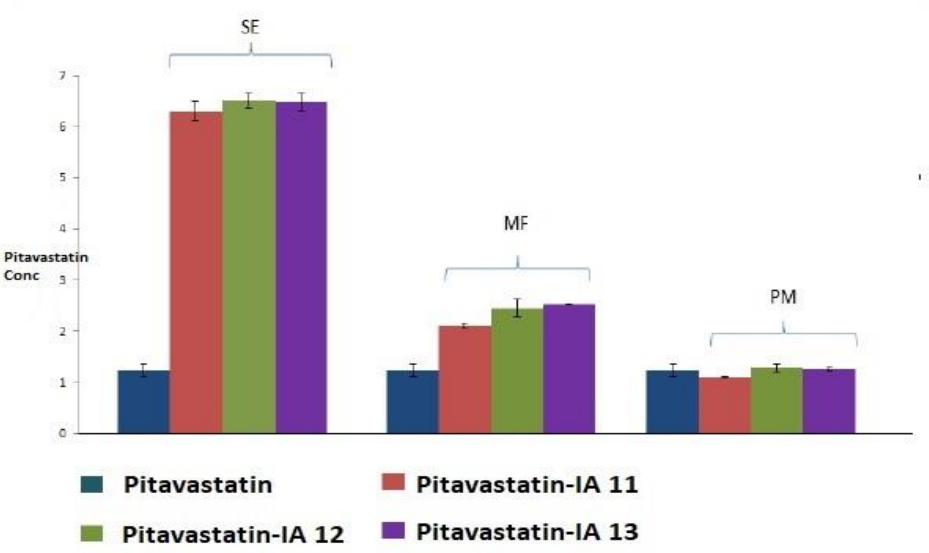

Figure 2 Saturation solubility of pitavastatin and pitavastatin cocrystals

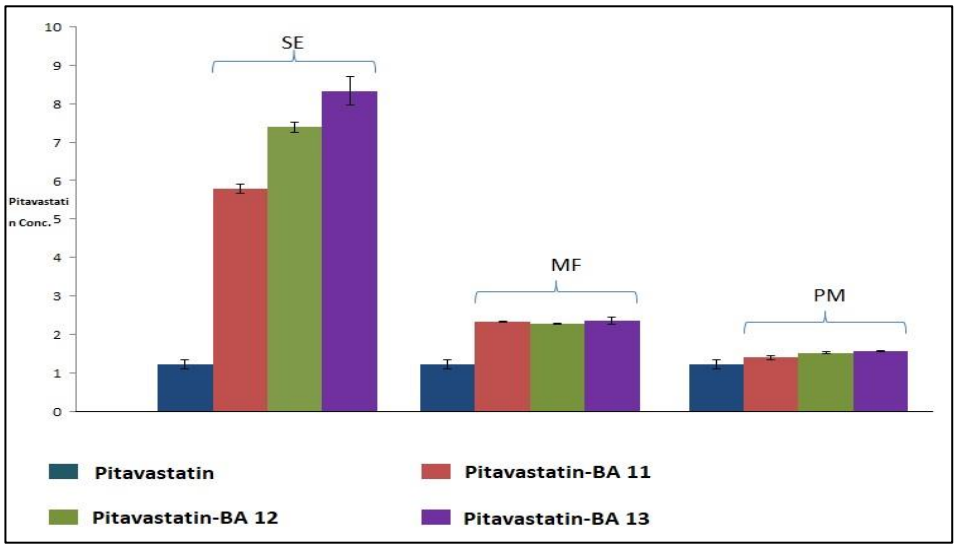

Figure 3 Saturation solubility of pitavastatin and pitavastatin cocrystals

Determination of drug content

Percentage drug contents of prepared cocrystals were found in the range of 90.18±1.07 w/w to 97.22±1.17 w/w.

Table 4 Drug content of cocrystals

Cocrystals	Drug Content (%)
Pitavastatin-BA11	95.00±0.94
Pitavastatin-BA12	97.17±1.17
Pitavastatin-BA13	96.56±1.07

Assessment of stability

Stability studies of optimized Pitavastatin-BA cocrystals were evaluated according to ICH guidelines. The cocrystals filled in glass vials were stored in 25ºC±2ºC/60%±5% RH and 40ºC±2ºC/75%±5% RH. Pitavastatin cocrystals were sampled after 1 month time point and evaluated for physical appearance and release profile.

Table 5 Organoleptic characteristics of cocrystals before and after stability studies

Cocrystals	Before stability	After stability 25^0C/60% RH ± 5% RH	After stability 40^0C/75% RH ± 5% RH
Pitavastatin-BA	White, shiny powder	White, shiny powder	White, shiny powder

Conclusion

The poor oral bioavailability of drug due to poor solubility and less dissolution rate is major constrain in oral delivery of most of drugs. Many scientific experts working in field of solubility enhancement have utilized various approaches for dissolution rate enhancement of drug. The formulation of cocrystals is recently explored way for solubility enhancement of drug. Thus, present study has planned to formulate pitavastatin cocrystals.
The cocrystals of pitavastatin were prepared using various coformer by solvent evaporation method. The formulated cocrystals showed enhanced saturation solubility than pure drug and more drug release than pure drug. Thus, cocrystals could be promising alternative for delivery of drug.

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