DEVELOPMENT, CHARACTERISATION AND EVALUATION OF SILDENAFIL ASPIRIN CO-CRYSTALS

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Sildenafil citrate is a selective inhibitor of phosphodiesterase type 5 enzyme (PDE5) extensively used for the treatment of erectile dysfunction (ED). Sildenafil citrate is BCS class II drug, practically insoluble in water. Its absolute bioavailability is 41% and half life is 4 hours. Aspirin is a Cyclooxygenase Inhibitors (COX-1 & COX-2) having a mechanism by dilating blood vessels in vivo, probably through direct effect on vascular smooth muscle. Therefore, the vasodilator action of aspirin which dilates the blood vessels gives synergistic effect to Sildenafil Citrate. The active pharmaceutical ingredients (APIs) in crystalline forms are more promising towards the stability, reproducibility and purification of the same in comparison to other types of solid forms, hence having the most priority in comparison to other forms. The properties like dissolution rate and intrinsic solubility of several crystalline forms are different and thus are playing an important role in enhancing the bioavailability. The stability with respect to temperature and humidity are more significantly depending on packaging of crystals. Sildenafil Aspirin Pharmaceutical co-crystals were successfully engineered with succinic acid as coformer under reflux condition. A new solid phase (cocrysaltals) was characterized by Fourier transformation infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), powder X-ray diffractometry (PXRD). Physical characterization showed that Sildenafil Aspirin cocrystalline phase had unique thermal, powder X-ray diffraction property. The physicochemical properties of pure Sildenafil and corresponding co-crystals were accessed in terms of melting point, drug content uniformity, dissolution studies. The rapid rate of dissolution of cocrystals was observed in initial 10 min. The extent of dissolution was also enhanced on account of cocrysatallization.

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INTRODUCTION

Erectile dysfunction (ED) is the persistent inability to achieve or maintain an erection sufficient for satisfactory sexual performance. According to data from the Massachusetts Male Aging Study, up to 52% of men between the ages of 40 and 70 are affected by ED.

So can say that erectile dysfunction (ED) is a complex condition involving psychosocial and biological factors. Erectile Dysfunction is a disorder of male sexual function, affecting all age groups people with a considerable impact on quality of life. Oral medicinal therapy with phosphodiesterase 5 inhibitors (PDE5I) has emerged as the first line treatment for male sexual dysfunction in a way that has transformed both the manner in which public views male sexual dysfunction and health care providers deliver care (Fazio and Brock 2004). Sildenafil (Viagra®), marketed by Pfizer, was the first PDE5 inhibitor on the market and was US FDA approved drug for the use in treatment of ED in 1998. Tadalafil (Cialis®), marketed by Lilly was launched in 2003 and vardenafil (Levitra®), marketed by Bayer, in 2003. Both have received US FDA approval for the treatment of male erectile dysfunction (Seftel et al. 2004).

ED is associated with chronic disease including cardiovascular disease, high blood pressure and diabetes (Diabetes Mellitus). Furthermore, ED may be an early warning sign of these chronic diseases. It can result from physical, medical or psychological factors, or a combination of factors. Long term use of drugs like anti depressants and nicotine consumption are the common causes of ED.

Aspirin has a unique vasodilator action in vivo, probably through direct effect on vascular smooth muscles giving synergistic effect with sildenafil Citrate also increasing its solubility in aqueous medium because Sildenafil Citrate is a BCS class II drug, difficulty in solubility. Hence, one such option is co-crystallization which is more promising towards the stability, reproducibility and purification.

Cocrystals are structurally homogeneous crystalline materials containing two or more components present in the definite stoichiometric amounts. Pharmaceutical Co-crystals are the nonionic supra molecular complexes and can be used to address the physical property issues such as solubility, stability and bioavailability in the pharmaceutical development without changing the chemical composition of the API. Cocrystal is a crystalline entity that formed by two different or more molecular entities where the intermolecular interactions are weak forces like hydrogen bonding and δ- δ stacking. Super porous systems, biodegradable hydrogel systems etc.

Co-crystallization alters the molecular interactions and composition of pharmaceutical materials, and is considered better alternative to optimize the drug properties. Co-crystals offer a different pathway, where any active ingredient regardless of acidic, basic, or ionic groups, could potentially be co crystallized. This aspect also helps in complement the existing methods by reintroducing molecules that had limited pharmaceutical profiles based on their non ionicable functional groups. The stoichiometric ratio of active ingredient and the cocrystal former in pharmaceutical cocrystal is mostly simple (1:1, 1:2, 1:3 or vice versa). Cocrystals with the same active pharmaceutical ingredient will have strikingly different pharmaceutical properties (melting point, solubility, dissolution, bioavailability, moisture uptake, chemical stability, etc.), depending on the nature of the second component. Some of the cocrystals formed had higher and some had lower melting points as compared to their pure components.

Crystal form can be crucial to the performance of dosage form which has intrinsic barriers to drug delivery, such as low aqueous solubility, slow dissolution in gastrointestinal media, low permeability, and first-pass metabolism etc. It can be prepared by melt crystallization, grinding, recrystallization from solvents. Sildenafil citrate is a selective inhibitor of phosphodiesterase type 5 enzyme (PDE5) which is extensively used for the treatment of erectile dysfunction (ED). Sildenafil citrate is BCS class II drug, practically insoluble in water. It has an absolute bioavailability of 41% and half life of 4 hours. Aspirin is a Cyclooxygenase Inhibitors (COX-1 & COX-2) having a mechanism by dilating blood vessels in vivo, probably through direct effect on vascular smooth muscle. Therefore, the vasodilator action of aspirin which dilates the blood vessels gives synergistic effect to the Sildenafil Citrate. Rapid onset and increased extent of bioavailability are desirable for PDE-5 inhibitor drugs. This inspired us to search for Sildenafil Aspirin cocrystals with special attention given to the cocrystallization with pharmaceutically acceptable cocrystal formers as a viable means to improve its dissolution rate and hence bioavailability.

MATERIALS AND METHODS

Pure drug sample of Sildenafil Citrate was kindly gifted by Intas Pharmaceuticals Ltd., Ahmedabad. Aspirin and Succinic acid were procured from Sulab and S.D. Fine respectively. Methanol (Sulab), Methyl P-Hydroxy Benzoate, Sodium Caboxy methyl cellulose, Potassium Chloride (KCI), Magnesium Chloride (MgCl2.6H2O), Calcium Chloride (CaCl2.2H2O), Di-potassium Hydrogen Phosphate (K2HPO4), Potassium Di-Hydrogen Phosphate (KH2PO4).

EXPERIMENTAL/METHODOLOGY

Cocrystals were prepared by recrystallization from solvents. Sildenafil-Aspirin-Succinic acid cocrystals were obtained by 1:1 molar ratio of the API (Sildenafil Citrate) and Coformer (Succinic Acid). 20 mL of solvent (Methanol) was added, and the slurry was transferred to the reaction vessel (round bottom flask) & stirred with magnetic stirrer. The complete assembly was set with reflux column on the magnetic stirrer with hot plate. The temperature was set at 70°C for 1 hour. The temperature was decreased in 10°C increments to induce precipitation in a stirred unseeded system. Finally iterate to enhance the solids recovery on decreasing the temperature.

Melting point determination

Melting points of co-crystal samples were determined using paraffin bath.
Fourier transform infrared spectroscopy

IR spectroscopy was conducted using a FTIR Spectrophotometer (Prestige-2100, Shimadzu Corporation, Kyoto, Japan.). Samples were distributed in KBr. The spectrum of drug alone, coformers and cocrystals was recorded in the wavelength region of 4000–400 cm⁻¹.

Differential scanning calorimetry

Thermal analysis of cocrystals were recorded on Metcor Toledo TGA/DSC. Powder samples i.e., 5-10 mg samples were placed in a sealed aluminum pan. The analysis was performed in a temperature range of 5 to 300°C with a heating rate of 10°C per minute.

Powder X - ray diffraction

The PXRD were undertaken to investigate the crystalline nature of Sildenafil, Aspirin and prepared cocrystal. Powder X-ray diffraction (Brukers D-8 Avance) analysis was performed at room temperature. Condition of measurement was set as follows: Cu metal target, Kα filter, voltage of 40 kV, 40 mA. The analysis was performed on the range of 2 theta of 5-40° C.

Spectrophotometric analysis and interference

Since the analysis of Sildenafil and Aspirin was done in the presence of the coformer Succinic acid, it was necessary to identify the spectrophotometric interference of Succinic acid with both Sildenafil Citrate & Aspirin. For this purpose three solutions were prepared, Sildenafil Citrate solution (10 µg/ml), Aspirin (10 µg/ml), and Succinic Acid (10 µg/ml). These solutions were scanned for UV absorption pattern between 200-400 nm. Overlaid zero order spectra of standard solution of Sildenafil citrate (10 µg), Aspirin (10 µg) are shown in Figure 1. Both the curves are identical so Derivative graphical method was used to estimate Sildenafil citrate and Aspirin. First order derivative overlaid spectra of Sildenafil citrate and Aspirin was shown in Figure 2. It was found to be appropriate for determination of Sildenafil citrate and Aspirin through first order derivative, having separate zero crossing point. The first derivative spectrum of Sildenafil has zero absorbance at 291 nm, where Aspirin gives significant derivative response while Aspirin has zero absorbance at 255 nm, where Sildenafil Citrate gives significant derivative response. Therefore 255 nm & 291 was selected for estimation of Sildenafil Citrate and Aspirin respectively. First order derivative spectra of Succinic acid showed zero absorbance at both the wavelength. So no interference of the co-former with the API evaluation.

![Figure 1: Overlaid zero order spectra of standard solution of Sildenafil citrate (10 µg) & Aspirin (10 µg).](image)

![Figure 2: - First order derivative overlaid spectra of Sildenafil citrate and Aspirin was shown in Figure 2.](image)
Determination of drug content

Drug content was determined by dissolving 10 mg of cocrystal in methanol. From the above solution, sample of conc. 10µg/ml was prepared. The samples were analyzed using UV spectrophotometer by first order spectroscopy and absorbance was measured at 255 nm & 291 nm.

Dissolution studies

Dissolution studies were carried out 14 min in USP (United States of Pharmacopoeia) dissolution test apparatus II (Electrolab, Mumbai, India). The dissolution rate studies were conducted in 900 ml of Artificial Salivary fluid, at 50 rpm maintained at 37±0.5°C. Drug (50 mg Sildenafil Citrate + 150mg Aspirin) and co-crystal (equivalent to 200 mg of drug) were added to dissolution medium in two different jars and samples were withdrawn at appropriate intervals. The volume of dissolution medium is adjusted by replacing with fresh 5ml buffer solution. The samples were filtered, suitably diluted and were analyzed by using first order spectroscopy at 255 nm & 291 nm. The obtained data was statistically validated.

RESULTS AND DISCUSSIONS

Melting point

Solid phases obtained from grinding based cocrystal screening were first evaluated by melting point determination as reported in literature. The melting point comparisons between starting materials and cocrystal are presented in Table 1. Melting points of Sildenafil Aspirin cocrystals were shifted from the melting point of drug Sildenafil Citrate and cocrystal former- Succinic acid. Determined melting points are also correlated well with the results of DSC analysis (Figure 6). Cocrystals were characterized by change in melting point as reported in literature.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Ingredient</th>
<th>Melting Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Aspirin</td>
<td>136º-138ºC</td>
</tr>
<tr>
<td>2</td>
<td>Sildenafil Citrate</td>
<td>187º-189ºC</td>
</tr>
<tr>
<td>3</td>
<td>Co-crystal</td>
<td>156-165°C</td>
</tr>
<tr>
<td>4</td>
<td>Succinic acid</td>
<td>184°C</td>
</tr>
</tbody>
</table>

Fourier transform infrared spectroscopy

The possible interaction between two drugs and coformer were studied by IR spectroscopy. The spectra of drug are shown in the Figure 5. IR spectrum of pure Sildenafil Citrate is characterized by the principal absorption peaks at 3300cm-1 (N-H stretching), 1730 cm-1 (C=O), 1616 cm-1 (C-N stretch), 2966 cm-1 (Aromatic C-H stretching). No change in the rest of peaks was noted indicating its intact structure in the cocrystal. Characteristic peaks of the Co-crystals: 1756 cm⁻¹, 1698 cm⁻¹, 1492 cm⁻¹, 1191 cm⁻¹, 2926 cm⁻¹ (CH₃ stretching), 22853 cm⁻¹ (CH₂ stretching), 1380 cm⁻¹ (OH in plane bending), 900 cm⁻¹ (OH Out of plane bending)
Figure 4: Interpretation of IR spectra of Sildenafil Aspirin Co-crystal.

Figure 5: FTIR spectral comparison of Sildenafil Citrate, Aspirin, Coformer (Succinic acid) & Sildenafil Aspirin cocrystals

The comparative FTIR spectra shows that, the FTIR spectra of Cocrystals have the characteristics peaks of Sildenafil as well as Aspirin and also co-crystal former Succinic Acid. There are some new peaks that show internal reactions and some peaks disappear from the pure compound shows bond formation between molecules.

Differential scanning calorimetry

DSC analysis has been shown to be a very useful thermoanalytical method in the characterization of the solid-state interactions between drug and coformers through the appearance, shifts or disappearance of endothermal or exothermal effects and/or variations in the relevant enthalpy. DSC experiments were carried out to study the thermal behavior of cocrystals with respect to the individual components. DSC thermograms of the Sildenafil Aspirin cocrystal presented in Figure 6. The DSC thermogram showed sharp melting peak at 136°C, 165.8, 187.2 and 234.1°C.
Figure 6: DSC thermogram of Sildenafil Aspirin Co-crystals.

The thermal analysis report of DSC shows characteristic peaks at 136°C (Aspirin), 165°C (Co-crystal), 187°C (Sildenafil Citrate) and 234°C temperature (Unknown).

The reported melting points of the Ingredients are: Aspirin = 136º – 138ºC, Sildenafil citrate = 187º-189ºC, Co-crystal [7]= 156 - 165ºC and Succinic acid = 184°C that are all shown in the graph. However at 234°C temperature, an unknown peak has been appeared which shows that there must be also any new compound formed.

Solubility

The Solubility studies have been performed as per the BCS guideline for the Sildenafil Citrate, Aspirin and Co-crystals of Sildenafil Citrate with Aspirin in Water as well as Artificial Saliva prepared in Laboratory. The results of the solubility studies have been reported under table 2.

Table No. 2: Solubility in water and Artificial Salivary Fluid.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sildenafil Citrate</th>
<th>Aspirin</th>
<th>Sildenafil Aspirin co-crystals (SIL)</th>
<th>(ASP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.5 mg ±0.5 mg / mL</td>
<td>6 mg ± 1 mg/ mL</td>
<td>Sil = 3 mg ± 1 mg / mL</td>
<td>Asp = 9 mg ± 1 mg / mL</td>
</tr>
<tr>
<td>Artificial Saliva</td>
<td>1 mg ±0.5 mg / mL</td>
<td>10 mg ± 1 mg/ mL</td>
<td>Sil = 5 mg ± 1 mg / mL</td>
<td>Asp = 15 mg ± 1 mg / mL</td>
</tr>
</tbody>
</table>

The Solubility of the Sildenafil Citrate has been increased up to 5 fold in Artificial Saliva.

Stability

The stability studies of the Cocrystals have been carried out to measure two parameters at Initial and 3 month time period. The sample analyzed was stored at room temperature (below 25°C). FTIR and Assay has been performed for the co-crystals.

Stability by FTIR

Figure 7: FTIR spectra of Cocrystals at Initial and 3 months.

There is no change in FTIR frequency after 3 months indicates that the co-crystals are stable for 3 months at room temperature.
% Assay

The Assay has been performed for the sample of the co-crystal that has been stored at room temperature (below 25°C) at initial and 3 month time period. The results have been reported under below table.

Table – 3: Results of the Assay of stability samples of Co-crystals.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Label claim (mg)</th>
<th>Amount found (mg)</th>
<th>% Label claim (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SIL</td>
<td>ASP</td>
<td>SIL</td>
</tr>
<tr>
<td>Initial</td>
<td>50</td>
<td>150</td>
<td>50.07</td>
</tr>
<tr>
<td>3Months</td>
<td>50</td>
<td>150</td>
<td>50.00</td>
</tr>
</tbody>
</table>

There is no significant change in % Assay after 3 months indicates that the co-crystals are stable for 3 months at room temperature.

Powder XRD (PXRD)

PXRD is a very reliable method to provide information of solid systems in terms of interaction between materials. Such interactions may produce new diffraction peaks as compared to the constituent materials. The PXRD pattern of pure Sildenafil Citrate, Aspirin and Succinic Acid showed intense peaks of crystallinity indicating its crystalline nature (Figure 8). A Cocrystals comprising Sildenafil Aspirin Cocrystals has been characterized with PXRD having characteristic peaks at 6°±0.2°, 16°±0.2°, 20°±0.2°, 22°±0.2°, 26°±0.2° and 27°±0.2°.

![Figure 8: PXRD spectra comparison of Sildenafil Citrate, Aspirin, Coformer (Succinic acid) & Sildenafil Aspirin cocrystals](image)

Dissolution studies

In vitro dissolution studies revealed that cocrystals showed enhancement in dissolution rate. This can be observed from comparative dissolution profile shown in Fig 9. Sildenafil Aspirin cocrystal showed 5 fold increased in dissolution rate than pure Sildenafil and Aspirin mixture within 10 min.
Figure 9: Comparative Dissolution study of Sildenafil Aspirin Co-crystals and mixture of Pure Sildenafil Citrate with Aspirin.

The faster dissolution of Sildenafil Citrate from the cocrystal might be attributed to alter crystallinity pattern, size, shape and crystal habit which lead to improved dissolution of cocrystal.

CONCLUSION

In the present investigation, Succinic acid was successfully used as coformer for the improvement of the physicochemical properties of Sildenafil & Aspirin via crystal engineering technology. Formation of new crystalline phase of cocrystals was indicated by DSC thermograms which exhibited a melting endotherm at a position different from that of Sildenafil Citrate, Aspirin and Succinic Acid. PXRD studies revealed these cocrystals to be different crystalline identity from the API and coformer used. Dissolution study showed 5 fold enhancements in dissolution rate in 10 min from the cocrystal preparation of Sildenafil & Aspirin. On the basis of these results, it could be concluded that, pharmaceutical co-crystallization of Sildenafil Citrate with Aspirin and could be possible and served as an alternative and synergistic effect approach for manipulation of physicochemical properties of Sildenafil Citrate. The prepared cocrystals can be further investigated for pharmacokinetic parameters.

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There is no conflict of interest in present work.

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