INTRODUCTION
Erectile dysfunction (ED) is a widespread condition with a negative impact on quality of life (QoL) [1]. There is increasing evidence that ED can be an early manifestation of coronary artery and peripheral vascular disease; thus, ED should not be regarded only as a QoL issue but also as a potential warning sign of cardiovascular disease [2]. Until recently, no effective oral therapy existed; the only treatments available were highly cumbersome or invasive, and men found them unacceptable solutions for the problem of achieving or maintaining a satisfactory erection. The oral treatment would have chiefly responsible for metabolism of cGMP in penis corpus carvernosum, leaving a high level of cGMP in penis [3]. Experience dysphagia (difficulty in swallowing) [4]. Hence, SC/Caffeine ODT is a promising dosage form for treating erectile dysfunction with avoiding side effects.

KEYWORDS: Sildenafil citrate, erectile dysfunction, oral disintegrating tablet, lyophilization

ARTICLE INFO
Received 4th Aug 2013
Accepted 20th Aug 2013
Corresponding Author:
Abdel Halim, SA
Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Cairo University, Cairo 11562, Egypt.

ABSTRACT
Sildenafil citrate (SC) oral disintegrating tablets (ODTs) have been prepared by freeze drying technique (lyophilization) using various excipients for improving their oral disintegration, dissolution and bioavailability in order to treat erectile dysfunction (ED). Caffeine was added to the best selected formula of the prepared SC ODTs to maintain normal blood pressure and prevent its decrease caused by administration of SC. The dissolution behavior of the all the prepared tablet formulae were evaluated. Moreover, SC bioavailability in human volunteers from the prepared ODTs was compared to that of the market product oral tablet (Viagra®). The effect of addition of caffeine to the best selected formula of SC ODTs on the blood pressure of human volunteers was also monitored. Using gelatin as a matrix former in oral disintegrating tablets together with tween80 as a solubilizer enhanced the dissolution rate and extent of SC with 100% of drug being dissolved after 7 minutes. Moreover, the in vivo study showed that the AUC0-12 of the lyophilized tablets SC ODTs and SC/Caffeine ODTs was higher than that of commercial oral product (Viagra®), with relative bioavailability values of 122% and 125% respectively compared to the commercial oral product (Viagra®). Addition of caffeine to the best selected formula of SC ODTs maintain blood pressure and inhibit severe decrease in the blood pressure which caused by administration of the best selected formula of SC ODTs and commercial oral product (Viagra®). Therefore, SC/Caffeine ODT is a promising dosage form for treating erectile dysfunction with avoiding side effects.

©2013, JPRO. All Right Reserved.
through the cheek allows the drug to bypass the digestive tract for rapid systemic distribution. Not all ODTs have buccal absorption and many have similar absorption and bioavailability to standard oral dosage forms with the primary route remaining GI absorption. However, a fast disintegration time and a small tablet weight can enhance absorption in the buccal area. Dissolution became more effective than effervescence through improved manufacturing processes and ingredients (such as the addition of mannitol to increase binding and decrease dissolution time) \[11\]. SC may cause temporary hypotension (low blood pressure) and is known to increase cardiovascular nerve activity, so it is prescribed with caution in men with a history of heart attack and chronic low blood pressure problems \[12\]. Caffeine stimulates the central nervous system, resulting in increased in alertness and wakefulness, faster and clearer flow of thought, increased focus, and better body coordination, and later at the spinal cord level at higher doses \[13\]. Once inside the body, it has a complex chemistry and acts through several mechanisms \[14\]. So caffeine and SC together in the oral disintegrating tablet may be useful for compliance of patient and prevent hypotention that may be caused after intake of SC.

**EXPERIMENTAL**

**MATERIALS**

Sildenafil citrate (kindly supplied from Copad Pharma, Egypt). Caffeine (kindly supplied from Egyptian International Pharmaceuticals Industries Co. (EIPICO)). Mannitol (Roquette Pharma, France). Gelatin, Glycine, Tween80, Sodium chloride and Potassium chloride (Advic, El-Nasr Pharmaceutical Chemicals Co., Egypt). Sodium Carboxymethyl cellulose (Na-CMC), Aerosil200, Aspartame and croscarmellose sodium (Kindly Provided from DELTA PHARMA). Xanthan gum (MP Biomedicals, Inc., France). Polyethylene glycol (PEG 400 and PEG 6000), polyvinylpyrrolidione (PVP K30) and (β-cyclodextrins), (Fluka AG, Buchs. Switzerland). Disodium hydrogen phosphate, potassium dihydrogen phosphate, methanol (Karl Fischer grade) and Hydranal Composite 5 reagent (Riedel-de Haen, Sigma-Aldrich. GmbH. Germany). Omeprazole (kindly supplied from Cobad pharma), Human plasma (Vacsera Blood Bank), Methanol and Acetonitrile HPLC grade (Scharlau, spain) and Ammonium formate (Sigma-Aldrich, Germany).

**METHODS**

I. Preparation of Sildenafil Citrate orally Disintegrating Tablets (ODTs)

Sildenafil citrate (SC) (ODTs) were prepared using four matrix formers, along with some other excipients, and collapse protectant; the four matrix formers were: gelatin (2% w/w), xanthan gum (2% w/w), Na-carboxymethyl cellulose (2% w/w), aerosil200 (2% w/w). Other excipients used were croscarmellose sodium as a super disintegrant, mannitol as filler, glycine as a collapse protectant and aspartame as a sweetener (as SC has bitter taste). An accurately weighed amount of SC powder was dispersed in an aqueous solution contain the matrix former and other excipients using magnetic stirrer (Thermolyn Corporation, Dubuque, Lowa, USA) to result in a dose of 70 mg of SC per one milliliter.

One milliliter of suspension was then poured into each of the pockets of a polyvinyl chloride blister pack resulting in a dose of 70 mg SC per tablet. The tablet blister packs were then transferred to a freezer at -22°C and kept in the freezer for 24 hours. The frozen tablets were then placed in a lyophiller for 24 hours using a Novalvphe-NL 500 Freeze Dryer with a condenser temperature of -45°C and a pressure of 0.07 mbar. Different formulae were also prepared after adding different solubilizers to the previously described aqueous solution such as: PEG 400, PEG 6000, PVP K30 and Tween80. Complex of sildenafil citrate with (β-cyclodextrin) (1:1 molar ratio) was prepared using the freeze drying method \[15\], these complexes were used instead of the plain SC in the preparation of ODT. The lyophilized tablets were kept in tightly closed containers in desiccators over anhydrous calcium chloride (29% relative humidity) at room temperature until further investigations. Sildenafil citrate/caffeine ODTs were also prepared after adding 70mg of caffeine to the best selected formula aqueous solution. Compositions of all tablet formulae are presented in table (1).

II. Preparation of the physical mixtures

SC powder was uniformly mixed with the excipients used in the formula in the same percentage used in the lyophilized tablets using a mortar and pestle. The physical mixture was prepared for comparison with the plain drug, and the lyophilized tablets.

III. Determination of SC and caffeine in ODTs in pharmaceutical mixture

A simple, accurate, sensitive and reproducible method has been developed and validated for the determination of caffeine and SC in pharmaceutical mixture. The method is based on isocratic high-performance liquid chromatography (HPLC) separation on a reversed phase. It was prepared by dissolving 70 mg of Sildnafil citrate and 70 mg of caffeine in mobile phase and was serially diluted with the mobile phase to give a final working concentration of 70 µg mL\(^{-1}\) of SC and 70 µg mL\(^{-1}\) of caffeine. Aglient (Germany) series LC system equipped with a degasser; solvent delivery unit and an auto-sampler was used to inject 20 µL aliquots of processed samples on a Hypersil BDS, 5 µ C18, (250 x 4.6 mm ID). All analyses were carried out at room temperature. The isocratic mobile phase (pH 4.5) consisted of acetonitrile and (0.05 M) phosphate buffer (30:70 V/V) and 1.0 ml of triethylamine delivered at a flow rate of 1.0 mL min\(^{-1}\) and detection at λ 230 nm.

IV. Characterization of the prepared ODTs

1. Uniformity of SC content:

   The test was carried out according to the European pharmacopoeia (2012) as follows: Ten randomly selected tablets from each formula were individually assayed for drug content uniformity. The mean value of ten tablets was estimated to calculate the percentage of SC content of the tablets (n=10).

2. Uniformity of Weight

   The test was carried out according to the European pharmacopoeia (2012) as follows: Twenty tablets, from each formula, were individually weighed. The mean of tablet weights was calculated. Not more than two of the individual weights may deviate from the average weight by more than 7.5% and none may deviate by more than twice that percentage.
determined using the USP dissolution tester type II (Pharma Test dissolution Tester, Germany). Dissolution media were 900 ml simulated saliva fluid (SSF) (pH=6.8) maintained at 37 ± 0.5°C with a paddle rotation speed at 50 r.p.m. The formula, were accurately weighed and placed in the drum of friabilator (Erweka tye, GmbH, Germany). The tablets were The dissolution profiles of SC in ODTs compared with the plain drug and market product (Viagra®) were recorded. The subjects were asked to spit out the content of the oral cavity after tablet disintegration and rinse their mouth with distilled water. The swallowing of saliva was prohibited during the test, and also saliva was rinsed from the upper surface of the tablet was noted as the wetting time. The time required for the dye to reach the European pharmacopoeia (2012). The time when there were no particles of tablets or only a trace amount of soft residue remains on the screen was selected as the disintegration time. The test results presented are the average of three determinations (n=3).

### In vivo disintegration time

The Oral disintegration time was tested on six healthy volunteers. The protocol of the study was reviewed and approved by the institutional review board of the Drug Research Center, Cairo, Egypt. Before the test, all volunteers received a detailed explanation of the purpose of the study and gave their written consent, selected of having no history of hypersensitivity to SC. Prior to the test, all volunteers were asked to rinse their mouth with distilled water. For the determination of the in vivo disintegration time of the prepared lyophilized tablets, each of the six subjects was given a coded tablet. Tablets were placed on the tongue and immediately the time was recorded. They were allowed to move the tablet against the upper palate of the mouth with their tongue and to cause a gentle tumbling action on the tablet without biting on it or tumbling it from side to side. Immediately after the last noticeable granule had disintegrated, the time was recorded. The subjects were asked to spit out the content of the oral cavity after tablet disintegration and rinse their mouth with distilled water. The swallowing of saliva was prohibited during the test, and also saliva was rinsed from the mouth after each measurement. Each subject was asked to test one tablet per day. The test results are presented as mean value ± S.D (n=6) [16].

### Wetting time

Ten milliliters of distilled water containing eosin (a water soluble dye) was placed in a petri dish of 10 cm diameter. The tablet was carefully placed in the centre of the petri dish and the time required for the dye to reach the upper surface of the tablet was noted as the wetting time. The test results presented are the average of three determinations (n=3) [17].

### Moisture analysis

The tablets were analyzed for their residual moisture content after lyophilization using a Karl Fischer titrator (Veego Matic-MD, Bombay, India). Each tablet was pulverized, inserted in the titration vessel containing dried methanol and titrated with hydralan composite 5 reagents after a stirring time of 3 minutes. The test results presented are the average of three determinations (n=3).

### In vitro dissolution studies

The dissolution profiles of SC in ODTs compared with the plain drug and market product (Viagra®) were determined using the USP dissolution tester type II (Pharma Test dissolution Tester, Germany). Dissolution media were 900 ml simulated saliva fluid (SSF) (pH=6.8) maintained at of 37 ± 0.5°C with a paddle rotation speed at 50 r.p.m. The

---

### Table 1: Composition of SC ODTs containing different matrix former, in addition to different solubilizers

<table>
<thead>
<tr>
<th>Formula</th>
<th>Matrix former (mg)</th>
<th>Filler (mg)</th>
<th>Super disintegrant</th>
<th>Collapse protector (mg)</th>
<th>Sweetener (mg)</th>
<th>Solubilizer (mg)</th>
<th>Caffeine (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G2</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G3</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G4</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G5</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G6</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X1</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X2</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X3</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X4</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X5</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X6</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A2</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A4</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A6</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>4</td>
<td>X</td>
<td>A</td>
<td>C</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All formulations contain 70 mg Sildenafil Citrate
* Different matrix former (G = Gelatin, X = xanthan gum, A = Aerosil 200, C = sodium carboxymethyl cellulose)
** β-CD = β- cyclodextrin

---

Halim et.al/ Formulation of New Sildenafil Citrate-Caffeine Orally Disintegrating Tablets: In Vitro And In Vivo Evaluation

48
amount of drug used was 70 mg sildenafil citrate equivalent to 50 mg sildenafil base. At specified time intervals (1, 2, 3, 5, 7, 10 and 15 min.), 3 ml of dissolution media were withdrawn, and replaced with an equal volume of the fresh medium to maintain a constant total volume. Samples were filtered through 0.45µm millipore filter and assayed for drug content using HPLC. Cumulative amount of drug dissolved in the preparations was calculated. Dissolution tests were performed three times per formulation (n=3). The market product Viagra® and SC plain powder were also tested in the same way.

V. Physicopharmaceutical characterization of selected SC ODTs

1. Differential Scanning Calorimetry (DSC):

DSC is the measurement of the energy change that occurs as a sample is heated at a constant rate [18]. The principal process involves the heating of two ovens to the same temperature at the same rate. One heater contains the sample in a sealed pan and the other containing an empty pan serving as the reference [19]. Approximately 2mg samples of SC, individual excipients and binary mixtures of SC and individual excipients in a 1:1 ratio were analyzed using DSC. DSC thermograms were generated at temperatures between 30 and 400ºC using a Model DS-60 (Shimadzu®, Tokyo, Japan) with equipment and PC control unit TAC 60 (Shimadzu®, Tokyo, Japan) at a heating rate of 10ºC/min and a nitrogen. Data analysis was undertaken using Pyris™ Manager Software.

2. Fourier-Transform Infrared spectroscopy (FT-IR):

The IR absorption spectra of SC and excipients were generated using a spectrum 100 FT-IR spectrophotometer (Perkin Elmer® Ltd, Beaconsfield, United Kingdom). The spectra were generated from samples of SC, individual excipients and binary mixtures of SC and individual excipients in a 1:1 ratio. A small amount of the mixture was placed on a diamond crystal and analyzed in the wave number range, 400-4000cm⁻¹ at a resolution of 4cm⁻¹.


X-ray diffraction experiments were performed using Cu Kα radiation with a nickel filter, a voltage of 45 KV and a current of 30 mA using X-ray diffractometer (X-Pert Graphics & Idenify, Philips Analytical, the Netherlands). Diffraction patterns for SC plain powder, and selected tablet formulae and their corresponding physical mixtures were obtained.

VI. Effect of storage on the prepared SC ODTs (accelerated stability):

Selected tablet formulae were stored in PVC blisters covered with aluminum foil at 75% relative humidity and at 40°C in a stability cabinet (accelerated stability), during a period of 6 months. Stability was assessed by comparing the results from the drug content, in vitro disintegration, in vivo disintegration, dissolution studies, as well as residual moisture content analysis to fresh prepared SC ODTs. Experiments were done at 0, 1, 3 and 6 months storage. The results were checked for statistical significance using the one-way analysis of variance (ANOVA) test for testing the equality of several means. P-value > 0.05 was considered statistically insignificant.

VII. Pharmacokinetic study of SC and SC/caffeine ODTs on healthy volunteers

1. Volunteer selection

The clinical trial was performed on 6 male volunteers aged between 20 and 40 years. All volunteers should have normal physiological examination. The subjects should be without known history of alcohol or drug abuse problems or chronic gastrointestinal, cardiac, vascular, hepatic or renal diseases and should preferably be non-smokers. The suitability of the volunteers would be screened using standard laboratory tests, a medical history, and a physical examination. If necessary, special medical investigations may be carried out before and during studies. Volunteers were excluded from this study if they had evidence of any clinically significant disease or abnormality, including asthma, eczema, drug hypersensitivity and/or a personal or family history of bleeding disorder, migraine or peptic ulceration. The protocol of the study was reviewed and approved by the institutional review board of the Drug Research Center (DRC), Cairo, Egypt. The research was carried out in accordance with the international clinical research guidelines, enunciated in the Declaration of Helsinki, adopted in Helsinki in 1964 and amended in Seoul, South Korea, October 2008 [20]. The purpose of this study was fully explained and the volunteers gave their written consent. The informed consent forms were carefully read before signing. All questions were discussed in detail with the clinical staff. No alcohol or xanthine-containing foods or beverages would be consumed for 48 hrs prior to dosing and until after the last blood sample is collected. Volunteers would take no medications two weeks prior to initiation of the study and until the study is completed. Water may be taken except for 1 hour before and after administration. All meals during the study would be standardized, and the same meals would be served during three phases of the study.

2. Study design

Randomized, single dose, three-way crossover open-label study was performed using the selected SC ODT formula, the SC/ Caffeine ODT formula compared to the conventional market product Viagra® 50 mg oral tablets (Pfizer, USA). Subjects are to be hospitalized at drug research centre (DRC), the nights before the date of phase I, phase II and phase III and during the clinical phase until blood sampling of 12 hours. A signed and dated registration form for each volunteer including time in, time out is applied. Following an overnight fast of at least 10 hours, subjects would be administered a single dose of the test or reference product and to continue fasting for about 4 hrs after administration of the test and reference treatment. All the volunteers are to be under complete medical supervision in the DRC. The clinical trial was performed on 6 male volunteers aged between 20 and 40 years. All volunteers should have normal physiological examination. The subjects should be without known history of alcohol or drug abuse problems or chronic gastrointestinal, cardiac, vascular, hepatic or renal diseases and should preferably be non-smokers. The suitability of the volunteers would be screened using standard laboratory tests, a medical history, and a physical examination. If necessary, special medical investigations may be carried out before and during studies. Volunteers were excluded from this study if they had evidence of any clinically significant disease or abnormality, including asthma, eczema, drug hypersensitivity and/or a personal or family history of bleeding disorder, migraine or peptic ulceration. The protocol of the study was reviewed and approved by the institutional review board of the Drug Research Center (DRC), Cairo, Egypt. The research was carried out in accordance with the international clinical research guidelines, enunciated in the Declaration of Helsinki, adopted in Helsinki in 1964 and amended in Seoul, South Korea, October 2008 [20]. The purpose of this study was fully explained and the volunteers gave their written consent. The informed consent forms were carefully read before signing. All questions were discussed in detail with the clinical staff. No alcohol or xanthine-containing foods or beverages would be consumed for 48 hrs prior to dosing and until after the last blood sample is collected. Volunteers would take no medications two weeks prior to initiation of the study and until the study is completed. Water may be taken except for 1 hour before and after administration. All meals during the study would be standardized, and the same meals would be served during three phases of the study.

3. Sample collection

Venous blood samples were collected in heparinized tubes before administration of the dosage form, and sampling time would be 0 (pre-dose), 5, 10, 15, 30 and 45 minutes, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours after drug administration. All samples were collected and plasma was immediately separated from blood cells by centrifugation at 3000 rpm for 10 min and stored frozen at ~20 °C until analysis.

4. Sample preparation

Appropriate number of disposable glass test tubes was placed in a rack. The tubes are numbered according to the order of the analytical runs and then blank and the volunteers human plasma samples (500ul) added into appropriate
tubes then the internal standard (50ul of omeprazole working solution 500 ng/ml) was dispersed and vortexed for 1 min. Then 1ml of acetonitrile was added to each and vortexed for 1 minute then centrifuge the samples at 4000rpm for 5 minutes and transfer clear supernatant layer to autosampler vial.

5. Determination of SC and caffeine in human plasma

A sensitive, selective and accurate Liquid Chromatography/ Mass Spectrometry / Mass Spectrometry (LC-MS/MS) method was developed and validated (data not shown) before the study for determination of SC and caffeine in human plasma. The method was validated following international guidelines [21]. Omeprazole internal standard (IS) stock solution was prepared by weigh accurately 10mg of omeprazole and then transfers it into a 100 ml volumetric flask. Add about 80 ml of Methanol. Sonicate for 10 minutes. Complete to volume with Methanol. This solution contains 100ug/ml omeprazole. Transfer quantitatively 0.5 ml of prepared solution into a 100ml volumetric flask and complete to volume with water to obtain 500ng/ml omeprazole. A Liquid Chromatograph: Agilent 1200 series, USA equipped with a degasser: Agilent 1200 series, USA, Mass Detector: Agilent 1200 series Triple Quad, USA and an auto-sampler Agilent 1200 series, USA was used to inject 10 ul aliquots of processed samples on Thermo, Hypersil Gold C8, 4.6 x 50 mm, 5.0 micron. All analyses were carried out at room temperature. Mobile phase consist of methanol:Ammonium formate (70:30) v/v delivered at a flow rate of 0.6 mL/ min into the mass spectrometer's electrospray ionization chamber. Quantitation was achieved by MS/ MS detection in positive electrospray ionization mode (ESI) for both SC and IS, using the Agilent 6410 mass spectrometer. Ion detection was performed in the multiple reaction monitoring (MRM) mode, monitoring the transition of the m/z 475 precursor ion to the 283 for SC and 346.1 precursor ion to the m/z 197.9 for IS. Analytical data were processed using Mass Hunter, Agilent system software.

6. Volunteers Monitoring:

Blood pressure would be monitored at 0 (pre-dose), 5, 10, 15, 30 and 45 minutes, 1, 1.5, 2, 3, 4, 6, 8 and 12 during the study. Subjects would be informed to report any unusual symptoms observed during the study. Subjects would be periodical questioned during each phase of the study for any unusual symptoms experienced after drug administration.

7. Pharmacokinetic and statistical analyses

Pharmaconcentration-time data of SC was analyzed for each subject by non-compartmental pharmacokinetic models using Kinetic@software (version 4.4.1). Peak plasma concentrations ($C_{max}$) and the time to peak plasma concentration ($T_{max}$) were directly obtained from the concentration-time data. The area under the plasma concentration-time curve ($AUC_{0-12}$) from time zero to the last measured. Relative bioavailability of SC ODT and SC/caffeine ODT compared to the commercial product (Viagra®) was calculated according to the following equation:

Relative bioavailability (%) = $\frac{AUC_{0-12} \text{(oral disintegrating tablets)}}{AUC_{0-12} \text{(Commercial oral tablets)}} \times 100$

Analysis of variance was used to assess the effect of the formulation on pharmacokinetic parameters. Differences between two related parameters were considered statistically significant for $p$-value equal to or less than 0.05.

RESULTS AND DISCUSSION

1. Uniformity of SC content

Results revealed uniform SC content in ODTs from formulæ [Gelatin (2%) (G1), xanthan gum (2%) (X1), Aerosil200 (2%) (A1) and sodium carboxymethyl cellulose (2%) (C1)] ranged from (98.80±1.08 to 99.13±0.21). These values were used to calculate the content of SC in the tablets. Results are presented in table (2)

2. Uniformity of weight

The average weight for ODTs formulæ (G1, X1, A1, C1) ranged from (199.2±0.95mg to 200.7±1.30mg), therefore all the tablets fall within the acceptable weight variation range; according to the European pharmacopoeia (2012), not more than two tablets deviated from the average weight by more than 7.5% and not deviated by more than twice this percentage. Results are presented in table (2).

3. Friability test

Table (2) reveals the friability results for the prepared ODTs. According to compendial standards (European pharmacopoeia 2012), the tablets comply with the friability test if the weight loss during the test was less than 1%, in addition, the tablets should not break or show any capping or cracking during the test. Results showed that ODTs formulated with gelatin (2%), Na-carboxymethyl cellulose (2%), xanthan gum (2%) and aerosil200 (2%) as a matrix former showed percentage fines within the acceptable range for tablets (less than 1%). Some ODTs were more friable than the others, as they showed higher percentage of weight loss. Tablet formulated with gelatin and aerosol 200 showed higher percentage weight loss than those formulated with Na-CMC and xanthan gum. ODT formulæ (G1 and A1) showed percentage weight loss of (0.91% and 0.95%) respectively while C1 and X1 ODT formulæ showed (0.30% and 0.40%) respectively.

4. In vitro disintegration time

Table (2) shows the average values of the in vitro disintegration times from the different ODT formulæ (G1, X1, A1 and C1). It was found that disintegration time is short; this may be due to the presence of super disintegrant in the tablets which rapidly uptake water and swell and exert sufficient pressure inside tablet to break apart into small segment (Malikarjuna Setty et al) [22] and super disintegrants which were added to the ODT formulæ facilitated the breakup or disintegration of tablet content into smaller particles that dissolved more rapidly [23].

Results showed also ODTs containing matrix former xanthan gum and Na-CMC have a longer disintegration times compared to tablets containing gelatin and aerosil200 as a matrix former. G1, X1, A1and C1 showed an average disintegration times of (12±1.00, 20±1.00, 18.6±1.53 and 20±2.00 seconds) respectively. These results were consistent with the results of friability testing where ODTs containing xanthan gum and Na-CMC as a matrix former are less friable than ODTs containing gelatin and aerosil200 as a matrix former. The shortest disintegration times was taken by the
formula containing gelatin as matrix former (G1) where (G1) disintegrated within 17±1.00 seconds. An addition of xanthan gum increase disintegration time and it could be due o the binding forces of xanthan gum which holds the drug and other excipients hence, prevented the breakdown of the formulae and it increases the disintegration time by resisting the breakup of tablet. Disintegration activity of xanthan gum formula at low concentration may be due to greater swelling and other excepients hence, prevented the breakdown of the formulae and it increases the disintegration time by resisting xanthan gum increase disintegration time and it could be due o the binding forces of xanthan gum which holds the drug formula containing gelatin as matrix former (G1) where (G1) disintegrated within 17±1.00 seconds. An addition of sodium carboxymethyl cellulose may be due to tremendous swelling of Na-CMC before disintegration.

5. In vivo disintegration time

Table (2) reveals the average oral (in vivo) disintegration time of SC ODTs. The in vivo results correlate with in vitro results where tablets containing xanthan gum and Na-CMC as a matrix former have longer disintegration time than tablets containing gelatin and aerosil200 as a matrix former. G1, X1, A1 and C1 showed an average disintegration times of (15.17±1.04, 18.16±1.04, 16.83±1.76 and 18.67±1.61seconds) respectively. The in vivo disintegration times were shorter when compared to the in vitro disintegration times, probably because of the gentle movement of the tablet inside the mouth and so gentle mechanical stress on the tablet. This agreed with the results obtained by Ciper and Bodmeier [27] in a study of the preparation of a fast disintegrating capsule for administration in the oral cavity.

6. Wetting time

Table (2) shows the average wetting times of the different formulae. The wetting time of all formulae (G1, A1, X1 and C1) was very short (8.3±0.58, 8.00±1.73, 9.67±1.53, 9.00±1.00) and did not exceeds 10 seconds. These results correlate with result obtained from friability, and disintegration time testing.

7. Moisture analysis

Table (2) shows the average percentage moisture content of different tablet formulae (G1, A1, X1 and C1). The residual moisture content in the lyophilized tablets was very small, not exceeding 2% ranging from (0.9 to 1.9%), indicating that lyophilization was efficient in removing water from the prepared ODTs.

8. In vitro dissolution studies

The cumulative SC dissolution as a function of time from ODTs (G1, X1, A1 and C1) compared to SC plain powder and the market product Viagra® are illustrated in figure (1). During first two minutes, the percentage of drug dissolved from formulations G1, X1, A1, C1, market product and plain powder were 54.9%, 49.17%, 47.80%, 49.10%, 7.25%, and 1.8% respectively. SC Plain powder yielded the slowest dissolution rate, with only 1.8% dissolved after 2 minutes. Its hydrophobicity caused the powder to float on the surface of the dissolution medium and prevented its surface contacting the medium. On the other hand, SC in the lyophilized tablet was immediately dispersed and almost completely dissolved in 15 minutes. Dissolution rate of SC in the lyophilized tablet increased markedly compared to SC powder alone. Results showed that the ODT formulae containing gelatin, xanthan gum, Aerosil200 and Na-CMC showed significantly higher percentage drug dissolved compared to SC plain powder and market product Viagra® (P<0.05). This may be attributed to the fast disintegration of the tablets and the great improvement in the wettability of SC in the ODTs.

Formula containing gelatin (G1) showed faster drug release than the corresponding formulae containing aerosil 200, xanthan Gum, and sodium carboxymethyl cellulose (A1, X1 and C1). Similar results obtained by Kallinteri and Antimisiaris [28] who studied the influence of gelatin on the solubility of several drugs. Their results showed that the solubility of all the tested drugs is significantly enhanced especially in presence of 0.5% Gelatin. They reported that there is a tendency for larger gelatin induced increases in solubility as the drug lipophilicity increases or aqueous solubility decreases. Statistical analysis revealed that formula containing 2% aerosil200 (A1) showed a significant decrease in the percentage of the drug dissolved after two minutes compared to the formula containing 2% gelatin (G1) (P<0.05).
result could be due to increased crushing strength upon addition of aerosil200 which slowed down the entrance of dissolution medium into the matrices [29]. Also Statistical analysis revealed that formulation containing 2% xanthan gum (X1) showed a significant decrease in the percentage of the drug dissolved after two minutes compared to the formulation containing 2% gelatin (G1) (P<0.05). This could be attributed to the hydration of individual xanthan gum particles results in extensive swelling and as a result of the rheological nature of the hydrated matrix, the swollen particles would coalesce. This resulted in a continuous viscoelastic matrix that fills the interstices, maintaining the integrity of the tablet and retarding further penetration of the dissolution medium (Rajat Kara and Snehamayee Mohapatraa) [30]. Also statistical analysis revealed that formula containing 2% Na-CMC (C1) showed a significant decrease in the percentage of the drug dissolved after two minutes compared to the formula containing 2% gelatin (G1) (P<0.05). This could be because of more polymer entanglement and more gel strength. Increase in gel strength would contribute to a lesser rate of polymer erosion. For all these reasons, the diffusion coefficient of the drug and dissolution through the matrix decreases and results in a lower drug release (Mukherjee Kaushik et al) [31].

9. Effect of addition of solubilizers

All The previous formulae (G1, X1, A1 and C1) were further tested in the presence of different solubilizers, in order to increase dissolution rate. Five solubilizers were used: PEG 400, PEG 6000, PVPK30 and tween80 (each in concentration of 2%w/w). Sildenafil citrate was also complexed with β-cyclodextrin (1:1 molar ratio).

The prepared tablets were examined in the same way as previously described under the methodology section including: uniformity of weight, friability test, drug content uniformity, in vitro disintegration testing, in vivo disintegration testing, wetting time, moisture content and in vitro dissolution studies. To study the effect of solubilizers on ODTs, statistical analysis was performed using a one - way analysis of variance (ANOVA) followed by a multiple comparison procedure (Dunnett’s test). Evaluation of SC Oral disintegrating tablets containing gelatin, xanthan gum, aerosol 200, Na-CMC as a matrix former, in addition to different solubilizers are presented in table (2).

a. Uniformity of SC content

The mean percentage of SC content in ODTs from all formulae containing gelatin, xanthan gum, aerosol 200 and Na-CMC as a matrix former after addition of solubilizers is high and uniform, ranged from (98.60%±0.10 to 100.30±1.89).

b. Uniformity of weight

The average weight for all tablet formulae after addition of different solubilizers ranged from (199.03±0.45mg to 200.13±0.21mg), therefore all the tablets fall within the acceptable weight variation range; according to the European pharmacopoeia (2012).

c. Friability

Friability results for the prepared SC ODTs ranged from (0.12% to 0.9). In addition, all the tablets did not break or show any capping or cracking during the test. Addition of solubilizers to different ODTs (G1, X1, A1, C1) did not show any significant effect on friability results (p>0.05).

d. In vitro disintegration time

Statistical analysis revealed that ODTs formulae G2 and G3 showed significantly shorter disintegration times (9.00±1.00 and 9.33±0.52 sec.) compared to ODT G1 (17±1.00 sec.) (p < 0.05). This could be due to the hydrophilic nature of the used solubilizers, which facilitates the penetration of water and so the faster disintegration to tablets. These results are in accordance with the results obtained by Ciper and Bodmeier [27], where they found that the addition of hydrophilic additives, such as PEG 400, PEG1500 and xyliitol decreased the disintegration time and increased the rate of dissolution of gelatin fast dissolving films. While the addition of other solubilizers to G1 formula (G4 to G6) did not affect disintegration time significantly with values ranged from (15±1.00 to 16±1.00 sec.). Addition of solubilizers to (X1, A1, C1) resulted in insignificantly shorter disintegration times (p > 0.05). Results are shown in table (2).

e. In vivo disintegration

The average values of the in vivo disintegration times of different gelatin tablet formulae containing solubilizers were compared to ODT G1. Statistical analysis revealed that ODTs formulae G2 and G3 showed significantly shorter disintegration times (9.00±1.00and 10.00±1.00) compared to ODT G1 (15.17±1.04) (p < 0.05). On the other hand, ODTs containing PVPK30, tween80 and SC complexed with (β - cyclodextrin) (G4, G5 and G6) showed insignificantly shorter in vivo disintegration time compared to ODT G1 (p > 0.05). Addition of different solubilizers to (X1, A1 and C1) did not affect the in vivo disintegration significantly. Results are shown in table (2).

f. Wetting time

Table (2) shows the average wetting times of the different formulae. The wetting time of all formulae after
addition of different solubilizers ranged from (2±1.00 to13±5.29). These results correlate with result obtained from friability, and disintegration time testing.

g. Moisture analysis

The average percentage moisture content of different ODTs formulae containing solubilizers not exceeding 3% ranged from (0.81±0.14 to 2.17±0.15%) for all formulae indicating that lyophilization was efficient in removing water from the tablets.

h. In vitro dissolution studies

Using five different solubilizers (PEG400, PEG6000, PVPK30, Tween80 and β-cyclodextrins) to increase the solubility of SC and increase its dissolution. As PEGs and PVPK30 improve wettability, local solubilization and reduce the possibility of forming large particle size drug crystal. Consequently, the practical size of drug crystal decreased, or more drugs were dispersed in the form of molecule and thus the drug dissolution rate increased [32,33]. Tween 80 has the ability to form micelles which enhances the solubility of the drug and as the micellar concentration increase the rate of release of the drug is increased; this increase in release is due to high absorption of the dissolution medium by the tablets and greater disintegration, which enhances the drug release and lower the surface tension, in order to make the drug to distribute evenly and prevent further agglomeration of drug particles [34]. Cyclodextrins in aqueous solutions are able to form inclusion complexes with many drugs by taking up a drug molecule or more frequently some lipophilic moiety of the molecule, into the central cavity. No covalent bonds are formed or broken during the complex formation and drug molecules in the complex are in rapid equilibrium with free molecules in the solution. The driving forces for the complex formation include release of enthalpy-rich water molecules from the cavity, electrostatic interactions, van der waals interactions, hydrophobic interactions, hydrogen bonding, release of conformational strain and charge-transfer interactions [35]. All these forces are weak and allow free drug molecules in solution to be in the equilibrium with drug molecules bound to cyclodextrin and increase its dissolution.

Figure (2) shows the dissolution profiles of SC from ODTs containing 2% gelatin as a matrix former (G1) and different solubilizers. Different grades of PEG as a solubilizer (G2 and G3) compared to G1 ODT. During 2 minutes, the percentages of drug dissolved from formula G2 (PEG 400), G3 (PEG 6000) were 66.67±0.57 and 69.43±1.26% respectively, compared to 54.87±0.15% from G1 ODT. It is evident that the addition of PEGs considerably enhanced the rate and extent of dissolution of SC. Similar observation by Vinay et al [32] in his study for enhancement the dissolution of fenofibrate using PEG 400 and PEG6000.

The addition of PVP K30 to G1 ODT showed a significant increase in the percentage of drug dissolved after two minutes when compared to G1 ODT (p<0.05). After two minutes, the percentages of drug dissolved from formula G4 (PVP K30) were 68.90±0.78% compared to 54.87±0.15% from G1 ODT. PVPs have been reported to improve the solubility and to enhance the rate and extent of many poorly soluble drugs [33,34]. This is in accordance with Chowdary and Naresh [35] who studied effects of PVPK30 on the solubility and dissolution rate of Efavirenz. They reported that solid dispersion of Efavirenz in PVPK30 were effective in improving the dissolution of Efavirenz, (2.49 fold), increase in drug dissolution was observed.

The dissolution of ODT G5 containing tween 80 as a solubilizer showed after two minutes, percentage of 77.07±2.10% of SC dissolved compared to 54.87±0.15% from G1 ODT. Results showed that addition of tween 80 significantly increase the percentage of drug dissolved after 2 minutes when compared to G1 ODT (p<0.05). This result comply with result obtained by Lakshmi AP et al [34] in his study to increase the solubility of poorly water soluble drug Irbesartan by using surfactant (2% tween80) resulting in enhancing the solubility of the drug and increasing the rate of release of the drug.

The percentage of drug dissolved from formula G6 after two minutes was 51.10±2.77% compared to 54.987±0.81% from G1 ODT. Dissolution results showed that complexation of SC with (β - cyclodextrin) didn't improve the dissolution rate of the drug compared to G1 ODT. The percentage of SC dissolved from lyophilized ODTs after two minutes can be arranged in the descending order as follows G5 > G3 > G4 > G2 > G1>G6.

Figure (2): Dissolution profiles of SC ODTs containing 2% gelatin with different solubilizers in SSF

Figure (3) shows the percentage dissolved of SC from ODTs containing 2% xanthan gum as a matrix former and two different grades of PEG and PVPK30 as a solubilizer (X2, X3and X4) compared to X1 ODT. After two minutes, the percentages of drug dissolved from formula X2 (PEG 400), X3 (PEG 6000) and X4 (PVPK30) were 49.57±0.81%, 56.00±1.00 and 56.30±0.98% respectively, compared to 49.17±0.61% from X1 ODT. The results showed that the drug dissolution was improved significantly with addition of PEG6000 and PVPK30. And this is in accordance with the result obtained by Sakeer K et al [37] where they found that the presence of PEG and PVP in xanthan-containing formulations...
induced an increase in dissolution rate of nystatin in buccoadhesive tablets during their study in the use of xanthan and its binary blends with synthetic polymers to design controlled release formulations of buccoadhesive nystatin tablets. The addition of tween 80 did not increase significantly the dissolution rate of SC from X5 ODT as it was 49.23±1.02 compared to 49.17±0.61% from X1 ODT after two minutes. The percent dissolved of X6 ODT containing SC complexed with (β–cyclodextrin) compared to X1 ODT showed after two minutes, 46.20±0.72% compared to 49.17±0.61% from X1 ODT. Dissolution results showed that complexation of SC with (β–cyclodextrin) didn’t improve the dissolution rate of the drug compared to X1 ODT. The percentage of SC dissolved from lyophilized ODTs after two minutes can be arranged in the descending order as follows X4 > X3 > X2 > X5 > X1 > X6.

Figure (3): Dissolution profiles of SC ODTs containing 2% xanthan gum with different solubilizers in SSF

Figure (4) shows the percentage dissolved of SC from ODTs containing 2% aerosil200 as a matrix former and two different grades of PEG as a solubilizer PEG400, PEG6000 (A2 and A3) compared to A1 ODT. After two minutes, the percentages of drug dissolved from formula A2 (PEG 400), A3 (PEG 6000) were 56.53±0.50% and 56.96±0.35% respectively, compared to 47.80±2.44% from A1 ODT. The results showed that the drug dissolution was improved significantly with addition of PEG400 and PEG6000 and this could be attributed to a reduction in particle size of the drug, its deposition on the surface of the carrier, and improved wettability [32,33] and this result comply with the result obtained by Shinde Sunita S. et al. [38] in a study on solubility enhancement of simvastatin using solid dispersion technology. Their study revealed that the dissolution profiles of the solid dispersions using PEG6000 and aerosil200 showed increase in the drug release where as plain simvastatin showed a poor dissolution profile. The percent dissolved of SC from A4 ODT containing PVPk30 as a solubilizer compared to A1 ODT after two minutes, A4 was 56.50±1.32%, compared to 47.80±2.44% from A1 ODT. The results showed that the drug dissolution was improved significantly with addition of PVPk30. Similar results obtained by Shinde et al. [38] in a study on improving the solubility and dissolution rate of poorly water soluble acedofenac by solid dispersion method followed by solvent evaporation method using hydrophilic polymer PVP-k30, HPMC E-5 and porous carrier aerosil 200. The percent dissolved of SC from ODT A5 containing tween80 as a solubilizer compared to A1 ODT after two minutes, A5 was 48.93±1.40% compared to 47.80±2.44% from A1 ODT. Results showed that addition of tween80 showed no significant difference in the percentage of drug dissolved after two minutes when compared to A1 ODT. The percent dissolved of SC after two minutes from A6 ODT complexed with (β–cyclodextrin) compared to A1 ODT was 46.40±0.56% and 47.80±2.44% respectively. Dissolution results showed that complexation of SC with (β–cyclodextrin) didn’t improve the dissolution rate of the drug compared to A1 ODT. The percentage of SC dissolved from lyophilized ODTs after two minutes can be arranged in the descending order as follows A3 > A2 > A4 > A5 > A1 > A6.

Figure (4): Dissolution profiles of SC ODTs containing 2% Aerosil200 with different solubilizers in SSF

Figure (5) shows the percentage dissolved of SC from ODTs containing 2% Na-CMC as a matrix former and two different grades of PEG as a solubilizer PEG400, PEG6000 (C2 and C3) compared to C1 ODT, after two minutes C2 (PEG 400), C3 (PEG 6000) were 56.86±0.23% and 57.93±1.27% respectively, compared to 49.10±0.26% from C1 ODT. The addition of PEGs significantly increase the drug dissolution rates and similar result were obtained by Hassan et al. [39] in a study on enhancement dissolution and the anti inflammatory effect of nimesulide, using liquisolid compact for oral application. Their study revealed that the tablets containing microcrystalline cellulose as a carrier and PEG400 produced faster dissolution rate in comparison with compressed tablets.
The percent dissolved of SC from C4 ODTs containing PVP K30 as a solubilizer compared to C1 ODT after two minutes; C4 was 49.23±0.35% compared to 49.10±0.26% from C1 ODT. Results showed that addition of PVP K30 to C1 ODTs showed no significant difference in the percentage drug dissolved after two minutes (p>0.05)

The percent dissolved of SC from OD C5 containing tween80 as a solubilizer compared to C1 ODT after two minutes were 55.33±0.58% and 49.10±0.26% respectively. Results showed that addition of tween80 showed significant increase in the percentage of drug dissolved after two minutes when compared to C1 ODT (p<0.05) and this result is in accordance with the result obtained by Dong Hoon Oh et al [40] who studied the effect of Na-CMC and tween80 on aqueous solubility of flurbiprofen and found that solid dispersions were formed by attaching hydrophilic carriers to the surface of drug without crystal change, resulting in changing the hydrophobic drug to hydrophilic form.

The percent dissolved of SC from C6 ODT containing SC complexed with (β–cyclodextrins) compared to C1 ODT were 49.13±1.50% and 49.10±0.26% respectively. Statistical analysis revealed no significant difference in the percentage of drug dissolved after two minutes from C6 ODT compared to C1 ODT (p>0.05). The percentage of SC dissolved from lyophilized ODTs after two minutes can be arranged in the descending order as follows C3 > C2 > C5 > C4 > C6>C1.

![Dissolution profiles of SC ODTs containing 2% Na-CMC with different solubilizers in SSF](image)

It is worthy to note that incorporation of gelatin with PEG6000 or PVPk30 or tween80 in oral disintegrating tablets together with SC gave a better extent and release rate than other 21 formulae as evidenced by the higher dissolution of G3, G4 and G5 ODTs. According to the above results, G3, G4 and G5 ODTs were chosen for x-ray diffraction with their physical mixtures and SC plain powder and also they were chosen for further accelerated stability studies.

10. Physicopharmaceutical characterization of SC ODTs

a. Differential Scanning Calorimetry (DSC):

The 1:1 w/w ratio was chosen because it maximizes the observation of any reaction. The incompatibilities were detected by appearance, shift or disappearance of the corresponding peaks of each substance. It is clear from figure (6) that SC has a main sharp characteristic endothermic melting peak with an onset at 184.76°C and endset at 208.93°C with a peak at 195.52°C. The sharp endothermic peak signifies that SC was in a pure crystalline state. It worthy to notice that there was no shift in SC peak upon mixing with Na-CMC, aerosil 200, xanthan gum, cross carmellose sodium, aspartame, PEG6000, PVPK30, glycine, caffeine and β-cyclodextrin as shown in figures (6 and 7). While the thermogram for mannitol reveals one endothermic peak at 171.51°C and the thermogram of the mixture of sildenafil citrate and mannitol displays a sharp peak at 169.66°C, indicating the presence of mannitol, in addition to a smaller and broad endothermic peak at 295.36°C. This interaction is more than likely due to a small amount of reducing sugar that may be present in mannitol and SC. However due to the fact that DSC analysis takes place at elevated temperatures, Fourier-Transform Infrared Spectroscopy (FT-IR) was used to determine whether that interaction also occurs at room temperature. Therefore, due to evidence of a potential interaction when using thermal analysis, long term stability testing may well be necessary for the formulae in which SC are included with excipients such as mannitol and to save time, accelerated stability study was done instead of long stability study [41].

b. Fourier-transform Infrared spectroscopy (FT-IR):

Figures (8 and 9) show possible main absorption peaks for correlation in the IR spectrum of sildenafil citrate and its near infrared spectrum include peaks at 1172 and 1359 cm⁻¹ for symmetric and asymmetric SO2, respectively, at 1581 and 1703 cm⁻¹ for symmetric and asymmetric COOH respectively, hydroxy (-OH) at 3614 cm⁻¹, NH symmetric and asymmetric stretching at 3296 cm⁻¹, C-H bond on benzene (=C-H) at 3028 cm⁻¹ and methyl and methylene (C-H) at 2962–2873 cm⁻¹.

The absorption spectrum for mannitol includes a number of characteristic peaks. The very broad peak at 3400 cm⁻¹ represents OH band stretching. The three peaks at 2983 cm⁻¹, 2947 cm⁻¹ and 2902 cm⁻¹ may be attributed to CH stretching while the peaks at 1282 cm⁻¹ and 1260 cm⁻¹ correspond to primary and secondary alcohol OH plane deformation. The peaks at 1080 cm⁻¹ and 1045 cm⁻¹ represent primary and secondary alcohol C=O stretching, respectively.

To ensure that no interaction between SC and mannitol occurred, an IR spectrum of a 1:1 binary mixture of SC and mannitol was generated as shown in figure (8) and revealed the presence of all characteristic peaks for SC and mannitol. It was thought that the Maillard reaction had occurred with reducing sugars found in mannitol, reacting with the secondary amine to form an imine. The spectrum highlights that no imine product was formed as a characteristic peak for this
Halim et al. Formulation of New Sildenafil Citrate-Caffeine Orally Disintegrating Tablets: In Vitro And In Vivo Evaluation

material would appear at a wave number of 1630 cm\(^{-1}\). The absence of this peak confirms the fact that at room temperatures a Maillard reaction is not likely to have taken place \[41\]. All characteristic peaks for SC appeared in 1:1 binary mixture of SC and other excipients indicating compatibility of SC with Na-CMC, aerosil200, xanthan gum, croscarmellose sodium, aspartame, PEG6000, caffeine, PVPK30, glycine, mannitol, gelatin and β-cyclodextrin.

Figure (6): DSC thermograms of sildenafil, different excipients and their physical mixture (1:1)
Figure (7): DSC thermograms of sildenafil, caffeine and their physical mixture (1:1)

Figure (8): FTIR spectra of a) SC b) xanthan gum c) SC and xanthan gum d) aerosil 200  e) SC and aerosil 200  f) Na-CMC  g) SC and NaCMC h) gelatin i) Sc and gelatin j) croscarmellose sodium k) SC and croscarmellose sodium, l) mannitol, m) SC and mannitol
c. Powder X-Ray diffraction (XRD)

The amorphous state of the drug is often preferred, since it shows improved solubility and dissolution rate in comparison to crystalline material. Glassy solutions, in which the drug is molecularly dispersed in the carrier, represent the highest level of particle size reduction and no energy is required to break up the crystalline structure as mentioned by Taylor and Zografi. SC was found to exhibit a strong and characteristic x-ray diffraction pattern, showing the crystalline nature of the powder as shown in figure (10). The x-ray diffraction pattern shows sharp peaks for SC at theta 7.3°, 8.07°, 10.27°, 14.4° and 19.8° indicating crystallinity of the drug. Figure (10) shows the powder x-ray diffraction pattern of SC powder, G3, G4, G5 ODTs and their corresponding physical mixtures. G3, G4, G5 ODTs and their corresponding physical mixtures showed a decrease in peak number and intensity in comparison with pure SC. These decreases indicate a reduction in SC crystallinity in the formulated ODTs and corresponding physical mixtures. Moreover, it might have dispersed as a microcrystalline form that enhances its solubility and dissolution.
11. Effect of storage on the prepared SC ODTs (accelerated stability study):

Storage at 40°C and 75% RH testing results for G3, G4 and G5 ODTs showed no significant difference in the mean percentage of SC content, in vitro and in vivo disintegration time during a storage period of six months (p-value > 0.05) (data not shown). There was no significant difference in the residual moisture content of SC ODTs G3 and G5 during a storage period of six months (p-value > 0.05) (data not shown). On the other hand, ODT G4 showed increase in the residual moisture content after six months storage (p-value =0.001). For SC ODTs G3 and G5 no significant difference in the percentage SC dissolved after 1, 2, 3, 5, 7, 10, 15 minutes during storage for 6 months (p-value >0.05) (data not shown) while for SC ODTs G4 the percentage of SC dissolved after two minutes was significantly decreased after storage for six months (p-value=0.019). This appears to be in accordance with results of the moisture content testing which revealed significant increase in the moisture content of the tablet during storage. As the moisture uptake by amorphous solids increases molecular mobility and consequently facilitates the recrystallization process (Ahlneck and Zeografi) [43].

Stability studies showed that SC ODTs G3 and G5 maintained their initial properties with respect to disintegration time, residual moisture and dissolution characteristics after 6 months storage at 40 ± 2°C and 75 ± 5% RH . ODT G4 showed significant change in the residual moisture content and dissolution characteristics during storage. It is worthy to note that after storage of six months percentage of SC dissolved from G3 and G5 ODTs after two minutes (69.30±1.13 and 77.23±1.93 ) respectively and after 15 minutes was (99.83±0.28 and 100.00±0.17) respectively. The previous results showed that ODT G5 can be selected for further in vivo study.

12. Addition of Caffeine

The best selected formula (G5) was further tested in the presence of caffeine, in order to maintain normal blood pressure and prevent its severe drop caused by SC administration.

The prepared tablets were examined in the same way as previously described under the methodology section including: uniformity of weight, friability test, drug content uniformity, in vitro disintegration testing, in vivo disintegration testing, wetting time, moisture content, and in - vitro dissolution studies.

To study the effect of caffeine addition on the prepared SC ODTs, statistical analysis of the in vitro disintegration time, in vivo disintegration time, wetting time, moisture content, and dissolution data was performed using independent-samples T test.

13. Evaluation of SC/ Caffeine ODTs

Uniformity of weight, friability test, drug content uniformity, in - vitro disintegration testing, in - vivo disintegration testing, wetting time and moisture content are found to conform (European pharmacopoeia, 2012) limits and showed no significant difference with results obtained by (G5) ODT. Evaluation of SC/Caffeine ODTs is presented in table (2)

a- In vitro dissolution studies for SC and Caffeine
Figure (11) showed the dissolution profiles of SC from SC/Caffeine ODT (F1) and (G5) ODT. After two minutes, the percentages of drug dissolved from formula F1 (SC/Caffeine) was 75.67±1.53, compared to 77.07±2.10% from G5 ODT. Results show that addition of caffeine to G5 ODT showed insignificant effect in the percentage of SC dissolved after two minutes when compared to G5 ODT (p<0.05). It is evident that the addition of Caffeine did not affect the rate and extent of the dissolution of SC from (G5) ODT. Figure (12) showed the dissolution profiles of caffeine from ODT (F1). Caffeine has high dissolution rate and extent with 100% of drug being dissolved after 7 minutes.

It is worthy to note that incorporation of caffeine with SC in oral disintegrating tablets together did not affect extent and release rate of SC or the characterization of the oral disintegrating tablets (G5) ODT. According to the above results (F1) ODTs would be introduced for x-ray diffraction with their physical mixtures and SC plain powder and also accelerated stability studies.

Figure (11): Dissolution profiles of SC from SC ODT (G5) and SC/Caffeine ODT (F1) in SSF

Figure (12): Dissolution profiles of caffeine from SC/caffeine ODTs (F1) in SSF

b- Powder X-Ray diffraction (XRD)
SC was found to exhibit a strong and characteristic x-ray diffraction pattern, showing the crystalline nature of the powder. The x-ray diffraction pattern shows sharp peaks for SC at theta 7.3°, 8.07°, 10.27°, 14.4° and 19.8° indicating crystallinity of the drug. Figure (13) shows the powder x-ray diffraction pattern of SC powder, F1 ODT and its corresponding physical mixtures. F1 ODT and its corresponding physical mixtures show a decrease in peak number and intensity in comparison with pure SC.

Figure (13): X-ray diffraction patterns of 1) SC, 2) ODT (F1) and 3) physical mixture of ODT (F1)
Pharmacokinetic parameters of caffeine after administration of SC/Caffeine ODTs (F1) to human volunteers are shown in Figure (14). The corresponding mean pharmacokinetic parameters calculated from the individual curves are collectively summarized in Table (3). Pharmacokinetic parameters of caffeine after administration of SC/Caffeine ODTs (F1) to human volunteers is shown in Table (3).

Table (3): Pharmacokinetic parameters of (SC and caffeine) and blood pressure measured after administration of G5, F1 and market product (Viagra®) to human volunteers.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>SC in G5</th>
<th>SC in F1</th>
<th>SC in Viagra®</th>
<th>Caffeine in F1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (ng mL⁻¹)</td>
<td>2.49±0.129.67</td>
<td>177.94±16.99</td>
<td>155.34±19.65</td>
<td>2210.2±1226.84</td>
</tr>
<tr>
<td>Tmax (h)**</td>
<td>0.63±0.10</td>
<td>0.88±0.12</td>
<td>1.08±0.16</td>
<td>0.79±0.07</td>
</tr>
<tr>
<td>AUC0-12 (ng mL⁻¹ h⁻¹)</td>
<td>655.03±1.78</td>
<td>673.18±38.27</td>
<td>536.72±28.44</td>
<td>4304.5±451.04</td>
</tr>
<tr>
<td>AUC0∞ (ng mL⁻¹ h⁻¹)</td>
<td>728.47±41.48</td>
<td>788.57±57.46</td>
<td>609.44±70.62</td>
<td>4790.7±399.80</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>5.06±3.20.21</td>
<td>6.2±0.48</td>
<td>5.89±0.44</td>
<td>4.54±0.34</td>
</tr>
<tr>
<td>L (h⁻¹)</td>
<td>0.19±0.01</td>
<td>0.17±0.01</td>
<td>0.19±0.02</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>AUC0∞ (h)</td>
<td>3.71±0.15</td>
<td>4.26±0.04</td>
<td>3.92±0.21</td>
<td>4.04±0.37</td>
</tr>
<tr>
<td>Average systolic B.P*</td>
<td>99.04±1.83</td>
<td>109.50±1.14</td>
<td>109.54±1.13</td>
<td>109.54±1.13</td>
</tr>
<tr>
<td>Average diastolic B.P*</td>
<td>68.67±4.77</td>
<td>75.45±1.42</td>
<td>68.97±7.21</td>
<td>68.97±7.21</td>
</tr>
<tr>
<td>Relative bioavailability (%)</td>
<td>122%</td>
<td>125%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Data are mean value (n=6) ± standard deviation.

*BP: Blood Pressure
** Median

Figure (14): Mean plasma concentration-time profiles of SC after ODTs oral administration of (G5), (F1) and market product (Viagra®) to human volunteers.

The plasma concentration-time profiles as well as the calculated pharmacokinetic parameters showed that the prepared oral disintegrating tablets (G5) improved the oral pharmacokinetic parameters of SC, expressed by higher Cmax (1.6 fold), shorter Tmax of oral disintegrating tablets (G5) compared to market product (Viagra®) with values (0.63 and 1.083 hrs) respectively. Moreover, the AUC0-12 of oral disintegrating tablets (G5) was higher than that market product (Viagra®), with relative bioavailability (122%). This may be due to the fact that the freeze-drying process imparts a glossy amorphous structure to the bulking agent and sometimes to the drug, with an increase in the surface area and hence the surface free energy, which result in an increase in the dissolution rate and thereby bioavailability. Also the plasma concentration-time profiles as well as the calculated pharmacokinetic parameters showed that the prepared oral disintegrating tablets (F1) improved the oral pharmacokinetic parameters of SC, expressed by higher Cmax (1.1 fold) compared to market product (Viagra®), shorter Tmax of oral disintegrating tablets (F1) compared to market product (Viagra®) with values (0.875 and 1.083 hrs) respectively. Moreover, the AUC0-12 of oral disintegrating tablets (F1) was higher than that market product (Viagra®), with relative bioavailability (125%). Addition of caffeine to SC did not affect significantly (p>0.05) the pharmacokinetic parameters of SC ODTs (Cmax, Tmax and AUC0-12).

The normal systolic blood pressure range from 90 to 120 mmHg while diastolic blood pressure range from 60 to 80 mmHg. The previous results showed that ODT F1 can be introduced for further in-vivo study compared to ODT G5 and market product (Viagra®) and to evaluate the effectiveness of caffeine to prevent decrease in blood pressure caused after administration of SC.
Results showed that average blood pressure for volunteers in three periods after administration of F1 ODT (109.50±1.41/75.45±1.42 mmHg) and for G5 ODT and (Viagra®) (99.00±1.83/68.67±4.77 mmHg) and (100.54±1.13/68.97±7.21 mmHg) respectively. Results revealed that addition of caffeine to SC prevent decrease in blood pressure insignificantly caused by administration of SC in G5 ODT and (Viagra®).

CONCLUSION

This comparative study reveals that freeze drying was a successful technique in preparing SC ODTs with accepted physical parameters (content uniformity, weight, friability, in-vitro disintegration time, in-vivo disintegration time, in-vitro dissolution studies and moisture content). These ODTs formulae were stable over a period of 6 months in accelerated stability studies. Formula of choice (G5) performed higher Cmax, higher AUC0–12, enhanced bioavailability and a rapid onset of action for treatment erectile dysfunction compared to the market product (Viagra®). Addition of caffeine to SC /ODT in (F1) formula prevented drop in blood pressure (as side effect) caused by administration of SC. (F1) is considered as a future promising formula for sildenafil citrate.

DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

ACKNOWLEDGEMENT

The authors are thankful to the management of Drug Research Center, Egypt, quality control department of El Deibeky pharma, Egypt and quality control department of DELTA pharma, Egypt for providing the necessary facilities and consultation for carrying out the in vitro and in vivo research work.

REFERENCES


12. Food and Drug Administration (FDA) for its Abbreviated New Drug Application (ANDA) for Sildenafil Citrate Tablets, 20 mg.


Nanotechnology 2011; 3 [4]: 1240-1251.


