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STUDY OF MICRONIZED GRADES OF TADALAFIL AND ITS EFFECT ON DISSOLUTION.

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ABSTRACT

BCS class II and class IV drugs are having poor solubility which ultimately shows poor bioavailability. To enhance their aqueous solubility various techniques have been proposed amongst which are micronization, solid dispersion, co-crystallization and many more. Micronization is one of the techniques which is adopted by various pharmaceutical companies in order to enhance the solubility of the drug belonging to the class of Phosphodiesterase inhibitor (PDE 5) and to have quicker release formulations. Tadalafil is one such drug which is used to treat the erectile dysfunction and it acts by inhibition of cyclic GMP. The current study aimed to study micronized grades of Tadalafil having varying d (0.9) values and its effect on dissolution. All micronized grades are not suitable for formulation development and this study can help to decide the suitable micronized grade for conventional Tadalafil formulation. The particle size of the drug is selected based on its release in reported OGD medium for Tadalafil Immediate release dosage form. The study showed that the higher micronization of Tadalafil [having d(0.9) value in range of 10-30 microns] lowers effective surface available for dissolution and it may possess formulation challenges for development of Tadalafil solid dosage form.

KEYWORDS: Micronization, PDE 5 Inhibitor, Tadalafil, Dissolution, Solid dosage form.

I. INTRODUCTION

Therapeutic effectiveness of a drug depends upon the bioavailability and ultimately upon the solubility of drug molecules. Solubility is one of the important parameter to achieve desired concentration of drug in systemic circulation for pharmacological response to be shown.[1] More than 40% of new drug molecules entering drug development fail to reach market because of non- optimal biopharmaceutical properties**.** Poorly water-soluble drugs involve

many difficulties in the development of pharmaceutical dosage forms for oral delivery systems due to their low bio- availability.[2] Tadalafil is an impotence agent. It is indicated for the treatment of erectile dysfunction Tadalafil is BCS Class IV drug having poor aqueous solubility with low membrane permeability.[3] It is a practically insoluble drug i.e. reported solubility is less than 1 µg/ml.[4] According to literature review, many techniques have been used for its solubility improvement, such as solid dispersion, micronization, self-emulsification, complexation with cyclodextrin.[5] etc. In the current research work, the approach of Micronization and its effect on dissolution was studied for Tadalafil. The different particle sizes of Tadalafil were evaluated for its effect on dissolution. The saturated solubility studies were carried out. The particle size studied were in the range of 10-120 microns. In each of these grade, Tadalafil particles size range was based on its d(0.9) values. The dissolution testing was studied in OGD dissolution Media. The particle size selected from this study used for formulation development. This research work focusses on micronization of Tadalafil and its impact on in vitro release. Active absorption from oral dosage forms depends on adequate release of the active pharmaceutical ingredient (API) from the product. Physico-chemical factors, such as dissolution or solubility of the API under physiologic conditions, and its permeability through the membranes of the gastrointestinal tract, play pivotal roles in this respect.[6,7] Due to the critical nature of these factors, dissolution of a pharmaceutical product *in vitro* can, in certain instances, be relevant to anticipate the *in vivo* characteristics/results.

II. MATERIALS AND METHODS

Tadalafil (Lot No: TDF/0912005, TDF-06 & TDF/0613001; SMS Pharma), Lot No: 1304000492, Alembic) Sodium Lauryl Sulphate (SD fine chemicals, Mumbai). All the reagents required for preparation of buffer solutions (pH 1.2, 4.5 & 6.8) were procured from SD fine chemicals. It includes sodium acetate glacial acetic acid, monobasic potassium phosphate, sodium hydroxide, potassium chloride.

2.1 Micronization

Micronized grade of Tadalafil API were available directly from the suppliers. The micronized grade were produced using milling process.

2.2 Particle Size Analysis

The particle size analysis after micronization was carried out using Malvern analysis.

2.3 Standard Calibration Curve for Tadalafil

Stock solution (200 µg/ml) was prepared using spectrophotometric grade methanol. Aliquots of 1,2,3, 4, 5,6, 7, 8 and 9 ml from stock solutions were transferred to series of 100 ml volumetric flask and volume was made up to give different concentrations, ranging from 2 to 18 µg/ml. Absorbance was measured using UV spectrophotometer at the absorbance maximum of 284 nm, which is characteristic peak for Tadalafil.[8] Calibration curve was constructed by plotting absorbance versus concentration of Tadalafil solutions and the regression equation was calculated. (**Table 2, Fig 1**).

2.4 Development of Dissolution Method for Tadalafil

Dissolution studies were performed on USP compliant dissolution apparatus (DBK Instruments, Mumbai) using method described in OGD medium for Tadalafil tablets.[9] The method consists of USP type II (paddle apparatus). The rotation speed was set at 50 RPM and temperature of vessels to 37±0.5°C. Dissolution medium comprised of 0.5% Dodecyl sulfate in 1000 ml of purified water.

5 ml of dissolution medium was sampled at 5,10,20,30,45,60,90 and 120 minutes. Each dissolution vessel was replaced with dissolution medium (5 ml) after sampling at each time point. OGD medium reports time points 10, 20, 30 and 45 minutes. Additional time points studied included 60, 90 and 120 minutes.

2.4.1 Sample preparation

Tadalafil API having 90% of particles in the range of 10-30µ, 20-50 µ, 60-90 µ & 90-120 µ (**Table 1**) were studied for dissolution. Sample preparation required was varied amongst the different particle sizes. Effect of sonication and effect of incorporation of solubilizer on release of Tadalafil from each particle size in OGD medium was studied.

2.4.2 Sample preparation for Tadalafil with sonication

20 mg of each lot of Tadalafil having d (0.9) in range of 10-30 microns and having d (0.9) in range of 60-90 microns, were weighed on calibrated electronic weighing balance (Model No: CA-224, Contech). Transferred to 10 ml volumetric flask, diluted with 1-2 ml of purified water. The each API lot were sonicated for 2, 4, 6 minutes (Orbital Shaker, Remi). Simultaneously, API dispersion prepared as such i.e. without sonication, was subjected to dissolution to study the effect of sonication and it acted as control sample. All the samples were transferred to dissolution bath and aliquots withdrawn at 5,10,20,30 and 45 minutes.

The 5 ml of aliquot was withdrawn at each time interval and replaced with dissolution medium. Stirring and temperature conditions of dissolution apparatus were maintained as per standard USP specifications.

2.4.3 Sample preparation of Tadalafil with solubilizer and sonication

20 mg of each lot of Tadalafil having d(0.9) in range of 10-30 microns and having d (0.9) in range of 60-90 microns, were weighed on calibrated electronic weighing balance (Model No: CA-224, Contech) and transferred to stoppered glass test tubes. 200 mg of PEG 400 was weighed and added to each test tube. 1-2 ml of purified water was added to it and all the samples were sonicated using orbital shaker for 2 minutes. The API dispersion was transferred to 6 dissolution bath (for each lot). Test tubes were rinsed with dissolution medium and added to dissolution bath to ensure complete transfer.

2.5 Dissolution studies for Micronized Tadalafil

Dissolution test was carried out for all the four available micronized API lots. 20 mg of Tadalafil from each lot were taken into stoppered test tube. 5 ml of purified water was added and API dispersion was transferred to dissolution bath. **TDF/0912005 having [d (0.9) value of 23 microns, required sonication of 6** minutes at 37$\pm$2 degree C as indicated in section 3.3. The aliquots of dissolution medium for each micronized tadalafil lot were removed at time intervals of 5,10,20,30,45,60,90,120 minutes. The dissolution medium was replaced with fresh medium at each time point to maintain the sink conditions in dissolution bath. The samples withdrawn from dissolution bath were filtered using whatmann filter paper (No. 1, pore size 11 µm). Absorbance of each solution was taken spectrophotometrically [**UV-vis Spectrophotometer, Model No: UV-1800, Shimadzu, Japan**] at 284 nm using filtered dissolution medium as Blank.

III RESULTS AND DISCUSSION

3.1 Micronization

The different micronized lots of tadalafil were studied. The Malvern data available from suppliers confirmed the range observed for micronized particles. Based on d (0.9) data, the API with their particle size ranges is presented in **Table 1.** Dissolution method was developed to understand *in vitro* release for the various tadalafil micronized grades.

Table 1: Micronized Tadalafil used for study.

Lot No of Tadalafil	d(0.9) value	Range in microns
TDF/0912005	23.7 µ	10-30
TDF/09/2005	40 µ	20-50
1304000492	Not Available	60-90
TDF/0613001	92 µ	90-120

3.2 Linearity of Tadalafil

Tadalafil exhibited linear response in the range of 6 to 16 µg/ ml at the λ_{max} of 284 nm (**Table 2, Fig 1**).

Table 2: Absorbance at 284 nm for Tadalafil

Concentration (mcg/ml)	Absorbance at 284.4 nm
0.0	0.000
2.0	0.098
4.0	0.181
6.0	0.266
8.0	0.344
10.0	0.424
12.0	0.501
14.0	0.587
16.0	0.664
18.0	0.740
20.0	0.810

The correlation coefficient value was found to be 0.9992, thus indicating the linearity in the selected range. (R^2 = 0.9992).

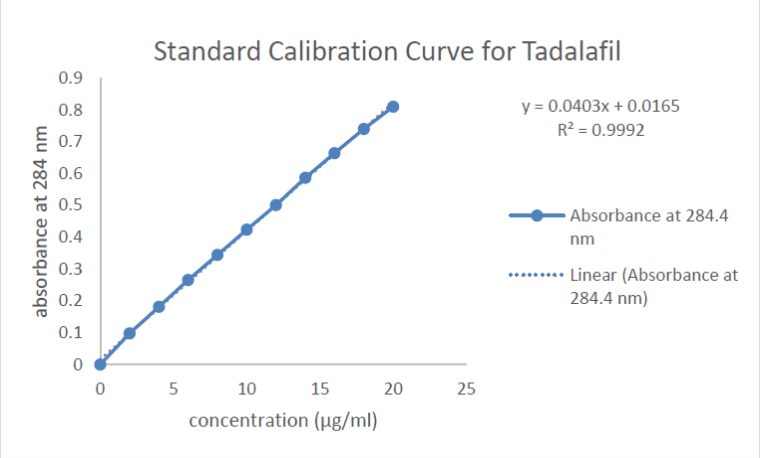

Fig 1: Standard calibration curve for Tadalafil.

3.3 Effect of sonication on release of Tadalafil

In order to understand the effect of Micronization on Tadalafil release, the effect of sonication was studied. Out of the four available micronized lots, each representative lot was selected based on the d (0.9) values. Thus, lot no. TDF/0912005 and 1304000492 were selected. TDF/0912005 representing the micronized grades of tadalafil, where d (0.9) lies in the range of 10-60 microns. Whereas, lot no. 1304000492 represented the micronized grade of Tadalafil, where d (0.9) lies in the range of 61-120 microns. Both the lots were subjected to sonication of 2, 4 and 6 minutes prior to dissolution.

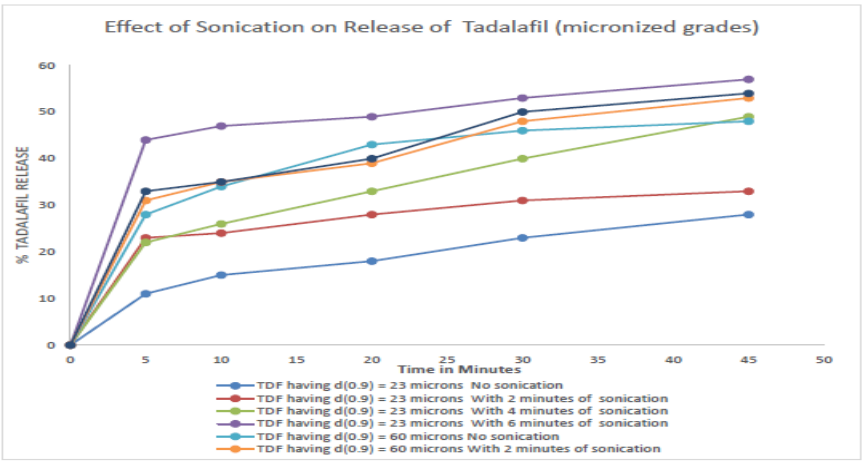

Fig 2: Effect of Sonication on Release of Tadalafil

The dissolution results (Fig 2) showed the requirement of sonication step for Tadalafil having d (0.9) of 23 microns. Whereas, not much effect is observed on sonication of Tadalafil having d (0.9) in the range of 60 microns. The probable reason for such difference is that micronized API may be having higher air entrapment thus sonication is required to break agglomerates.

Whereas, sonication has no effect on release of Tadalafil having particle size of 60 microns.

3.4 Effect of solubilizer and sonication

Since Polyethylene glycol (PEG) 400 works as solubilizer, it may help in increasing the dissolution rate of API. The dissolution testing was performed by subjecting samples to sonication and PEG 400 was used during sample preparation. From earlier studies, it was shown that Tadalafil having particle size in the range of 10-60 microns required sonication to break agglomerates and had no effect on dissolution rate. Therefore, sonication was included along with PEG 400. The dissolution was performed on each representative lot, having d(0.9) in range of 23 microns & 60 microns. The **fig 3** shows the combined effect of solubilizer and sonication on percent release of micronized grades of Tadalafil.

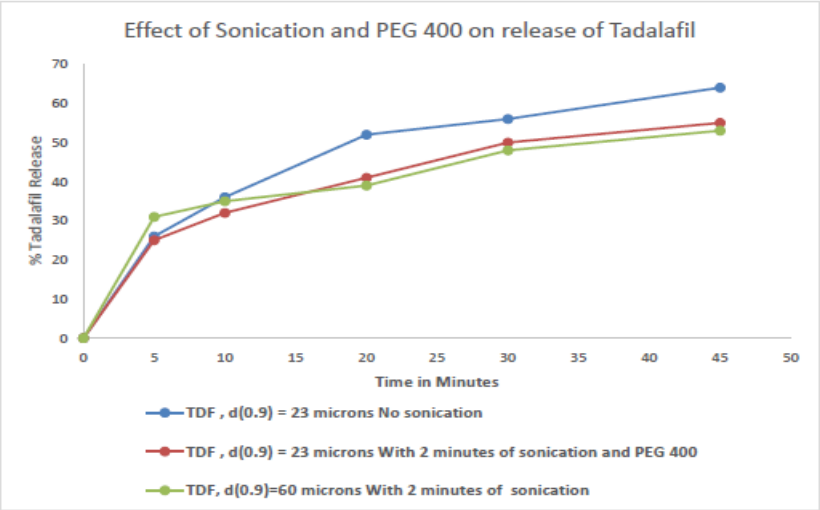

Fig 3: Effect of solubilizer and sonication on release of Tadalafil.

3.5 Dissolution results of micronized grades using developed method

Based on the results of sonication and PEG 400 (Refer section 3.3 and 3.4), sample preparation was modified to include sonication for Tadalafil having d (0.9) of particles in the range of 10-60 microns. Especially, Lot no. TDF/0912005, having d (0.9) in the range of 23 microns, required sonication of 6 minutes. Whereas, Tadalafil having d (0.9), having particles in the range of 60- 120 microns did not require sonication during sample preparation. The results are presented in **Table 3.**

Table-3: Dissolution data of API of different particle size.

Time (minutes)	(Lot No: TDF/0912005) Average % release of TDF, 23 µ	(Lot No. 1304000492) Average % release of TDF, 40 µ	(Lot No. TDF-06) Average % release of TDF, 60 µ	(Lot No. TDF/0613001) Average % release of TDF, 92 µ
5	18 ± 0.23	20 ± 0.17	26 ± 0.27	17 ± 0.18
10	22 ± 0.39	31 ± 0.7	39 ± 0.11	28 ± 0.18
20	25 ± 0.17	46 ± 0.22	49 ± 0.45	45 ± 0.25
30	27± 0.54	58 ± 0.81	60 ± 0.12	56 ± 0.81
45	31 ± 0.4	62 ± 0.5	69 ± 0.2	68 ± 0.5
60	32 ± 0.9	75 ± 0.92	81 ± 0.89	80 ± 0.16
90	38 ± 0.55	82 ± 0.2	88 ± 0.65	86 ± 0.12
120	41 ± 0.11	85 ± 0.41	90 ± 0.13	88 ± 0.23

The below fig 4 shows the *in vitro* release of micronized grades of Tadalafil in OGD medium reported for tadalafil immediate release tablets. There is no significant difference in release from tadalafil containing d (0.9) in the range of 40 microns to 92 microns. However, Tadalafil having d(0.9) value (90% of particles) of 60 microns showed comparatively faster release. The tadalafil containing d (0.9) in the range of 23 microns showed release only upto 41%. The sonication and incorporation of solubilizer, did not help in improving its release in OGD medium.

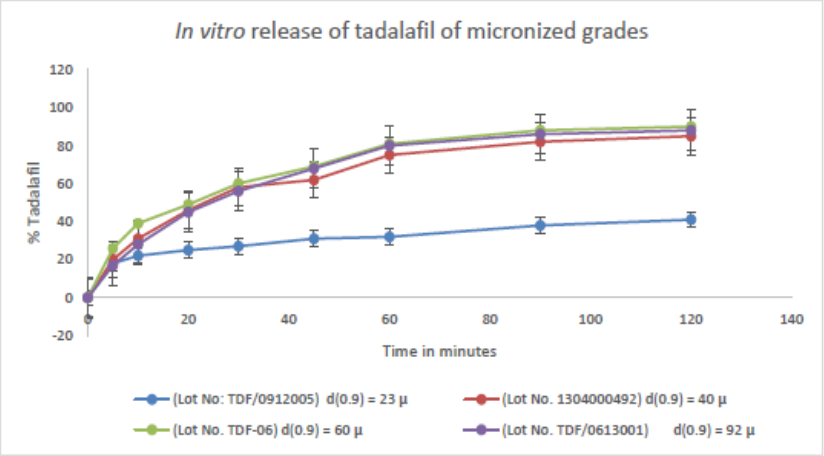

Fig 4: In vitro release of Tadalafil micronized grades

CONCLUSION

The research work involved the study of Micronized Tadalafil and its effect on dissolution. Being BCS class IV, micronization of tadalafil helps in improving solubility and hence

overall it may contribute in improvement of bioavailability. However, the excessive micronization of drug may actually reduce the effective surface area available, thereby creating air interface between particles and prevents its solubilization. In this study, the micronized API having d(0.9) of 23 microns, required higher sonication time for sample preparation. The probable reason may be due to decrease in effective surface area available for dissolution. Literature reports the decrease in effective surface area for micronized drug, it generally adsorbs air and floats on the dissolution medium.[10] Performing dissolution of micronized drugs without proper dispersion or wetting may result in erroneous results. Thus, in this case, systematic understanding of sonication, solubilizer and its effect on release was studied. Due to application of these modified procedures for sample preparation of micronized API, tadalafil drug particles were properly wetted and no floating or agglomerates was observed in dissolution bath. Thus, based on study, it can be concluded that micronized tadalafil having d (0.9) in the range of 40 microns and below may show lower release and hence it may require special solubilization techniques for its formulation development. The micronized tadalafil having d (0.9) in the range of 60-120 microns were found to be promising for formulation development using conventional formulation approach.

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