FORMULATION AND EVALUATION OF NANOSUSPENSIONS OF TADALAFIL USING DIFFERENT STABILIZERS

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In the present study, an attempt was made to prepare oral nanosuspension of Tadalafil. Tadalafil is a PDE5 inhibitor used for treating erectile dysfunction (ED) and pulmonary arterial hypertension. Tadalafil nanosuspensions were prepared by nanoprecipitation method using different polymers (such as sodium lauryl sulphate (SLS), TWEEN-80, TWEEN-20, Pluronic F127) and acetone. Estimation of Tadalafil was carried out spectrophotometrically at 285nm. The oral nanosuspension were evaluated for various physical and biological parameters, drug content uniformity, particle size analysis, zeta potential, invitro drug release, short-term stability, drug-excipient interactions (FTIR). Out of the formulation from F1 to F12, F11 containing TWEEN-20 (0.3 %) showed 99.74 % release at the end of 30min and follows first order drug release kinetics.

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INTRODUCTION

Over the past few decades the major challenging task faced by the formulators is poor solubility of the drugs. Oral delivery of these drugs is problematic owing to their poor solubility which consequences in low bioavailability and lack of dose proportionality. To overcome these challenges, formulators come up with some technologies like solid dispersions, complexation, co-solvency, micellar solubilization etc.[1-3]But these technologies are lagging in terms of universal applicability. Then formulators developed novel technologies like lipid based drug delivery systems, micronization, nanonization, combination of two technologies etc.[4,5]

Tadalafil is a phosphodiesterase-5 (PDE-5) inhibitor indicated for the treatment of erectile dysfunction and was officially approved by FDA in 2003. It is the most potent PDE-5 inhibitor which exhibits 5000 times more affinity to PDE-5. Tadalafil has gained much attention and wide clinical acceptance owing to its longer duration of action and minimal ability to cause vision abnormalities. Tadalafil is a BCS class II drug with low solubility and high bioavailability which consequences in poor dissolution with variable bioavailability.

In the present research work an attempt was made to improve the solubility and dissolution rate of model drug tadalafil.

Experimental

Tadalafil was obtained as a gift sample from Dr Reddy's Laboratories. Sodium lauryl sulphate, TWEEN-80, TWEEN-20, Pluronic F127 and all other chemicals and solvents used were obtained from Rankem, Mumbai.

Preparation of Tadalafil nanosuspension

Nano suspensions were prepared by the nanoprecipitation technique. Tadalafil was dissolved in acetone at room temperature (organic phase). This was poured into water containing different polymers like TWEEN-80, SLS, TWEEN-20 or Pluronic F127, maintained at room temperature and subsequently stirred on magnetic stirrer which is stirred at rpm 800-1000 for 30 min to allow the volatile solvent to evaporate. Organic solvents were added by means of a syringe positioned with the needle directly into stabilizer/surfactant containing water. Organic solvents were left to evaporate off under a slow magnetic stirring of the nanosuspension at room temperature for 1 h followed by sonication, later the nanosuspension was collected and evaluation tests were performed.[6-9] The composition of the formulations is given in **Table 1**.

Evaluation parameters of Tadalafil nanosuspensions

Drug content uniformity

10 mL of each formulation was taken and dissolved in 10 mLof isotonic solution and kept overnight. 10 mg (similar as in formulation) of drug was taken and dilution was made to 10 μgmL^{-1}. The dilutions were filtered and analyzed using UV for their content uniformity. The absorbance of the formulations were read using one cm cell in a UV-Vis spectrophotometer. The instrument was set at 285 nm. The drug content in each formulation was calculated based on the absorbance values of known standard solutions.[10-12]

Table 1. Composition of nanosuspension of Tadalafil.

Ingredients mg/tablet	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
Tadalafil	10	10	10	10	10	10	10	10	10	10	10	10
SLS (mg)	10	--	--	--	20	--	--	--	30	--	--	--
Pluronic F127 (mg)	--	10	--	--	--	20	--	--	--	30	---	--
TWEEN-20(w/v)	--	--	0.1 %	--	--	--	0.2 %	--	--	--	0.3%	--
TWEEN-80(w/v)	--	--	--	0.1%	--	--	--	0.2%	--	--	--	0.3%
Acetone (mL)	5	5	5	5	5	5	5	5	5	5	5	5
Water (mL)	40	40	40	40	40	40	40	40	40	40	40	40

Entrapment efficacy

The freshly prepared nanosuspension was centrifuged at 10,000 rpm for 20 min at 5°C using cool ultracentrifuge. The amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted 25 mL of supernatant solution at 285 nm using UV spectrophotometer against blank/control nanosuspensions. DEE was calculated by subtracting the amount of free drug in the supernatant from the initial amount of drug taken. The experiment was performed in triplicate for each batch and the average was calculated.[13-15]

The entrapment efficiency (EE %) could be calculated by eqn. (1)

$$EE\ \% = \text{Drug content x } 100/\text{Drug added} \quad (1)$$

Zeta potential

The zeta potential is defined as the difference in potential between the surface of the tightly bound layer (shear plane) and the electro-neutral region of the solution. The potential gradually decreases as the distance from the surface increases. The most widely-used theory for calculating zeta potential was developed by Smoluchowski in 1903. The theory is based on electrophoresis and can be expressed as eqn. (2), where (μ) is the electrophoretic mobility, (ε) is the electric permittivity of the liquid, (η) is the viscosity and (ζ) is the zeta potential. [16-18]

$$\mu = \zeta\varepsilon/\eta \quad (2)$$

Particle size and shape

Average particle size and shape of the formulated nanosuspensions was determined by using Malvern Zetasizer ZS using water as dispersions medium. The sample was scanned 100 times for determination of particle size.

***In-vitro* drug release study**

In-vitro dissolution studies were performed in USP apparatus-II (LAB INDIA DS 8000), employing paddle stirrer at rotation speed of 50 rpm and 200 mL of pH 6.8 phosphate buffer as dissolution medium. Accurately weighed bulk drug and nanosuspensions were dispersed in dissolution medium. The release study is performed at 37 ± 0.5°C. Samples of 5 mL are withdrawn at predetermined time intervals and replaced with fresh medium to maintain sink condition.[19-22] The samples were filtered through 0.22 µm membrane filter disc (Millipore Corporation) and analyzed for Tadalafil, after appropriate dilution, by measuring the absorbance at 285 nm.

RESULTS AND DISCUSSION

Tadalafil is a BCS class-II drug having low solubility and high permeability. Thus, it is challenging to enhance the solubility of tadalafil in an aqueous solution. Solvent evaporation with precipitation has been employed to produce nanosuspension of Tadalafil.

Solubility of tadalafil was carried out to determine the vehicle for evaluation. Tadalafil showed highest solubility in phosphate buffer pH 6.8 as represented in **Figure 1**. Spectral analysis was carried out and the λ-max was found to be 285 nm.

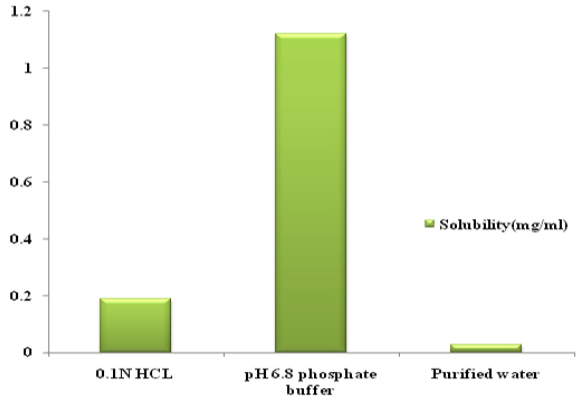

Figure 1. Solubility of Tadalafil in different solvents

Compatibility studies were performed using IR spectrophotometer. The IR spectrum of pure drug and physical mixture of drug and excipients were studied. The shift in the finger print region of tadalafil optimized formulation is within the range as that of the tadalafil pure drug as depicted in **Figure 2**.Thus it clearly indicates that there is no chemical interaction between the drug and excipients and they can be further employed for the development of nanosuspensions.

Nansuspensions of tadalafil were prepared by nanopreciptation technique using different polymerslike TWEEN-80, SLS, TWEEN-20, Pluronic F127 as stabilizers and acetone as solvent. Twelve formulations were developed and evaluated for various parameters.

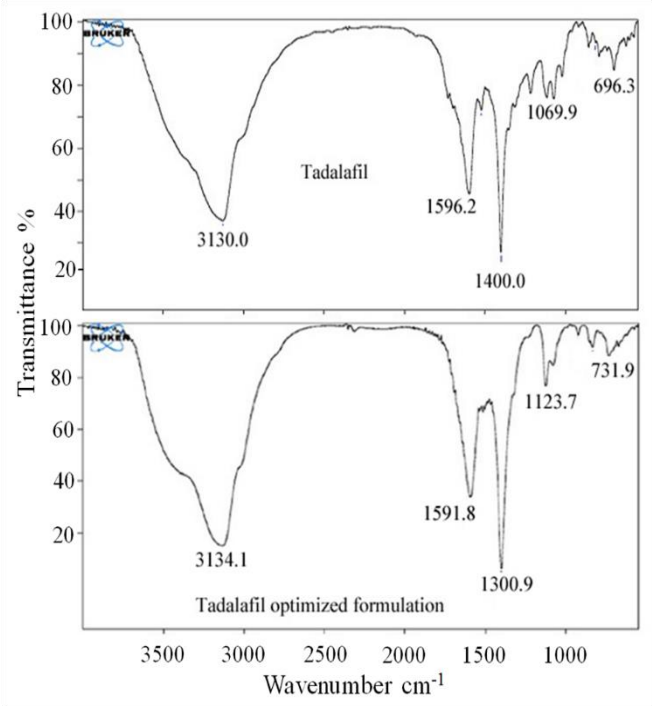

Figure 2.FTIR spectrum of Tadalafil and tadalafil optimized formulation.

The drug content of the tadalafil nanosuspension was found in the range of 76.14 %to 95.46% respectively. The entrapment efficacy of the formulated nanosuspension was found to be in the range of 87.30%-97.77%respectively. The values were reported in the Table 2**.** Based on the results it was observed that higher drug content was reported with good entrapment efficiency indicating less drug loss during formulation.

Table 2. Drug content and entrapment efficiency of tadalafil formulations.

Formulation code	Mean % drug content* ± S.D (CV)	Mean EE %* ± S.D
TF1	79.31±0.14	89.11±0.87
TF2	81.06±0.07	90.12±0.15
TF3	84.16±0.47	91.45±0.42
TF4	76.14±0.36	87.30±0.33
TF5	82.64±0.55	90.63±0.21
TF6	85.33±0.15	92.44±0.77
TF7	88.79±0.23	94.40±0.44
TF8	83.02±0.74	89.97±0.89
TF9	86.45±0.86	93.36±0.98
TF10	89.91±0.98	95.64±0.02
TF11	95.46±0.70	97.77±0.10
TF12	84.47±0.11	92.50±0.09

Further *in-vitro* drug release studies were carried out for F1 to F12formulations. Compared to all other formulations, formulation containingTWEEN-20 as stabilizer (F11) shows immediate drug release of 99.74 % drug within 30 min and it was selected as promised formulation as shown in **Figure 3**.

This formulation was selected for further analysis like SEM, zeta potential, particle size determination and stability studies were carried out.

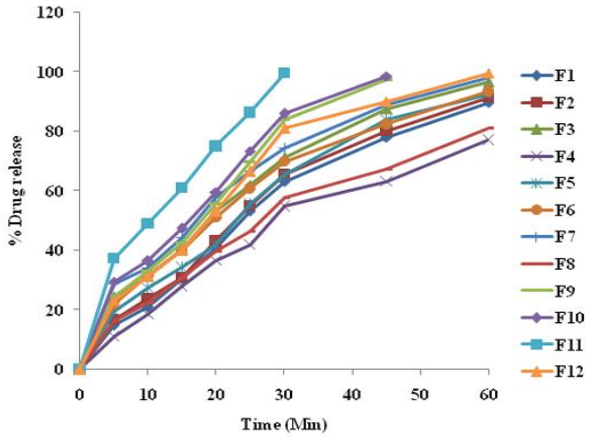

Figure 3. Percentage drug release of tadalafil from nanosuspension formulations.

From the SEM analysis it was observed that the particles were round, discrete and of nano range as shown in figure 4. The zeta potential of F11 was found to be within the acceptable limits as shown in **Figure 5.**Average particle size of nanosuspension of optimized formulations (F11)was found to be at a range of 126.6nm as depicted in **Figure 6**.

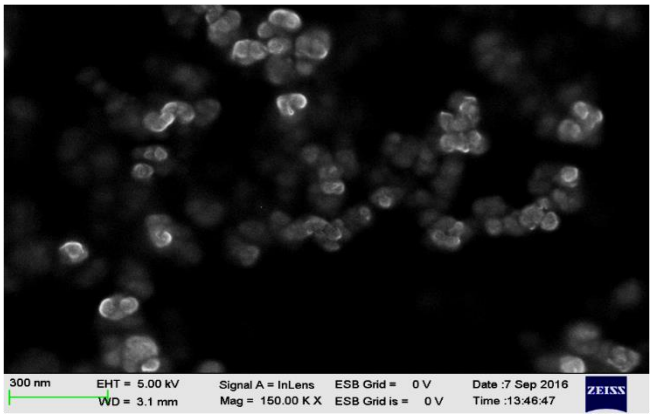

Figure 4.SEM analysis of Tadalafil optimized formulation, F11.

Table 3. In vitro drug release data of the stability formulation F11.

Time (min)	Cumulative % drug released ± S.D at 40±1°C			
	Day 1	Day 30	Day 60	Day 90
05	37.26	36.88	37.10	37.20
10	49.06	48.49	48.80	48.98
15	61.09	61.12	61.13	61.01
20	75.04	74.49	75.03	74.97
25	86.34	86.20	86.41	86.25
30	99.74	99.56	99.70	99.69

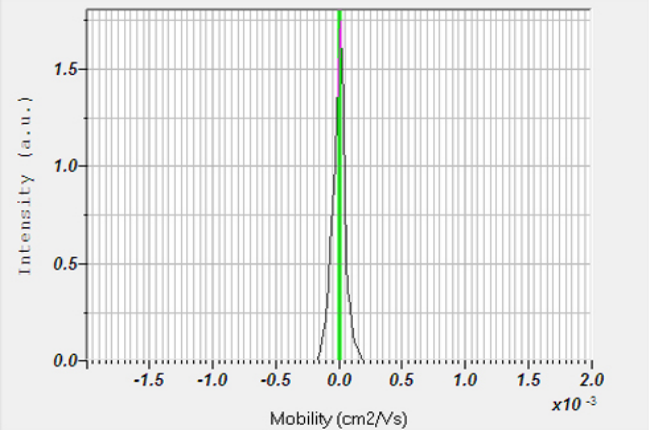

Figure 5. Zeta potential of F11 formulation.

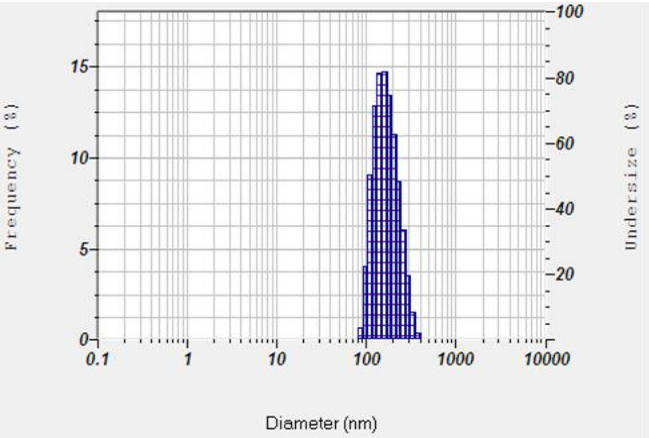

Figure 6. Particle size distribution of F11 formulation.

From the stability data obtained after 90 days study, it was inferred that there is no major difference in the drug release from the F11 formulation indicating the stability of the nanosuspension as given in Table 3.

CONCLUSION

Oral Nanosuspension of Tadalafil can be prepared by precipitation method using Tween 80, Sodium lauryl sulphate, TWEEN-20, Pluronic F127, and Acetone. When comparing all the results obtained nanosuspensions prepared using TWEEN-20 (0.3%) shows better results among all the formulations. The optimized formulation shows 99.74% of drug release by end of 30 minutes and follows first order release kinetics having R^2 value of 0.972. Finally by comparing all the formulations we can say that as the amount of polymer increases, the drug release rate increases, whereas nanosuspension strength increases.Thus nanosuspension can be a better alternative for the delivery of tadalafil.

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