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Terbinafine Hydrochloride Loaded PLGA Nanoparticles for Topical Administration

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Abstract - Terbinafine hydrochloride (TBF-HCl) loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) were produced by emulsification/solvent evaporation method and were characterization for pH, size, polydispersity index, zeta potential, encapsulation efficiency and *in vitro* release. The effect of polymer concentration was studied. The particle size analysis indicated a unimodal particle size distribution in all systems, with a mean diameter of 229-250 nm. Polydispersity index lower than 0.2 were identified, indicating a narrow size distribution. The measured zeta potential of the NP surface was approximately −10-12 mV indicating a strong negative charge at the particle's surface. In terms of encapsulation efficiency (%), high values were achieved (98%) for prepared all formulations. Drug released from loaded PLGA NPs (≈80%) was for 24 hours. By *in vitro* drug release studies, the formulations showed controlled release characteristics following Non-Fickian type of diffusion controlled release. It is concluded from the present investigation that PLGA NPs of TBF-HCl could be a potential alternative for the treatment of topical fungal infections.

Keywords**:** Nanoparticulate Drug Delivery System, Emulsification/Solvent Evaporation Method, Controlled Release, PLGA, PVA, Terbinafine Hydrochloride.

1. Introduction

With approximately 40% of candidate products now under clinical evaluation, topical therapy is considered the most innovative research area in drug delivery. It offers the following advantages over the oral route: avoidance of hepatic first-pass metabolism, self-administration with ease, convenience, and generally good acceptance by patients [1].

Terbinafine hydrochloride (TBF-HCl), a synthetic allylamine, exerts potent broad-spectrum fungicidal activity by inhibiting squalene epoxidase. It has a broad-spectrum activity against yeast, fungi, molds, and dermatophytes and is indicated for both oral and topical treatments [2]. TBF-HCl has very poor water solubility and is highly lipophilic (log P 3.3) in nature. The benefits of topical administration of TBF-HCl include direct delivery and targetability to the affected area of the skin, low dose requirement and minimized drug related toxicities [3].

Poly (lactic-co-glycolic acid) (PLGA) has been extensively studied in topical drug delivery applications because it is biocompatible, biodegradable, widely used in medical devices and pharmaceutical formulations approved by the FDA, commercially available with different molecular weights and copolymer ratios, able to encapsulate a wide range of drugs from hydrophobic to lipophilic, and capable of surface modification for site-specific drug delivery. It can be formulated as nanoparticles (NPs) using several different methods, with sizes ranging from 10 to 1000 nm. Also, it is especially suitable for controlled drug delivery applications [4]. During the NP formulation process, amphiphilic substances called surfactants are frequently used to stabilize the NPs and reduce their surface tension. Polyvinyl alcohol (PVA) is one these surfactant, commonly used in the formulation of PLGA NPs. PVA, a water-soluble polymeric surfactant, is known to form PLGA NPs with uniform size distribution. The objective of this study was to prepare and evaluate the *in vitro* characteristics of TBF-HCl loaded PLGA NPs for topical application as controlled drug delivery system.

2. Materials and Methods

2.1. Materials

TBF-HCl Terbinafine HCl (TBF-HCl) was a gift from Santa Farma Drug Company (Istanbul, Turkey) PLGA (Resomer® RG 502H) and PVA (Mw 30000-70000, 87-90% hydrolyzed) were purchased from Sigma-Aldrich (St. Louis, MO). All other chemicals were analytical grade.

2.2. Formulation of PLGA NPs

The PLGA NPs were prepared by an emulsification/solvent evaporation method. Briefly, an organic solution, TBF-HCl and PLGA was dissolved in 5 mL of acetone. Aqueous solution, 10 mL of PVA 3 % (w/v) was prepared using heated magnetic stirrer. Then PVA solution was brought to room temperature. PLGA solution was added to PVA solution. The mixture was homogenized using homogenizer (Silverson L5M) at 10000 rpm for 2 minutes. 15 mL ultrapure water was added to mixture and O/W emulsion formed. This system was stirred with magnetic stirrer for evaporate organic solvent at room temperature during 24 hours. The resulting mixture was centrifuged at 4750 rpm for 90 minutes. After centrifugation, the supernatant was separated. For removal of excess PVA, 10 mL ultrapure water was added and centrifuged at 4750 rpm for 30 min. After centrifugation, the supernatant was separated and re-suspended by adding 20 mL of ultrapure water to the NPs. The compositions of formulations are given in Table 1.

Table 1: The composition of NPs.

Formulation Code	TBF-HCl (%)	PLGA solution (w/v)	PVA solution (w/v)
F1	1	1	3
F2	1	1.5	3
F3	1	2	3

2.3. Characterization of Formulations pH

Thermo Scientific pHmeter was used for measuring the pH at room temperature. All experiments were replicated at least three times.

2.4. Measurement of Particle Size and Polydispersity Index

The particle size (PS) and polydispersity index (PI) were measured at 25°C using a Nano-ZS Zetasizer (Malvern Instruments, Malvern, UK). The PS and PI values were obtained by averaging ten measurements at an angle of 173° using disposable cells. The PS measurements were performed with undiluted and diluted formulations. NPs were diluted with distilled water in the ratio of 1:1, 1:5 and 1:10 (n=5).

2.5. Measurement of Zeta Potential

The zeta potential (ZP) of the NPs was measured using disposable plain-folded capillary zeta cells (Malvern Zetasizer Nano-ZS) at room temperature. The ZP was calculated from the electrophoretic mobility using the Helmholtz–Smoluchowski equation under an electrical field of 40 V/cm. The processing was done using the software included within the system (n=5).

2.6. High-Performance Liquid Chromatography Analysis

The amount of TBF-HCl were analyzed by using a high pressure liquid chromatography (HPLC) system (Agilent 1100 Series) consisting of a UV detector and ACE 5® C18 column (Aberdeen, Scotland) with the following specifications: length of 25 cm, an internal diameter of 4.6 mm, a particle size of 5 µm, and a pore size of 110 Å. A filtered and degassed solution containing 75% (V/V) acetonitrile, 20% (V/V) KH_2PO4 buffer (pH 4.5) and 5% (V/V) tetrahydrofurane was used as the mobile phase at flow rate of 0.9 ml/min. The UV detection was at 224 nm. The stock solution was TBF-HCl in dimethyl sulfoxide and dilutions were prepared in PBS:Ethanol (pH 7.4) (1:1). [2].

2.7. Drug Entrapment Efficiency (EE)

NP dispersion was ultracentrifuged for 90 sec at 10,000 rpm. The supernatant was used for TBF-HCl analysis by HPLC and the quantity of free drug was determined. The drug loading of NPs was determined by dissolving 0.5 mL of the nanosuspension in 10 mL acetone. All experiments were replicated at five times. The percentage of entrapment efficiency was calculated by using Equation 1.

$$EE\ (\%) = \frac{Total\ amount\ of\ drug - the\ amount\ of\ free\ drug}{Total\ amount\ of\ drug} \times 100 \quad (1)$$

2.8. *In Vitro* Release of TBF-HCl from NPs

The release of TBF-HCl from NPs was assessed using a dialysis bag (cellulose membrane, MW cut off 12-14,000, Spectrum, Canada) into phosphate buffer saline (PBS, pH 7.4):ethanol (1:1) at 32±0.5 ºC for 24 h. The amount of the drug in the receiving solution was analyzed by validated HPLC as described above.

2.9. Determination of Release Mechanism

The dissolution data were fit to Peppas equation, and best-fit parameters were calculated to determine the release mechanism of NPs. The parameters were estimated to determine the drug release mechanism with Equation 2 [5].

$$\log(M_t \div M_\infty) = \log k + (n \times logt) \quad (2)$$

Where, Mt /M∞ is the fraction of drug released at time, k is the drug release rate constant and n is the release exponent that characterizes the drug release mechanism.

2.10. Statistical Data Analysis

Statistical data analysis was performed by using the Student's ttest with $p<0.05$ as the minimal level of significance.

3. Result and Discussion

In this study, TBF-HCl loaded PLGA NPs with PVA were successfully prepared using emulsification/solvent evaporation method. This technique involves mainly two steps, first step is emulsification in which organic solvent loaded with the drug is dispersed in an aqueous phase containing suitable stabilizer to form an emulsion and in the second step organic solvent was evaporated to form the NPs stabilized by surfactant. Organic solvent selection is very critical in producing unimodal NPs because formation of NPs depends on the evaporation of organic solvent, due to which solvent influences the particle mean diameter and PI significantly. Acetone, a water-soluble solvent, gives a small sized, uniform, unimodal population even in the absence of homogenization through a nanoprecipitation mechanism. Acetone rapidly diffuses into the aqueous phase resulting in the precipitation of the polymer (PLGA) that forms NPs. Emulsification is crucial step in this method, so polymer concentration in the internal organic phase of the emulsion is one of the paramount parameter. In this study, three different concentration of PLGA (1%-1.5%-2%) were investigated. PVA polymer was used stabilizing agents in PLGA NP preparation. The formulations compared them in terms of their NP characterizations such as pH, PS, PI and ZP.

The pH values of the NP formulation were found to be 7.04, 6.95 and 7.09, respectively (Table 2). Neutral pH values indicated the formulation could be used as topical delivery with low risk skin irritation.

Table 2: pH and EE (%) oF NPs.

Code	pH	EE (%)
F1	7.04	98.190±0.952
F2	6.95	98.398±1.053
F3	7.09	98.600±1.415

PS is one of the most important parameters determining biocompatibilities and bioactivities of NPs. Also, PS has a direct relevance to the stability of the formulation. Larger particles tend to aggregate to a greater extent compared to smaller particles, thereby resulting in sedimentation [6]. The PS values of NPs were greatly increased by the increment of PLGA concentration in formulations. Lowest PLGA ratios produced smaller NPs. This result was consistent with literature [7]. However, no significant differences were observed PS value with dilution ($p \geq 0.05$). PS, PI and ZP of the NP formulations are given in Table 3.

The PI of all formulations was low (PI<0.2), showing that this method of preparation results in highly uniform NPs.

Higher negative values of ZP were obtained for NPs formulation due to the presence of terminal carboxyl groups in the polymer. In case of charged particles, as the ZP increases, the repulsive interaction will be larger, leading to the formation of more stable particles with more uniform size distribution. The negative ZP is decreased, means increasing the NPs stability. PLGA NPs were found negative zeta potential between -10 and -13 mV.

Table 3: PS, PI, and ZP of NPs.

Code		PS(nm)±SD	PI±SD	ZP (mV)±SD
F1	undiluated	229.3±0.83	0.055±0.010	-12.5±0.5
	1:1	228.7±1.59	0.083±0.014	-
	1:5	230.1±1.80	0.062±0.038	-
	1:10	227.2±1.03	0.057±0.012	-
F2	undiluated	229.7±0.70	0.063±0.018	-11.4±0.7
	1:1	228.5±0.704	0.069±0.021	-
	1:5	224.9±1.550	0.064±0.015	-
	1:10	227.74±0.902	0.063±0.027	-
F3	undiluated	253.5±1.11	0.062±0.038	-10.1±0.4
	1:1	253.3±2.91	0.086±0.001	-
	1:5	252.5±1.53	0.075±0.026	-
	1:10	254.3±1.79	0.087±0.032	-

A HPLC method for quantitative analysis of TBF-HCl in NP formulations was used. The peak area correlated linearly with TBF-HCl concentrations in the range of 0.01–5 µg/ml (r^2 = 0.996). The chromatogram of the TBF-HCl standard presented a peak in the retention time of 16.737 minutes, and total analysis time was 20 minutes (Figure 1).

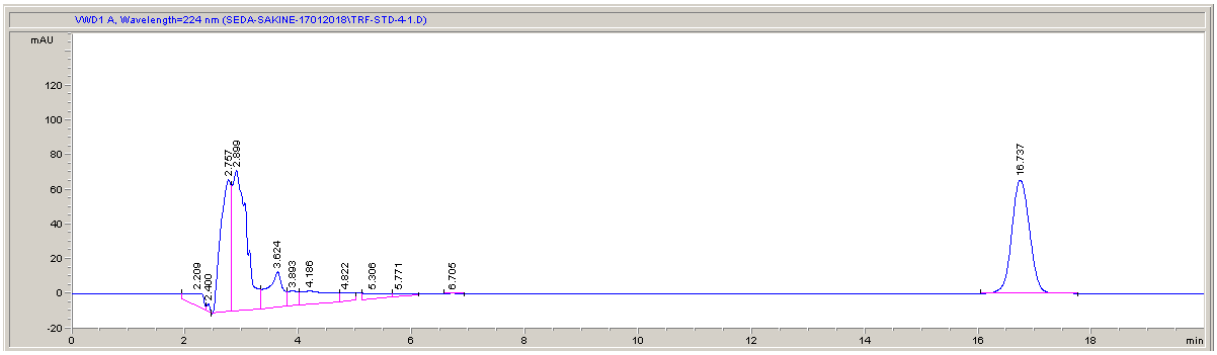

Fig. 1: The chromatograms obtained of TBF-HCl.

The EE% of TBF-HCl from the NPs (the percentage of TBF-HCl encapsulated with respect to the total amount of TBF-HCl added to the system) was as high as 98.190%–98.600%. EE% capacity of the NP formulations are given in Table 2.

Release rates are important parameters in NP-mediated drug delivery to optimize the therapeutic efficacy of the encapsulated drug.

Drug release from a polymer follows different mechanisms such as drug diffusion, swelling of polymer matrix and hydrolysis or degradation of polymer. The *in vitro* release profiles reflected the trend observed with the physicochemical properties i.e. particle size, drug loading and morphology. Figure 2 shows that prepared all TBF-HCl loaded NP formulations controlled drug release. No burst effect has been observed, indicating that TBF-HCl was homogeneously dispersed in the all NP dispersions and that no significant amount of drug was adsorbed onto the NP surface. The release profiles of all the formulations prepared with different PLGA concentration were similar which is in accordance with other physicochemical properties. However, F1 formulation was accompanied by a decrease of the percentage of TBF-HCl release.

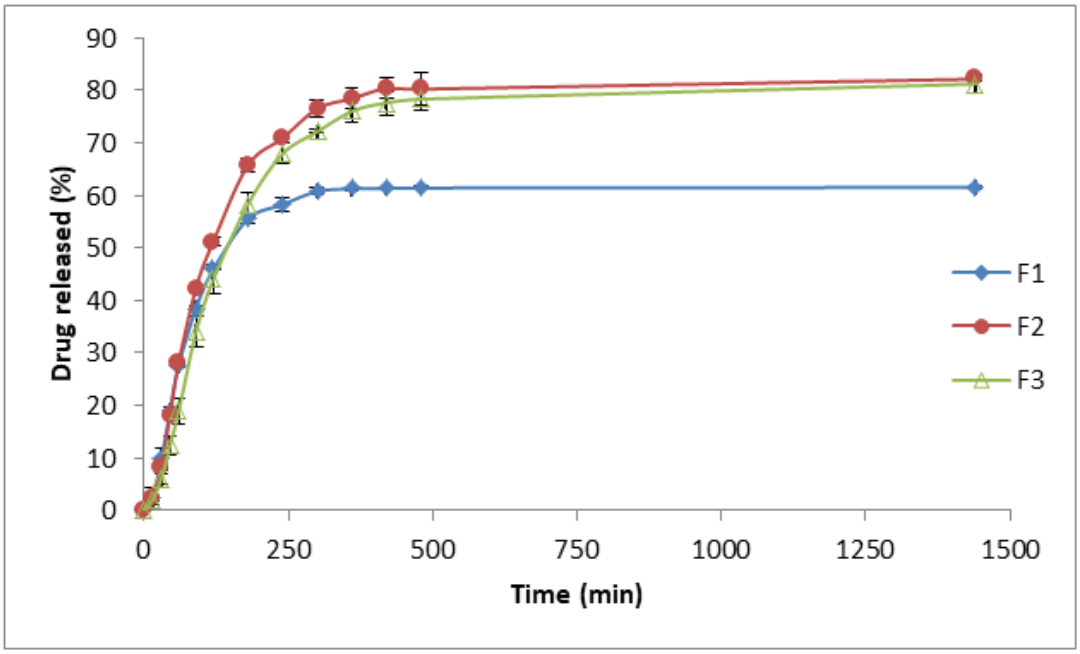

Fig. 2: *In vitro* release of TBF-HCl loaded NPs.

The theory of the determination of drug release mechanism from NPs is based on an empirical equation proposed by Ritger and Peppas [5]. When n=0.45, this indicates case I (Fickian) diffusion or square root of time kinetics; when 0.45<n<0.89, this indicates anomalous (non-Fickian) diffusion; when n=0.89, this indicates case II transport; and when n>0.89, this indicates super case II transport. The n values obtained from the release equation proposed by Peppas are shown in Table 4. In this study, the values of n fell within the range of 0.814–0.877, indicating that the drug release from the NPs is non-Fickian. Non-Fickian release refers to the combination of both diffusion and erosion mechanisms of controlled release. Similar kinetic results have been investigated with PLGA NPs in the literatures [8, 9].

Table: Release parameters of TBF-HCl from NPs.

Formulation Code	n	Log k	r^2
F1	0.856	0.264	0.899
F2	0.877	0.207	0.909
F3	0.814	0.134	0.929

4. Conclusion

In this study, TBF-HCl loaded PLGA particles were produced using an emulsification/solvent evaporation method. According to characterization studies it was seen that the polymer concentration did not affect parameters significantly. As it was indicated in the introduction part the aim of study is to prepare a controlled release system of TBF-HCl for topical delivery. And *in vitro* release studies showed that TBF-HCl NPs exhibited a controlled release. It is concluded that topical PLGA NPs containing TBF-HCl may effectively deliver the drug to the fungal infection and formulations will significantly improve patient compliance by reducing dosing frequency from conventional doses. In the near future, we will aim to work on *in vitro* antifungal activity.

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