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Preparation and Characterization of Electrospun Ketoconazole Loaded Nanofibers as Dermal Patch

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Abstract

Objective: This study aims to formulate a ketoconazole patch containing electrospun nanofibres and enhance the solubility due to the increased particle surface using the electrospinning method.

Methods: The Design-Expert version13 software was used in experimental designing, to ensure an efficient nanofiber preparation process. Several nanofiber formulations were prepared with different concentrations of drug and polymers. In this study, Ketoconazole was selected as the model drug, ketoconazole is widely used as an antifungal drug in the treatment of fungal infections. Eudragit and Polyethylene glycol polymers were used with ketoconazole to formulate electrospun nanofibers. Then Ketoconazole nanofibers were investigated and evaluated chemically and morphologically by Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM).

Results: Utilizing the electrospinning method, Ketoconazole electrospun nanofiber was successfully fabricated. The produced ketoconazole-loaded nanofibers mat was formulated as a dermal patch. The Eudragit and polyethylene glycol polymers revealed good characteristics that led to the production of uniform ketoconazole nanofibers. The prepared patch showed good physical and mechanical properties. The SEM results Images for the produced nanofiber mat showed good nanofiber distribution and no beads formation with several fiber diameter sizes, while Fourier Transform Infrared Spectroscopy findings revealed the conjugation between polymers and ketoconazole pure powder, the major characteristic peaks of ketoconazole appeared in the FTIR spectrum. Formula 7 showed minimum nanofibers diameter with maximum entrapment efficiency.

Conclusions: In this study, the use of the electrospinning method completed the formulation of a dermal patch containing antifungal nanofibers successfully. Using design expert software to ensure maximum optimization, two biocompatible polymers were used, Eudragit E100 and PEG 600 to formulate ketoconazole nanofibers. The formulated dermal patch could be promising for pharmaceutical antifungal applications.

Keywords: Eudragit, PEG, electrospinning, design-expert, antifungal, SEM

Introduction

Pharmaceutical nanotechnologies' role is very important in improving the problems of traditional dosage forms, these enhancements may lead to improved patient compliance.[1] Preparation of substances at Nano-size may improve their chemical, physical, and biological characteristics. So, it's convenient to prepare Nano-size structures to improve or modificate the physicochemical properties in order to obtain the desired characteristics, electrospinning technology is widely used for fiber fabrication with different diameters ranging from Nano to micro-scale, this method utilizes different types of polymers synthetic and natural. [2] The idea for nanofibers is to formulate a fiber structure with high surface area which provides a good release profile and efficient carriers for the medications.[3] Ketoconazole medication belongs to a family called imidazole antifungal drugs which is used in the treatment of infectious diseases caused by fungi, Ketoconazole drug was approved for topical and systemic use in the treatment of fungal infectious disease.[4] ketoconazole is used widely, especially for skin infections caused by fungi such as tinea versicolor.[5] There are a lot of advantages when considering dermal drug delivery such as the need to decrease the liver first pass metabolism, a wearable skin patch can be a convenient dosage form for drug delivery through the skin.[6] The study aims to formulate a patch containing ketoconazole nanofibers as antifungal medication using the electrospinning method by incorporating two biodegradable and biocompatible polymers (Eudragit E100 and PEG 600). The use of Design-Expert software is to ensure fewer experimental attempts, cost-effective design, and determine the variable influence on experimental design.[7] The study findings could be promising for advancing antifungal skin therapies using ketoconazole as a dermal patch considering drug delivery systems to improve treatment outcomes.

Materials and Methods

Materials

Ketoconazole was procured from MERYER, China. Eudragit E100 and Polyethylene glycol, PEG 600 were procured from Baoji Guakang, China. Absolute Ethanol was purchased from Changshu Hongsheng, China.

Methods

Ketoconazole calibration curve

The ketoconazole calibration curve in ethanol was conducted from 25 ml stock solution. The range of ketoconazole concentration from 1.25 to 40 µg/ml was taken from the stock solution. After preparing a series of 6 dilutions by taking 2 ml of ketoconazole in ethanol stock solution and diluting it with 2 ml of absolute ethanol and measuring each concentration spectrophotometrically at λ max of 270 nm using (UV-1800 series), (Shimadzu, Japan).[8]

Electrospun solution preparation

The electrospun solution of two polymers PEG 600 and Eudragit E100 alongside ketoconazole was prepared at different amounts by dissolving the ketoconazole powder and the two polymers in absolute ethanol separately, and the solution for each substance was stirred at room temperature for 12 hours. Eudragit E100 and PEG600 polymers solution alongside the ketoconazole were mixed at a ratio of 8:1:2 respectively. The final solution was stirred for 1 hour and ready for the electrospinning process.[9,10]

Design-Expert software proposed 8 runs of different concentrations of both the drug and polymers according to the input information of minimum and maximum amounts of the 3 factors. Table 1 illustrates the eight runs.

Electrospinning apparatus setting

Several parameters are involved in the electrospinning process setting which can affect the nanofibers fabrication. The syringe pump flow rate was stable and set at 0.5 ml/hr. The tip-to-collector distance was 15 cm; the ranging temperature at 25–30°C, the gauge of the needle was G18, and the applied voltage was 15 KV.[11]

Ketoconazole fibers investigation

The collected ketoconazole electrospun nanofibers were investigated by several techniques such as Fourier transform infrared spectroscopy FTIR for analyzing the drug-polymers conjugation, scanning electron microscopy SEM to determine the morphology of the produced nanofibers and fiber diameter.[9]

Fourier-transform infrared spectroscopy FTIR of Ketoconazole

The ketoconazole (FTIR) spectrum was investigated to detect the drug and polymer peaks individually and combined in the final formula at different bandwidth positions. FTIR analysis technique was performed to determine whether there is a chemical interaction or not between polymers (E100, Peg 600) and ketoconazole in the optimized formula and also to detect the compatibility. Using an FTIR spectrophotometer from (Shimadzu-Japan) this process was conducted at 400 to 4000cm^{-1} band length.[12]

SEM analysis for ketoconazole nanofibers

The produced ketoconazole nanofiber morphology, uniformity, and diameter size were all analyzed for each nanofiber formula using scanning electron microscopy (SEM) (USA, FEI Company), also the optimized formula was analyzed. The mean nanofiber diameter was calculated for each formula calculated.[13]

Table 1. **The proposed eight formulas by the Design-Expert software**

Run	Ketoconazole mg/ml	Eudragit E100 mg/ml	Peg (600)/ml
1	2	40	1
2	2	40	0.5
3	1	40	0.5
4	2	80	0.5
5	1	80	1
6	1	40	1
7	2	80	1
8	1	80	0.5

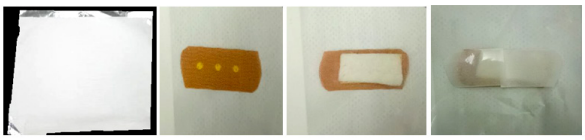

Fig. 1 **Patch preparation process, (A) ketoconazole nanofiber mat on aluminum foil, (B) prepared backing membrane, (C) applying nanofibers on patch, and (D) applying linear.**

Patch preparation method

The backing membrane was made from a 5*5 cm piece of aluminum foil, plastic sheet also 5*5 cm attached to the backing membrane using adhesive. After that, a 1*2 cm piece of plastic sheet was fixed. The ketoconazole nanofiber mat was placed over the small sheet area and wrapped with nylon mesh.[14] Figure 1 shows the prepared patch.

Patch evaluation

The produced ketoconazole nanofiber patch was subjected to some evaluation tests to determine whether the patch preparation process was efficient or not, in this study the patch evaluation involved a weight uniformity test, patch thickness test, and folding endurance test. Thickness was measured at the different areas by digital micrometer, while for weight uniformity the average weight for different pieces was calculated after weighing using digital balance, for folding endurance a patch strip piece was cut and repeatedly folded until the strip broke, and the number of folding required for without braking represent the folding endurance.[14,15]

Drug release

In vitro ketoconazole release from the patch was accomplished by immersing small pieces (3 cm circle) of the patch in ethanol and phosphate buffer of 5.5 PH separately, each 15-minute samples were taken and refilled by fresh medium with the same amount and then examined using a spectrophotometer at 270 nm wavelength to detect the concentration.[16,17]

Results

Ketoconazole Calibration Curve

The ketoconazole concentration range was (1.25 – 40 µg/ml), 6 dilution series was achieved and analyzed the sample by UV spectrophotometer at 270 nm λ max. The regression coefficient value (R^2) was 0.9991 and the equation was y = 0.0131 X – 0.0078. Figure 2 shows the calibration of ketoconazole.

Fourier-transform Infrared Spectroscopy FTIR of Ketoconazole

The (FTIR) spectrum of pure ketoconazole was investigated as well as the polymers. FTIR analysis was accomplished in order to detect the polymer and drug and peaks separately for each one and combined in the final formulation. Ketoconazole

FTIR analysis showed the presence of C-H stretching at 2958.80 cm^{-1} wave number, C-O aromatic at 1249.87 cm^{-1}, C=N stretching at 2362.80 cm^{-1}, C-CL at 817.82 cm^{-1}, N=H stretching at 3417.86 cm^{-1}, and C=O at 1645.28 cm^{-1}.

While Eudragit E100 FTIR revealed C-O-C stretching at 1145.72 cm^{-1} wave number, dimethyl amino at 2771.71 cm^{-1} and 2823.79 cm^{-1}, C=O stretching at 1732.08 cm^{-1}, and methylene at 2953.02 cm^{-1}. PEG 600 polymer FT-IR spectrum peaks were 3383.14 cm^{-1} related to OH absorption broad, C-H at 2873.94 cm^{-1}, C-H bending vibration at 1462.04 cm^{-1} and C-O stretching at 1107.14 cm^{-1}. Figure 3 shows the FTIR findings.

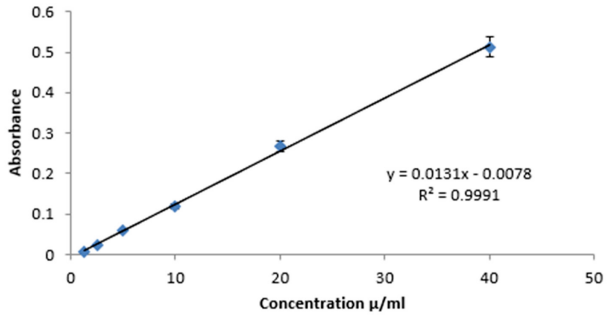

Fig. 2 **Ketoconazole pure powder calibration curve in ethanol.**

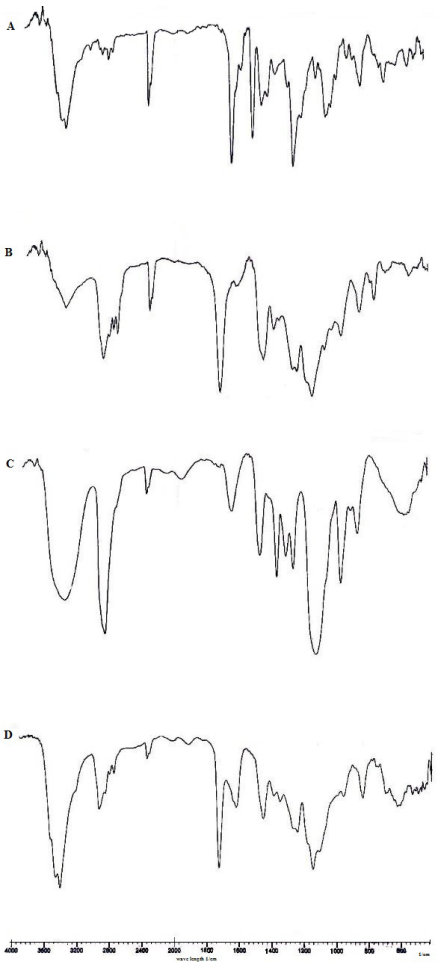

Fig. 3 **FTIR Findings. (A) ketoconazole; (B) Eudragit E100.; (C) PEG 600.; (D) Nanofiber loaded with ketoconazole; The Measurements range was (400–4000 1/cm).**

SEM Analysis Outcomes for Ketoconazole Nanofiber

The SEM images demonstrated high resolution and accuracy. The SEM images for the prepared 8 formula and the final nanofibers optimized formula showed good entanglement and good homogeneity, also no beads or imperfection formation. Figures 4, 5, and 6 illustrate the results.

Patch Evaluation

The obtained thickness average result for the prepared patch was 1.36 mm, while the folding endurance test value was 105 which means that the prepared patch has good mechanical flexibility. The value of weight uniformity was 0.029 g.

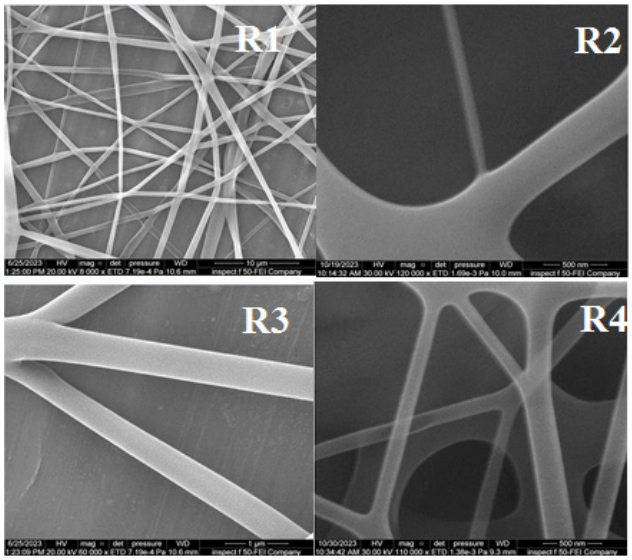

Fig. 4 **SEM results. The diameter average was (R1) 516 nm, (R2) 240 nm, (R3) 111 nm, and (R4) 160 nm.**

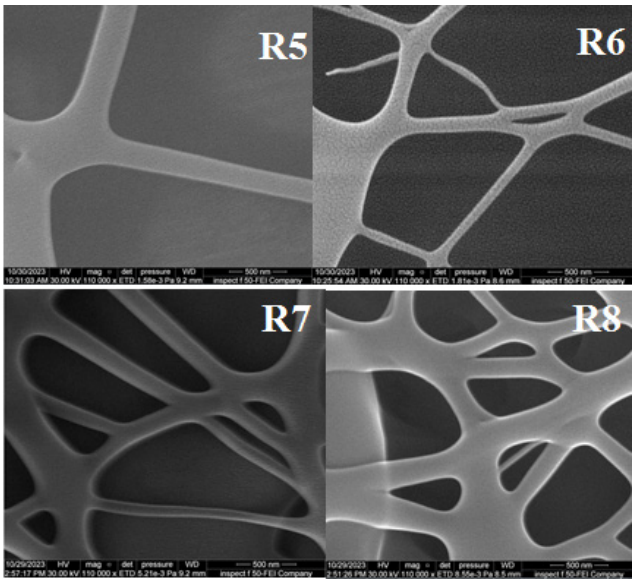

Fig. 5 **SEM results. The diameter average was (R5) 245 nm, (R6) 127 nm, (R7) 118 nm, and (R8) 136 nm.**

Statistical Analysis

The experiment's statistical analysis was achieved using Design-Expert software and Excel software. Several data and factors were analyzed by figures using the previously mentioned software. The *P*-value was less than 0.05 indicating the process was statically significant. Different factors were analyzed using design expert software such as entrapment efficiency and fiber diameter as in Table 2. Figures 7 and 8 illustrate the results.

Drug Release

The ketoconazole release process was accomplished for both pure drug and final nanofiber formula at two different mediums (phosphate buffer ph. (5.5) and ethanol) to investigate the release profile at different mediums, four patch formulations were prepared for this purpose. Figure 9 shows the release profile for the 4 formulated ketoconazole patches.

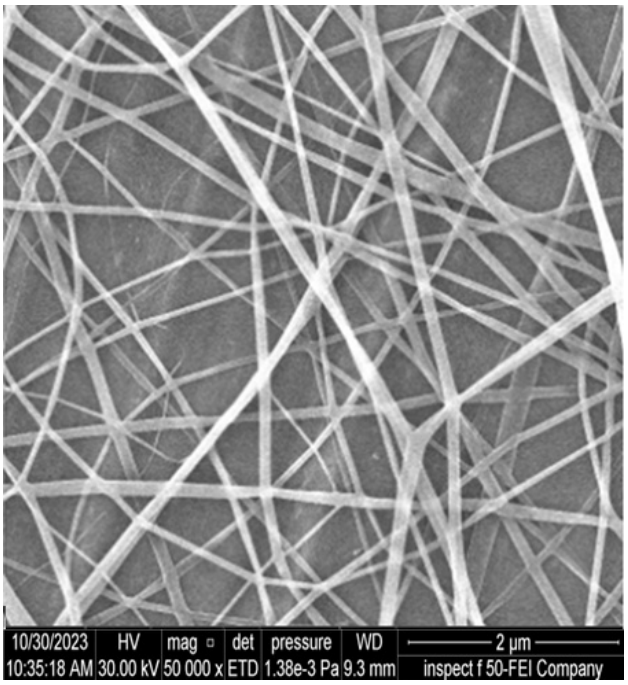

Fig. 6 **SEM result for the final formula.**

Table 2. **Diameter size and Entrapment efficiency values by Design-Expert**

Response 1 dimeter size Nm	Response 2 Entrapment efficiency %
516	55
240	49
111	47
160	78
245	88
127	54
118	97
136	63

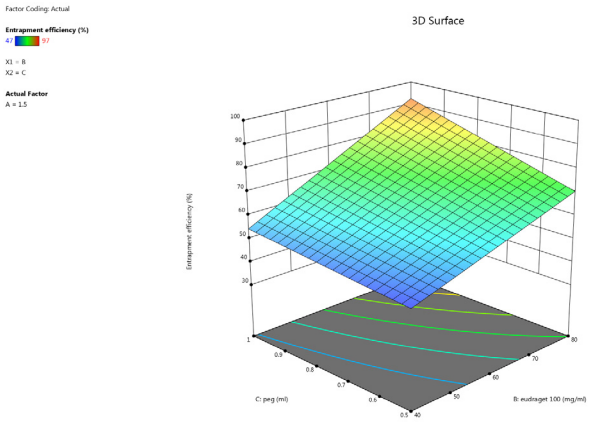

Fig. 7 **Three-dimension surface graph. Factor B (E 100) and C (PEG 600) effects on entrapment efficiency.**

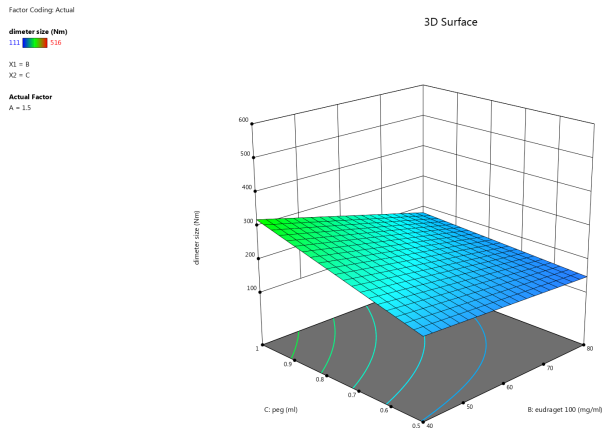

Fig. 8 **Three-dimensional surface graphs. The effect of Factor B (E 100) and C (PEG 600) on fiber diameter.**

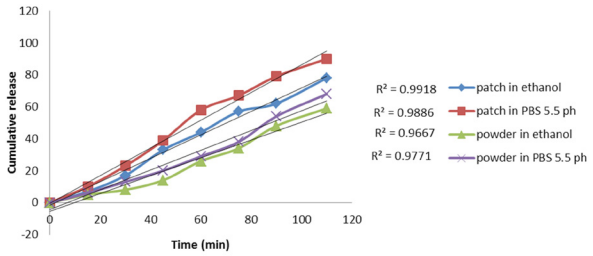

Fig. 9 **Release profile of ketoconazole as patch-loaded nanofiber and as patch containing pure powder in ethanol and phosphate buffer.**

Discussion

In this study, the aim was to fabricate a nanofibers-loaded ketoconazole as an antifungal drug in the form of a dermal patch. In calibration curve calculation the regression coefficient was (0.9991) with linear equation y = (0.0131 X – 0.0078). These results agree with the reported findings about R^2 (0.999) by Ramavath N., et al., (2019).[18] The FTIR results that are illustrated in Figure 3 indicate the purity of the materials used (Ketoconazole, PEG600, and Eudragit E100) because it shows peaks band agreement with the references.[19–21] The final formula FTIR spectrum in Figure 3 D indicates the minor

conjugation between drug and polymers and there is minor shifting in peak position also FTIR outcomes for ketoconazole loaded nanofiber formula show most of the characteristic peaks which means low or no chemical interaction.[22,23] The SEM images show that the 8 formula proposed by Design-Expert software and the final formula was beads free and uniform nanofibers with a diameter ranging from 111 to 516 nm, the controlled viscosity, electrospun solution concentration, type of polymer, and device-related parameter among several factors that lead to getting this uniform nanofiber mat, the predicted fiber diameter by Design expert software for the optimized formula was 118 nm while the experimental actual diameter was 108 nm value for the optimal nanofiber formula was 108 nm which indicates 91,52% accuracy, the increase in viscosity to certain limits and the use of E100 and PEG 600 may leads to produce of uniform and beadless nanofiber.[24–26]

For the patch evaluation process, the results indicate there is a small variation in the thickness of the patch because of the occupied volume by ketoconazole, the value of folding endurance indicates the flexibility of ketoconazole loaded nanofibers patch, the average weight was similar to most pieces weight which indicates the well-distributed fiber and fiber uniformity.[27–29]

The three-dimensional graph in Figure 7 shows that Eudragit E100 has a positive effect on entrapment efficiency by which increasing the concentration will lead to an increase in the drug entrapment, PEG600 has a positive effect but less than Eudragit. While Eudragit E100 has a negative effect on fiber diameter which means an increase in the concentration leads to a decrease in the fiber diameter, and PEG has a positive effect on fiber diameter, also in this study increasing or decreasing ketoconazole concentration will affect the fiber diameter as shown in three-dimensional graph, Figure 8. The type of polymer can affect the drug release, E100 polymer is considered a cationic polymer that may lead to an enhanced rate of dissolution at acidic PH, Figure 9 shows that the release in phosphate buffer is better than ethanol, PEG 600 can be soluble in a variety of solvent and can be a carrier for hydrophobic medication (ketoconazole) increases which facilitate the rate of dissolution, the release of ketoconazole loaded nanofibers patch is better than patch with pure powder and this is because the dissolution is better when the fiber at Nano size.[30–33]

Conclusion

The final formula of ketoconazole-loaded nanofibers was prepared with success using the electrospinning method. The two used polymers (E100 and PEG600) and the model drug (ketoconazole) were biocompatible. SEM results showed that the 8 runs were uniform and without beads formation, formula number 7 showed a good fiber diameter and maximum entrapment efficiency. The patch evaluation tests confirmed that the patch has good mechanical flexibility and weight uniformity, which means that the preparation process of this patch was efficient, the release was enhanced when comparing the nanofibers with the pure powder due to the enhanced dissolution rate by increasing the surface area using electrospinning technique and also due to using suitable polymers that can enhance the dissolution rate.

Conflict of Interest

None.

Funding

Nil.

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