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Fabrication and Evaluation of Itraconazole Loded Collagen Scaffolds

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***Abstract*: The collagen scaffolds are of greater importance in tissue engineering (or) tissue regeneration process. Itraconazole is a triazole type antifungal agent selected and drug candidate has a broad spectrum of activity and is well tolerated. In this study, itraconazole was incorporated into the collagen matrices to fabricate the scaffold by freeze drying (Lyophilization) technique. The fabricated scaffolds were evaluated for morphology by SEM, water uptake study, folding endurance, in-vitro drug release and drug release kinetics. The results showed the physical entrapment of drug in fibrillar networks with less pore size formation, 70% drug water absorbing property, stable without any cracks during folding at 228 times, 97.68% drug release at 4th hour. The drug release from scaffold followed first order kinetics due to the porous fibrillar drug entanglement. Hence, the itraconazole loaded collagen scaffold could support the rapid wound healing process followed with skin regeneration in damaged site.**

***Keywords*: Collagen, Itraconazole, Scaffolds.**

1. Introduction

Collagen scaffold is a three-dimensional porous solid material which is the most common protein in the body. It provides strength and structural stability to tissue in the body. Including skin, blood vessels, tendons, cartilage, and bone. The collagen scaffolds are of greater importance in tissue engineering / tissue regeneration process. Itraconazole is a triazole type antifungal agent selected as a drug candidate and has broad spectrum of activity. Itraconazole with collagen fabricated as a drug delivery to treat the fungal disease followed by tissue regeneration process.

2. Methodology

A. Fabrication of Itraconazole Loaded Collagen scaffold

Collagen was dissolved in 0.5 M acetic acid. Solutions with different collagen concentration were prepared. These solutions with collagen concentration of 5 mg/mL, 10mg/mL and 20mg/mL were injected into a mold (diameter: 1.5cm; depth: 1cm). Samples were frozen at 20^0C for 1 hour and then lyophilized for 24 hours to obtain a porous structure. For Scaffold Preparation, 45mg of lyophilized collagen was dissolved in 15 mL of 0.05 M acetic acid. To the collagen solution, 300mg of itraconazole was added followed by homogenization for 15min to disperse the drug homogeneously throughout the solution. The homogenized foam solution was immediately poured into the 90mm sterile petridish, stored overnight at -70^0C and freeze dried.

3. Results and discussion

A. Standard curve of Itraconazole

Itraconazole is a white fine powder which is practically insoluble in water. Though several methods are reported for its estimation, the UV- spectrophotometric method is employed in the study. Accurately weighed quantity of 10mg of Itraconazole was transferred in to 10 ml of volumetric flask and dissolved and diluted up to the mark with Methanol to give a stock solution having strength 1000 µg/ml. Absorbance of the prepared solutions determined by spectro photo metrically at 255nm. Methanol was used as blank.

B. FTIR (Fourier transform infrared spectroscopy)

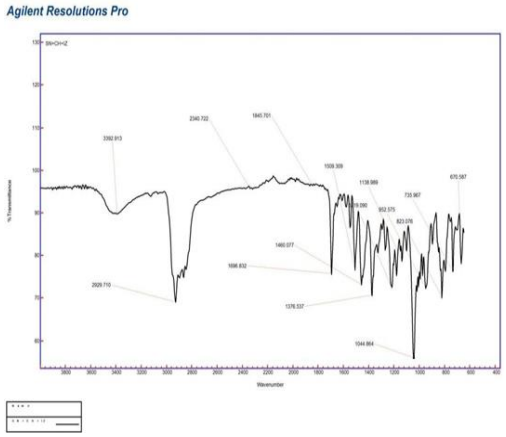
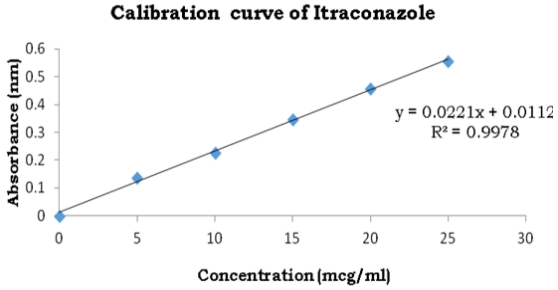


Fig. 1. FTIR spectrum of Itraconazole



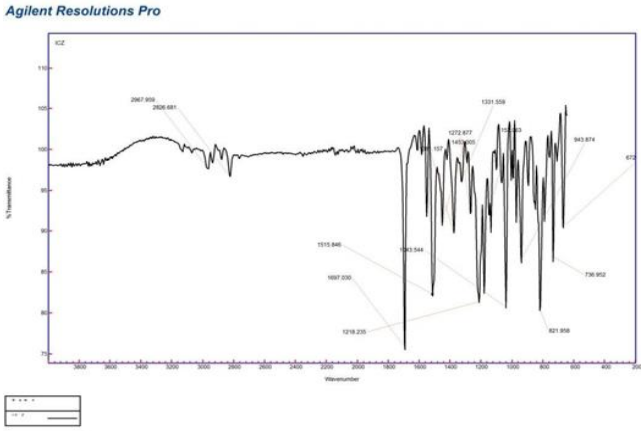


Fig. 2. FTIR Spectrum of physical mixture

Table 1
FTIR data of itraconazole pure drug and physical mixture

S.No.	Functional group	Characteristic peaks	Observed peaks	
			Itraconazole	Physical Mixture
1.	C=C	1800-1600	1453	1696
2.	C=N	1600-1700	1697	-
3.	C=O	1800- 1600	1697	1696
4.	C-H	3500-3000	2967	1460
5.	C-C	1200-800	1521	1509
6.	O-H	3500-3000	-	3392

The FTIR spectra of dry collagen preparation were measured using the FTIR spectrometer. In each measurement, 64 scans were collected with a resolution of 4cm-1and the range of 4000-750cm-1. After measuring all FTIR spectra corresponding to a selected strain and background subtraction, the average spectrum was calculated. The spectrometer was purged with dry nitrogen to diminish the negative influence of watervapour.

C. Melting point

Although melting points do generally increase with increasing molecular weight, the pure collagen was melted at the temperature of about 45^0C and succinyl collagen was observed at about 50^0C. Based on the result, succinyl collagen can withstand the temperature up to 50^0C.

D. SDS-PAGE (Sodium dodecyl sulphate-polyacrylamidegel electrophoresis)

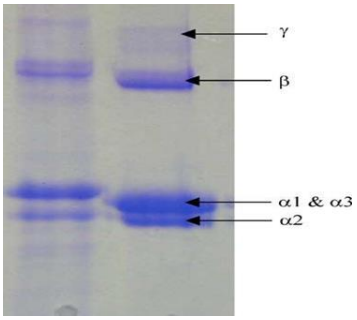


Fig. 3. SDS PAGE of itraconazole collagen scaffold

The electrophoretic pattern of pepsin soluble collagen. Collagen precipitated with 0.7M NaCl includes Type-I as observed the ratio of α1: α2 is more than 2:1 which confirmed the presence of Type-I collagen. The electrophoresis patterns of type 1 collagen are showed in fig. Collagen displayed one β=band and two α-bands (α1, α2) which were the unfolding poly peptide chains of triple helix.

E. Morphology by SEM

The SEM images show that the drug material was physically entrapped in the scaffold layers with the entanglement between the fibrillar networks. With the less pore size formed in the collagen scaffold, drug release from the fibrillar network may be prolonged for sustained delivery. The morphological features of the scaffold with drug load were depicted in the fig. 3.

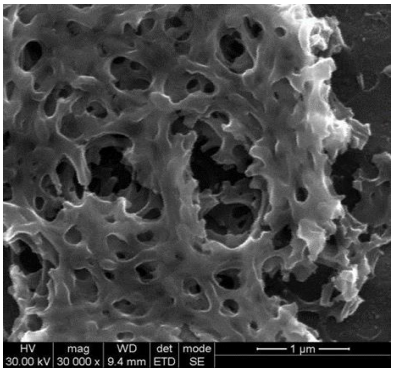


Fig. 4. SEM images of physical entrapment of drug in collagen scaffold

F. Measurement of diameter and thickness of multilayered scaffold

The data obtained for uniformity of diameter and thickness showed in the table and it was revealed that the gel was uniformly layered in the plastic moulds for lyophilization.

Table 2
Thickness data of Itraconazole collagen scaffold

Scaffold Zone(s)	Diameter (mm)	Thickness (mm)
1	15.06	3.14
2	15.18	3.10
3	15.02	3.05
4	15.17	3.10
5	15.14	3.2

G. Water up-take study

The results reveal that the prepared scaffolds were dried uniformly and were having uniform in weight. The scaffolds has very good water absorbing property as it received more than 70% of water in the study stated. This will be more helpful to absorb wound secretions and for drug release.

Table 3
Water up-take study of Itraconazole collagen scaffold

Contour No.	% of water uptake
1	378
2	374
3	380
4	375
5	376

H. Percentage of moisture content

The above results stated in the table indicate that the scaffolds were completely dried. The percentage of moisture content is less than 10% and it is required for scaffold integrity as per the dermal delivery patches standards.

Table 4
% Moisture content of collagen scaffold

Scaffold	Initial Wt. (gm)	Final Wt. (gm)	% moisture content
1	0.833	0.803	4.0
2	0.830	0.794	5.0
3	0.828	0.796	5.0
4	0.829	0.800	4.0
5	0.826	0.791	5.0

I. Folding endurance

The Itraconazole loaded collagen scaffold has sufficient texture and it was stable without any cracks or pinholes formed during folding at many times. The data was given in the table 5.

Table 5
Folding endurance of Plain and drug loaded collagen scaffold

Strip(s)	No. of folding's
Plain Scaffold	250±2.31
Drug Loaded Scaffold	228±1.86

J. In-Vitro drug release studies

Table 6
In-Vitro drug release studies

S. no.	Time (mins)	% Drug Release
1	5	14.325
2	30	20.432
3	60	44.786
4	120	63.574
5	180	76.133
6	240	97.683

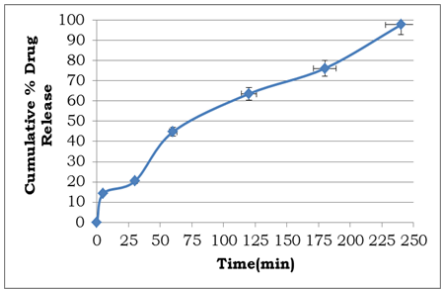


Fig. 5. In-vitro drug release profile of optimized itraconazole collagen scaffold

K. Drug Release Kinetics

Table 7
Pharmacokinetic models of Itraconazole loaded collagen scaffold

Model of kinetics	R	K
Zero order	0.9837	0.1090
T-test	12.22	(Passes)
1st order	0.9904	-0.0012
T-test	16.043	(Passes)
Matrix	0.9722	1.4209
T-test	9.283	(Passes)
Peppas	0.9737	0.5792
T-test	9.563	(Passes)
Hix.Crow.	0.9885	-0.0004
T-test	14.59	(Passes)

By observing the pharmacokinetic models of itraconazole loaded collagen scaffold by *in-vitro* drug release. The maximum R value (0.9904) noted on 1st order so it follows first order pharmacokinetics.

4. Conclusion

The fabrication & evaluation of itraconazole loaded collagen scaffolds were successfully achieved. Physical entrapment of drug in fibrillar network with less pore size and 70% drug water absorbing property, stable without any cracks during folding at 228 times, 97.68% of drug release at 4th hour. The drug release from scaffold followed first order kinetics due to the porous fibrillar drug entanglement. Hence, the itraconazole loaded collagen scaffold could support the rapid wound healing process followed with skin regeneration in damaged site.

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