Formulation Development and evaluation of a Nanofibrous Gel Containing Ketoconazole and Clotrimazole.

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Abstract---

Topical drug administration is a localized method of delivering drugs to specific areas of the body via topical channels such as ophthalmic, rectal, vaginal, and cutaneous. The major route of topical medication delivery is through the skin, which is one of the most easily accessible organs on the human body for topical drug administration. The present investigation involves formulation of topical Nanofibrous gel using Ketoconazole and Clotrimazole. For the treatment of fungal Infection, Topical Ketoconazole and Clotrimazole is used to treat Fungal Infection. Ketoconazole and Clotrimazole belongs to the antifungal family of drugs. It works by reducing fungal infection. Topical Nanofibrous gel of Ketoconazole and Clotrimazole was prepared by using High molecular weight water soluble polymer Hydroxy propyl methyl cellulose such as K35 grade and other excipients including carbopol, methyl paraben, Glycerin and purified water were reported in the formation of Nanofibours gel. In the present investigation combination of Ketoconazole and Clotrimazole Nanofibrous gel.The formulated gel was evaluated for pH, viscosity, Spreadability, extrudability, conductivity, particle size, zeta potential, in vitro drug diffusion studies. Among the formulated gel batch 3 has met all the specifications and was formed to be optimized Efficient delivery of drug to skin application was found to be highly beneficial in localizing the drug to desired site in the skin and reduced side effects associated with conventional treatment.

Keywords -ketoconazole, clotrimazole, fungal , topical

Introduction

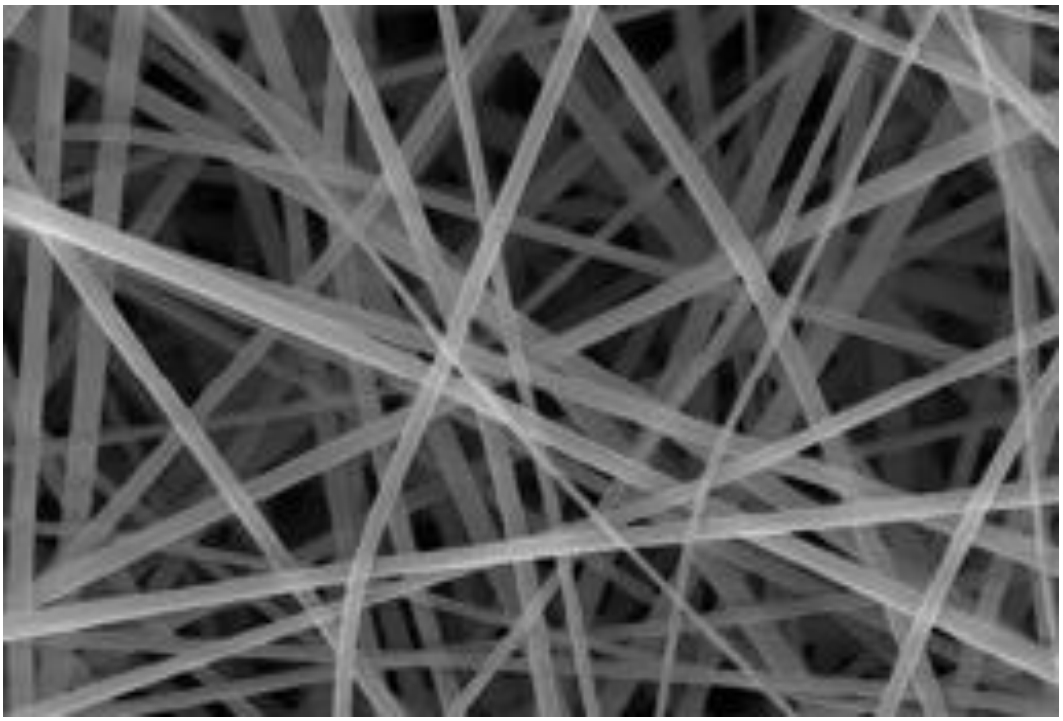
- Nanofibers are **fibers with diameters in the nanometer range** (typically, between 1 nm and 1 μm).
- Nanofibers can be generated from different polymers and hence have different physical properties and application potentials.
- There exist many different methods to make nanofibers, including drawing, electrospinning, self-assembly, template synthesis, and thermal-induced phase separation.
- Electrospinning is the most commonly used method to generate nanofibers because of the straightforward setup, the ability to mass-produce continuous nanofibers from various polymers, and the capability to generate ultrathin fibers with controllable diameters, compositions, and orientations.

Electrospinning method for Both the Solutions

All spinning solutions were prepared at room temperature under stirring for 24 h to ensure their homogeneity. Electrospinning was conducted using a *γ*-High Voltage Research DC power supply generator with a maximum voltage of 50 kV. The electrospinning solutions were fed through the tip of the needle by syringe pump with a stable flow rate (0.5 mL/h). Meanwhile, high voltage (15 kV) was applied to the needle and the nanofibers were concurrently collected on the grounded and steady metal plate which was placed at 15 cm from the needle and covered with Al foil. Temperature and relative humidity were 20±2°C and 60±5%, respectively.

5μM

Sample 1



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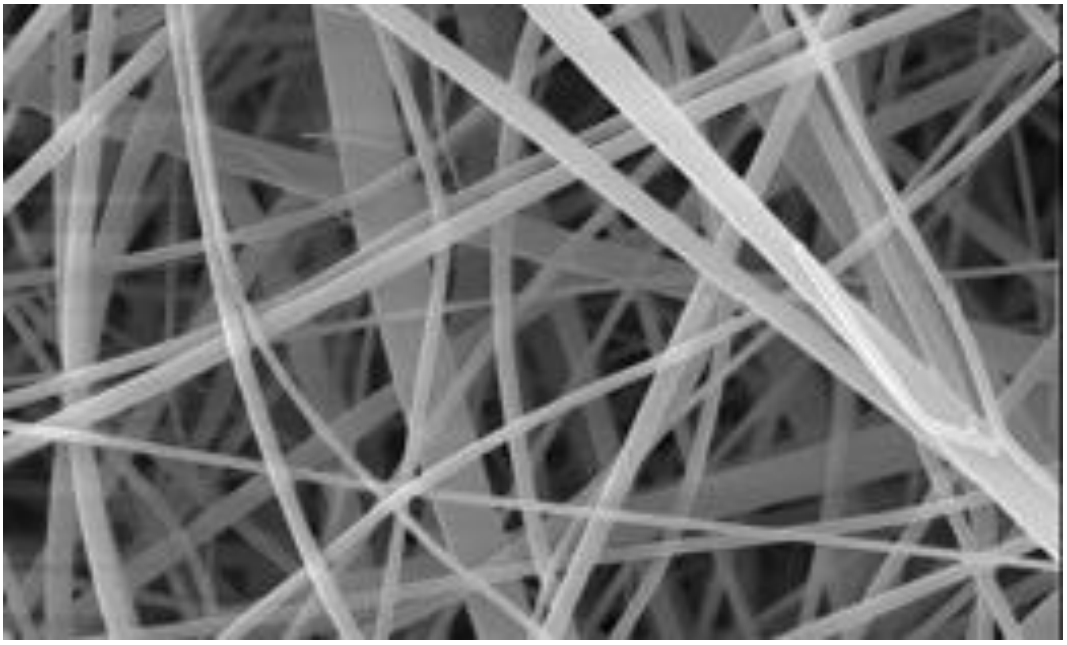


Sample 3

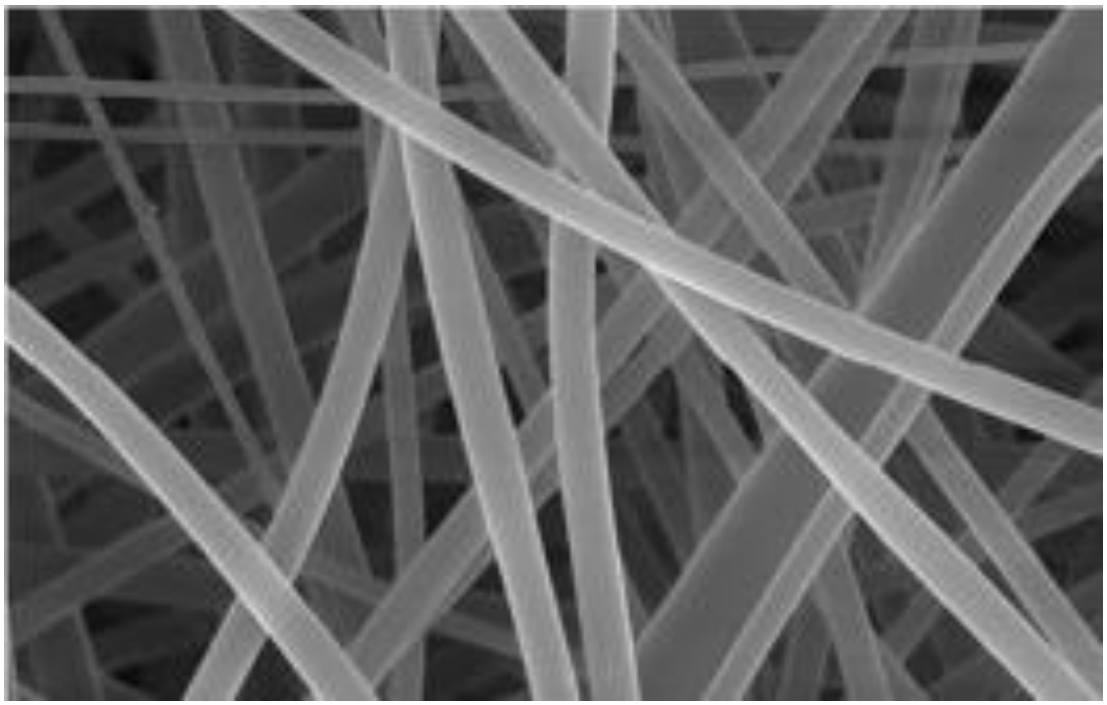


Table 1 . The effect of electrospinning parameters on the size of the nanofibers.

Effect of flow rate		Effect of distance		Effect of Volt	
Flow rate (mL h−1)	**Size (nm)**	**Distance (cm)**	**Size (nm)**	**Voltage (kV)**	**Size (nm)**
0.3	**415±102**	**5**	**458±108**	**10**	**412±115**
0.5	**201±112**	**10**	**315±156**	**20**	**358±123**
0.7	**517±251**	**15**	**158±253**	**30**	**195±147**
0.9	**602±62**	**20**	**641±45**	**50**	**174±125**

ELECTROSPINNING

As illustrated, the basic setup for electrospinning is rather simple, making it accessible to almost every laboratory.[1,2]The major components include a high-voltage power supply, a syringe pump, a spinneret (usually, a hypodermic needle with blunt tip), and a conductive collector. The power supply can be either direct current (DC) or alternating current (AC). During electrospinning, the liquid is extruded from the spinneret to produce a pendant droplet as a result of surface tension. Upon electrification, the electrostatic repulsion among the surface charges that feature the same sign deforms the droplet into a Taylor cone, from which a charged jet is ejected. The jet initially extends in a straight line and then undergoes vigorous whipping motions because of bending instabilities. As the jet is stretched into finer diameters, it solidifies quickly, leading to the deposition of solid fiber(s) on the grounded collector. In general, the electrospinning process can be divided into four consecutive steps: (i) charging of the liquid droplet and formation of Taylor cone or cone-shaped jet; (ii) extension of the charged jet along a straight line; (iii) thinning of the jet in the presence of an electric field and growth of electrical bending instability (also known as whipping instability); and (iv) solidification and collection of the jet as solid fiber(s) on a grounded collector.,[2,3,4.] A more detailed discussion of these four

Materials and Methods

Ketoconazole was purchased from Yarrow chem Products (Pune,India). Clotrimazole was purchased from Yarrow chem Products. Methyl paraben was purchased from Research-lab Fine Chem Industries (Mumbai, India). HPMC K35 was purchased from Ashland Inc. Netherland. All the chemicals were of analytical grade. Polyvinyl Alcohol(PVA) was purchased from Research-lab Fine Chem Industries (Mumbai, India). Poly Lactic co Glycolic Acid(PLGA) was purchased from Research-lab Fine Chem Industries (Mumbai, India).

Methods [5,6]

Identification of pure drug

Identification of pure drug was carried was by Fourier Transform Infra-red Spectrophotometry (Shimadzu 8400s) scanned in the range of 400-200nm.

Drug-excipient compatibility study

Studies of drug-excipient compatibility are important to ascertain drug and excipients are compatible with each other. DSC graph and IR spectra are used to study drug-excipient compatibility.

FTIR study

FTIR (Shimadzu 8400s)spectrophotometer were used in the range of 400-4000 cm-1 using potassium bromide discs (Mixing ratio1:1) The samples were hermetically sealed in aluminium pans and heated at a constant rate of 10°C/ min over a temperature range of 40 to 300°C.

FTIR spectroscopy The FTIR spectrums of pure Ketoconazole as well Clotrimazole and physical mixtures of drugs and polymers were studied separately as per the excipients used in the formulation. It was observed that there were no major shifts in the main peaks of either drug. This indicates that there were no compatibility problems with the drug with the polymers and excipients used in the formulation. Ketoconazole had peaks at 1643 (C=CO stretching), 817 (C-Clstretching), 1512(C=Cstretching), 2841(C-H stretching), and 1041(C-O stretching), while Clotrimazole showed characteristic peak values at 756 (C-Cl stretching); 2940 (C-H stretching); 1535 (C=C stretching) and 1620 (C=N stretching). These peak values were in accordance with previously reported spectra of Clotrimazole(Fig. 2)

Fig.1 FTIR of Ketoconazole

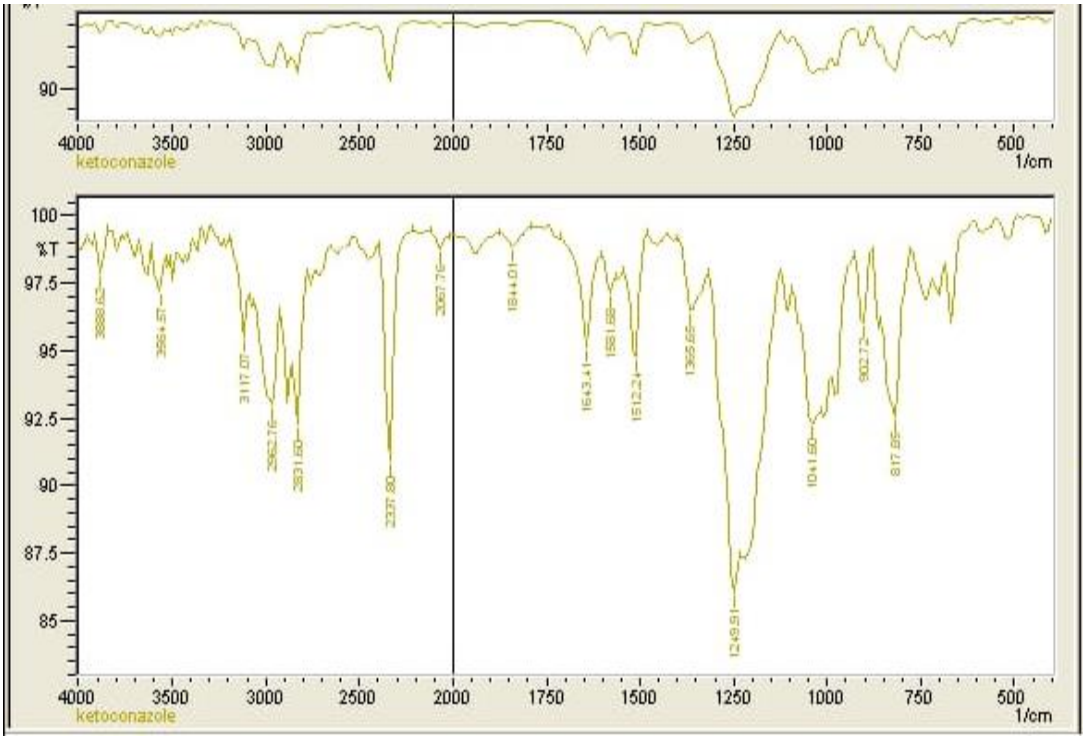
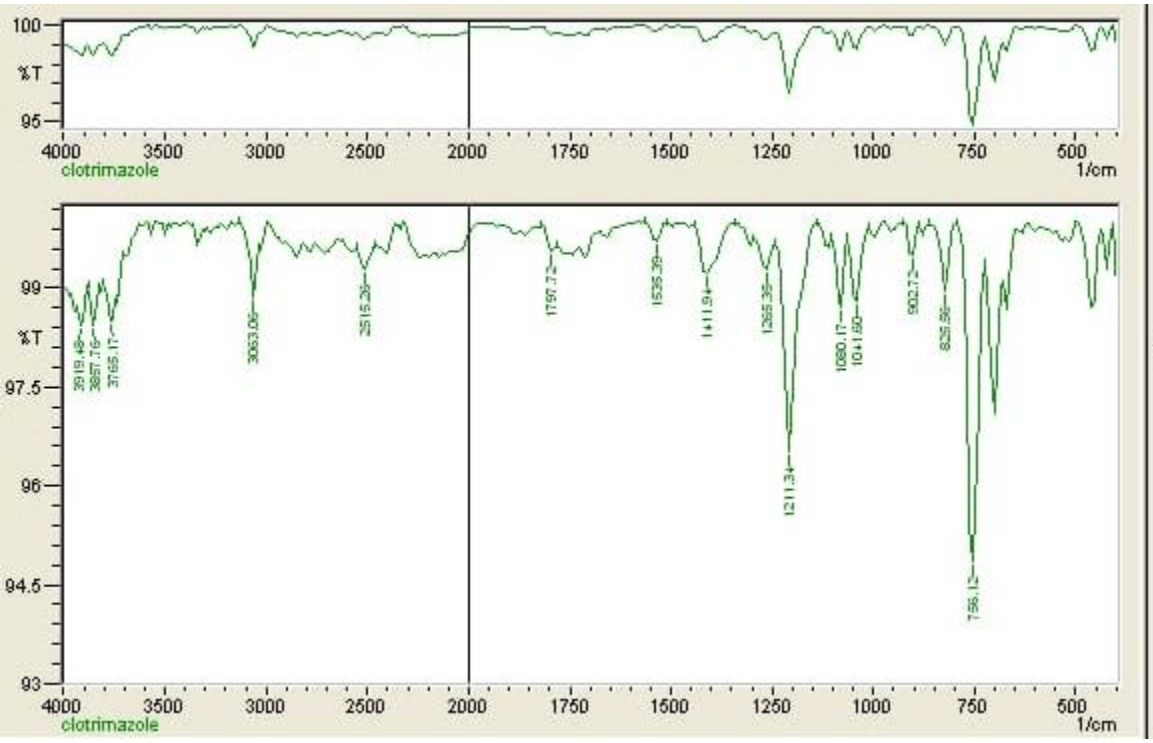


Fig.2 FTIR of Clotrimazole



UV spectroscopy

The linearity of the responses of both drugs was verified at 2–10 µg/ml concentrations. The calibration curve was obtained by plotting the absorbance versus the concentration data and was treated by linear regression analysis. The equation of the linearity curve for Ketoconazole obtained was y = 0.1995x+0.533 The linearity curve was found to be linear in the a for mentioned concentrations (the correlation coefficient (r^2) of determination was 0.9994) (Fig.1). Similarly, the equation of the linearity curve for Clotrimazole obtained was y= 0.132x + 0.0515. The linearity curve was found to be linear for mentioned concentrations. (the correlation coefficient (r^2) of determination was 0.995) (Fig.2)

Fig 3.Calibration Curve of Ketoconazole

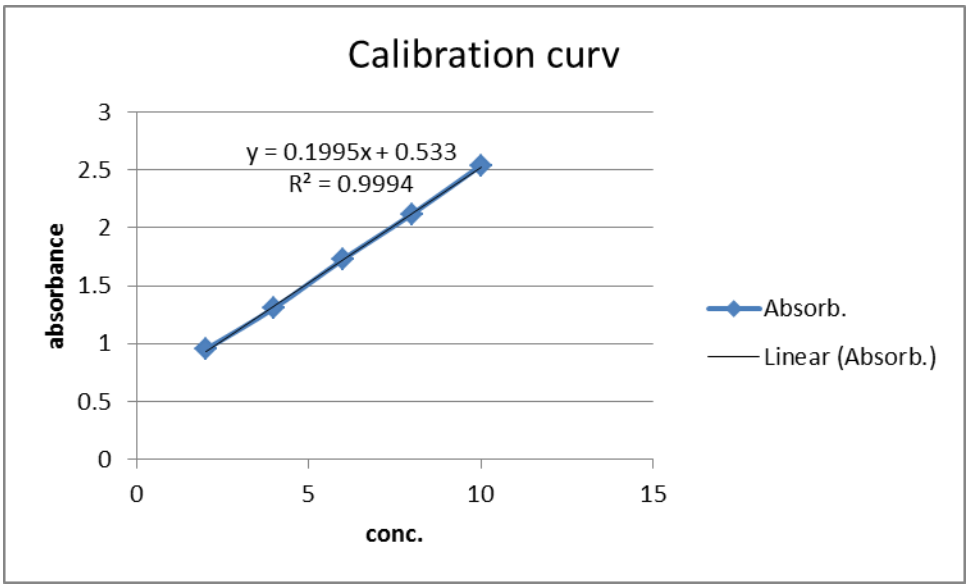
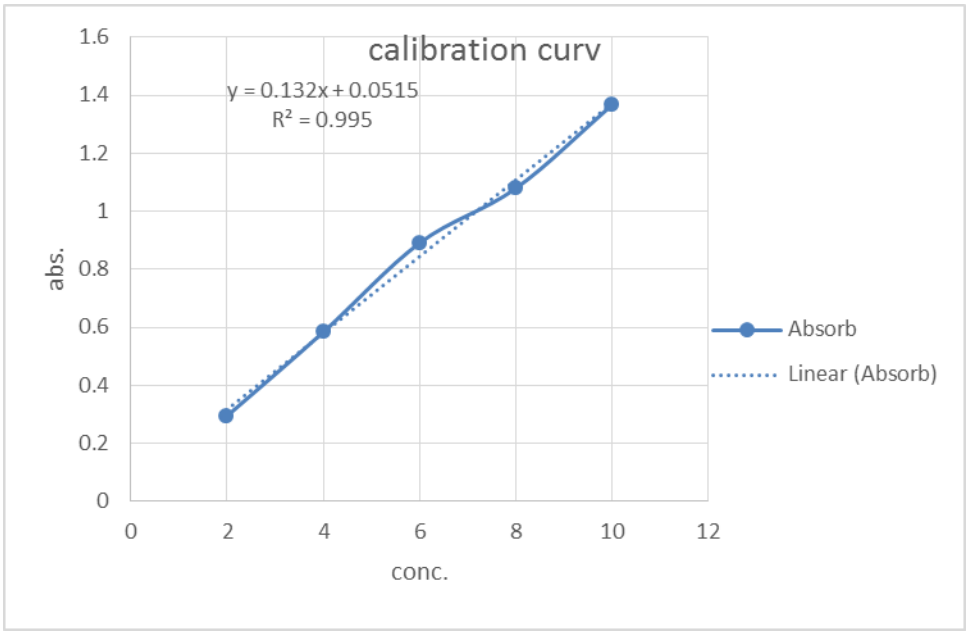


Fig 4.Calibration Curve of Clotrimazole



Preparation of Gel

To obtain O/W gel formulation HPMC K35 polymer was solubilized in purified water with constant trituration. In polymer dispersion Carbopol was added slowly with continuous stirring along with Ketoconazole, Clotrimazole and metyl paraben and was mixed well by continuous trituration. Finally gel was made by adjusting the pH of the mixture to 7.0 and resulted in desired gel consistency good homogeneity and spreadibility.

Table 2- Composition of gel (KC1-KC3)

Ketoconazole	10mg	10mg	10mg
Clotrimazole	10mg	10mg	10mg
Methyl Paraben	0.025	0.025	0.025
HPMC K35	200mg	220mg	240mg
Carbopol	0.025	0.025	0.025
Glycerin	Q.S	Q.S	Q.S
Purified water	Q.S	Q.S	Q.S

Evaluation of Gel

1. Appearance - Patient compliance is aided by the use of colour. The prepared gels were examined visually for clarity, colour, and particle presence.

2. pH [7,8] :- A digital pH metre (Model EQ-610) was used to determine the pH of the gel. In distilled water, 1 gm gel was swirled until a homogeneous suspension was obtained. The volume of the solution was increased to 100 mL, and the pH was determined. The pH of each formulation was done in triplicate and average value are determine.

3. Viscosity[9,10]:- The gel's viscosity was determined using a (LV) Brookfield viscometer. The spindle no. 96 is utilised since the system is nonNewtonian. The viscosity was tested for 2 minutes.

4. Conductivity[11]:- A direct reading digital conductivity meter (Systronics model no. 304) and dipping type conductivity cell.

5. Zeta Potential[12]:- The charge on the surface of particles is characterized by the HORIBA Scientific SZ-100 by measuring the zeta potential of a gel. The sample is injected into a

disposable cell and a measurement of the particle electrophoretic mobility results in the calculated zeta potential.

6. Particle size[13]:- Horiba sz-100 windows [z type] were used to investigate the particle size (PS) of the gel. Particle size and zeta potential were measured in triplicates after dilution with distilled water, and the average values ±SD were recorded.

7. Spreadability[14,15]:- Excess sample was sandwiched between the two glass slides, and a 100 g weight was used to compress the sample to a uniform thickness for 5 minutes. The pan was filled with weight (250 g). The time it took to separate the two slides in seconds was used as a measure of spreadability.

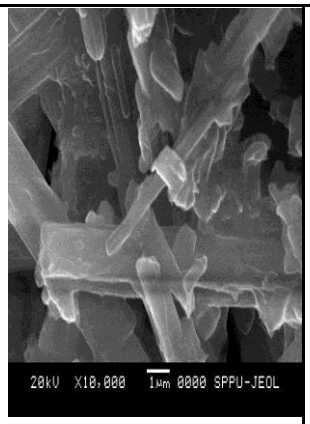
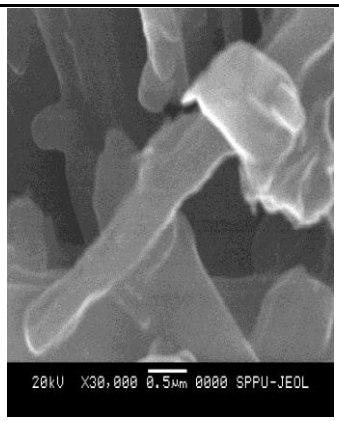
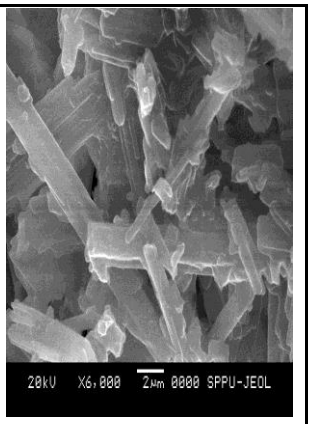
8. Extrudability[16,17]:- Measure the force required to extrude the material from tube. Extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminium collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. +++ excellent ++ very good + average

9. Scanning electron microscopy[18]:- Scanning electron microscopy (SEM) provides high-resolution imaging that may be used to evaluate diverse materials for surface cracks, defects, contaminants, or corrosion. When a focused stream of secondary electrons interacts with atoms in the sample, multiple signals are produced that include information about the surface topography and sample composition using the Nova NanoSEM NPEP, all pictures were scanned at 10000x with a 5 m dimension scale 303.

10. Content uniformity[19]:- Drug content of gel was determine by dissolving accurately weighed 1gm of gel in methanol. After suitable dilution absorbance was recorded by using UV- visible spectrophotometer (UV – 1800 Shimadzu, Japan) at 290 and 225nm. Drug content was determined using slope of standard curve.

11. Diffusion studies[20,21]:- The study was performed using Franz diffusion cells with dialysis membrane with the use of the instrument Jasco V-630 model no V-630. Here the microemulsion based gel equivalent to10mg of Ketoconazole was placed on the donor compartment and the receptor compartment was filled with mixture of phosphate buffer solution (pH 7.4) and 30% methanol, maintained at 37 ±10 °C for in vitro diffusion studies, artificial dialysis membrane was soaked in the same buffer solution for 24hrs before mounting on the diffusion cells. Receptor liquid was withdrawn after each hours and sink condition was maintained by replacing liquid kept at same temperature. Ketoconazole concentration was assayed using UV spectrophotometer. Using the photometric mode for noting absorbance of UV/VIS bandwidth was taken at wavelength of Clotrimazole was taken at wavelength of 263nm and diffusion study of gel was performed.

Particulars	KC	KC	KC
Appearance	Off-white to yellow gel with suspended particles	Off-white to yellow gel with suspended particles	Off-white to yellow gel with suspended particles
Fill volume (gm)	10	10	10
pH	6.8-7.2	6.8-7.2	6.8-7.2
Conductivity **1)200ms 2)20ms 3)2ms 4)200µs 5)20µs**	1)0 2)00.7 3)0.67 4)1 5)1	1)0 2)00.6 3)0.69 4)1 5)1.0	1)0 2)00.1 3)0.12 4)148 5)1
Zeta potential (mV)	-20.7mV	-22.3mV	-23.7mV
Particle size (nm)	333.1nm	339.1nm	244.3nm
Spredability (gm.cm/sec)	3.2±0.0156	4.1±0.0264	3.9±0.057
Extrudibilty	++	+++	++

Scanning electron microscopy			
Uniformity of content(%)1) Ketoconazole	94.6	96.1	98.4
Clotrimazole	93.5	95.4	97.3

In vitro Diffusion studies

Table 3- Data of In-vitro drug release studies of gel of Ketoconazole (%)

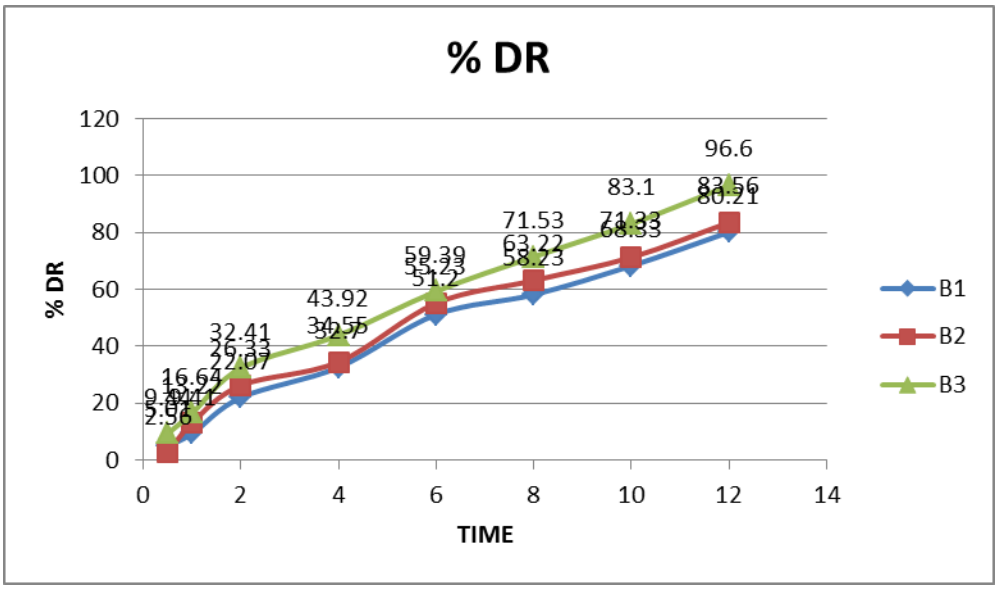
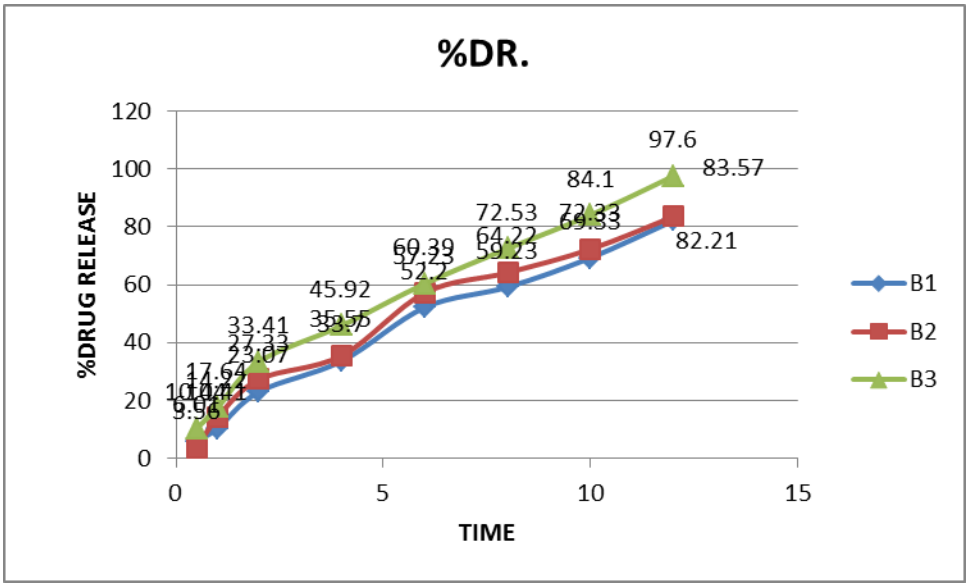


Table 4- Data of In-vitro drug release studies of gel of Clotrimazole(%)



Viscosity results KC1 (Spindle number-96)

RPM	Surface(cP)	Middle(cP)	Bottom(cP)
20	8766	9281	9563
30	6844	7656	7594
50	4331	4931	5006
60	3000	4375	4578

Viscosity results KC2 (Spindle number-96)

RPM	Surface(cP)	Middle(cP)	Bottom(cP)
20	7313	9094	9188
30	5063	6781	7375
50	3938	4294	5139
60	2313	2531	3038

Viscosity results KC3 (Spindle number-96)

RPM	Surface(cP)	Middle(cP)	Bottom(cP)
20	7453	9328	9757
30	7969	7344	7438
50	5044	5100	5456
60	6047	6422	7422

Conclusion

- It can be concluded that from the experimental study carried out that the formulation of a Nanofibrous gel containing Ketoconazole and Clotrimazole drug yields a formulation with spherical and smooth surface, nano in size range. The prepared Nanofibrous was smooth without any lumps, particle and aggregates. So, all the formulations are homogenous. Based on all the factors the nanofibrous gel drug delivery system B3 shows good drug content compared to other. The particle size of the nanofibrous gel formulation is optimum and it is less than 1000 nm. So, it concluded that the particles are in tiny and nano in size range. All nanofibrous gel formulations shows pH in the range of 5.5 to 7. Based on the Spreadability diameter study it shown the nanofibrous gel is having good Spreadability. Nanofibrous gel formulations shown viscosity range from 5000-50000 cps. It concluded that they are stable in nature. Formulation Batch-3 shows highest percentage of drug release compare to other formulations. In-vitro diffusion studies show Batch-3 formulation shows controlled release pattern of drug from the formulation. Zeta potential of batch B-3 shows -22.3mV. High zeta potential values shows there will be no particles coming together and no flocculation. Hence it is conclude that formulation Batch-3 is an optimized batch containing optimum HPMC and Carbopol.

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