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Development, characterization and evaluation of anti-fungal activity of miconazole based nanogel prepared from biodegradable polymer

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Abstract: Topical candidiasis is a known skin fungal infection which is usually treated by conventional dosage forms such as cream, gel, emulgel which are having numerous adverse effects on skin. To overcome such disadvantages, different novel drug delivery systems have been considered. Polymer based nano-particulate systems have shown good skin penetration after topical application. Therefore, in the present study the main focus was on the pathology, pathogenesis, and consequently topical treatment of candidiasis. Nanogel containing miconazole have been prepared from the natural polymers i.e. gelatin and chitosan. The nanogel of miconazole (100 mg) nitrate was formulated by modified emulsification-diffusion technique and characterized for different parameters. From all the seven nanogel formulations named as F1 to F7, F1 (Gelatin and Chitosan in the percentage of 82.85 and 17.15 respectively) have been selected as model formulations. The reason behind that was as per ICH stability guideline, the formulations F1 was found optimum and stable. Miconazole nanogel formulations F1 also showed the maximum release i.e. 78 % approximately. XRD showed the formulated nanogel was in crystalline shape. In summary, the miconazole nanogel drug delivery systems have two main advantages i.e. they are topical preparation as well as nano sized. It can be postulated that nanogel may be a best approach to treat the fungal skin diseases.

Keyword: Gelatin, nanogel, miconazole, anti-fungal, topical.

INTRODUCTION

Fungal infections are common medical issue in Pakistan. These infections include candidiasis, congenital candidiasis, intertrigo, dermatitis and dermatophytosis. Many fungal diseases are treated with either oral or parenteral dosage forms while limited topical formulations are available to treat those (Kang *et al*, 2019). Similarly nanoparticles are now-days being formulated and tested for oral and parenteral administration and a limited work has been done with reference to the topical administration (Ali *et al*, 2014; Badri *et al*., 2014).

Nanoparticle based drug delivery system got a great focus to deliver drugs to desired specific site in the body which is a challenging task (Babaei *et al*., 2008). Thus, to overcome these issues, several strategies for the site specific targeting for these drugs have been reported in the literature. A major benefit of these nano particulate systems is their small size and large surface area which favors the uptake at cellular level (Li *et al*., 1998).

Nanotechnology has an important role in medicine and formulations research particularly in the protection of earlier drug degradation and development of potential targeted and site-specific delivery system with increased absorption into a specific tissues and improvement of intracellular penetration with decreased drug toxicity and enhanced efficiency (Lu *et al*., 2004).

First step is to prepare of gelatin based nanogel by using three different processes namely two step desolation, nano precipitation and inverse miniemulsion (Balthasar *et al*., 2005). The most important characterization need to be performed for nanogel include rheological behavior, surface morphology, particle size, zeta potential, drug loading and release features of the loaded antifungal drugs (Mukherjee *et al*., 2009).

Nanoparticle are highly being studied in drug development process as their size is very small and thus help in efficient targeted drug delivery especially of those drugs which are not effectively delivered by conventional delivery methods(Singh *et al*., 2009). Antifungal drug i.e. miconazole have been loaded in gelatin nanogel for topical/dermal route of delivery for its effective

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penetration which has not been reported previously. Most of the antifungal drugs are available either as oral or parenteral formulations which have serious side effects. The objective of this research work was to prepare nanogel with maximum drug loading for effective skin penetration (Tan *et al*., 2011).

Biodegradable polymer gelatin is a natural polymer and is considered to be more biocompatible and nontoxic in nature (Bartosova *et al*., 2012). Antifungal agents were explored for loading in gelatin based nanogel. Antifungal agents are mostly given by oral or parenteral route while the current project focuses on the topical application to bypass the possible side effects and only target delivery to the infected site.

MATERIALS AND METHODS

Poly (ethylene glycol) (PEG, Mn of 10,000 g/mol), Gelatin, and Chitosan were from Sigma Aldrich source, Methacrylic acid, Dichloromethane, Miconazole, Ethanol were from Merck, USA source. PEG 6000, acetone, acetonitrile were supplied from Musaji Adam and sons. All chemicals and reagents were used as received without further purification unless stated otherwise. *Candidiasis albicans* was obtained as gift from University of Veterinary and Animal Sciences, Lahore.

Preparation of nanogel

The nanogel of the miconazole nitrate was formulated by the method of modified emulsification-diffusion. 100 mg miconazole dissolved in 20 ml ethyl alcohol containing gelatin already dissolved by the continuous stirring. The prepared organic phase dispersion was introduced into 40 ml of aqueous phase. Meth acrylic acid (MAA) was added in the aqueous phase on a high speed homogenizer at 8000 rpm approximately. The organic solution was introduced in the aqueous phase containing MAA. The resultant dispersion was stirred continuously at 10000 rpm for 15 minutes and followed by the sonication of the dispersion for 15-20 minutes. Double distilled water (DDW) was also introduced into the prepared dispersion with continuous stirring for 45 minutes so that organic phase can be easily diffused into the aqueous phase. This was resulted in the formation of the nanodispesion. Gels were formulated from the prepared nanodispersion by the introduction of gelling agent, lecithin. The process was carried out under constant stirring. pH of the prepared nanogel was adjusted to 7 (Wu *et al*., 2010). Seven different formulations were prepared by changing the ratio and percentage of polymers as shown in table 1.

Characterization of nanogel

Rheology

To assess the viscoelastic properties of the miconazole loaded nanogel Brookfield Rheocalc V 32 Rheometer was used. Data analysis of the nanogel was generated by Rheocalc software. Nanogel was studied for shear rate at an increasing order of 0 to 50 D [1/s]. All the readings were taken in triplicate at 25ºC (Wang *et al*., 2009).

HPLC analysis

HPLC system with a reverse phase analytical column C18 was used for the investigation of nanogel. The mobile phase was comprised of acetonitrile as solvent 1 and distilled water as solvent 2 and they were run in ratio of 65:35 to 35:65 solvent 1:2. The flow rate was set 1.2 mL/min. Miconazole in the sample was calculated at the 250 nm (Erk *et al*., 2001).

In vitro drug release

To analyze release pattern of drug from the prepared nanogel *in vitro* drug release studies were performed using Franz diffusion cell with a porous membrane. All seven nanogel formulations from F1 to F7 were applied on the surface of the membrane. Receiver compartment of the Franz diffusion cell was pre filled with the 1% w/v phosphate buffer saline having pH of 7.4, stirred at 350 rpm. Temperature of the Franz diffusion cell was maintained at 37ºC. The samples were drawn after specified time intervals i.e. 0.5,1 , 2, 4, 6, 8, 12 and 24 h. 0.5 ml of sample was drawn after each moment and Is replaced with the same amount of fresh buffer solution. HPLC method was used to analyze the drug sample (Indulekha *et al*., 2016).

Stability studies

ICH guideline was used for stability study. All the seven nanogel samples were filled in glass vials separately and stored at temp 30ºC and 65% RH i.e. ambient conditions and 40ºC & 75% RH i.e. at accelerated conditions for 3 months. After that period the appearance and clarity of the nanogel was analyzed by visualizing the vials (Raemdonck *et al*., 2009).

In vitro antifungal activity

Miconazole loaded nanogel was checked for their anti-fungal action against *Candidiasis albicans* by the method reported previously by (Özcan *et al*., 2009) with a few modifications. The mean inhibition zone (MIZ) was calculated. The value of MIZ was used as an indicator to assess antifungal activity.

Fungal inoculum

Culture of *Candidiasis albicans* was taken and left overnight in the laboratory. After 12 hours it was diluted with 0.9 % saline solution (sterile) to get cultures having 106 colony forming units/mL of the microorganism.

Plate diffusion method

Miconazole loaded nanogel was compared to its placebo sample. Petri dishes were prepared having 20 mL dextrose agar and *Candidiasis* inoculum (1% w/w) was seeded. 200 µL of the *candidiasis* inoculum was

introduced in the petri dishes. The plates were dried at room temperature for 30 minutes after the application of inoculum. 75 mg of miconazole nanogel were placed in each well after cutting the wells. The plates having wells and miconazole nanogel were placed at 36ºC±2ºC for one day. Inhibition zone of the microorganism around the wells was measured and calculated for results. All the measurements were made thrice.

Fourier transforms infra-red spectroscopy (FT-IR)

FT-IR spectra of miconazole raw, gelatin and miconazole loaded nanogel were chronicled using FT-IR spectrometer. Drug, polymer and nanogel were scanned in the range of 4000 cm^{-1} to 400 cm^{-1} with 32 scans per sample (Bhuptani *et al*., 2019).

Scanning electron microscopy (SEM)

The superficial structure and shape of miconazole loaded nanogel was assessed by scanning electron microscopy. Nanogel was powdered and then dispersed this dried powder in water (1 mg/ml) by sonication for 10min and dispersion was casted on a glass slide and dried. Its shape and surface morphology was predicted by scanning electron microscope by applying an acceleration voltage of 10 kV (Kuckling *et al*., 2002).

Average particle size, polydispersity index (PDI)

Malvern Mastersizer 2000 MS was used to evaluate the mean particle size and polydispersity index and size distribution of the miconazole loaded nanogel. The mean particle size and size distribution of the prepared samples were recorded in triplicate (Sanap *et al*., 2013).

X-Ray Diffraction (XRD)

The structural analysis of gelatin, miconazole raw and nanogel was performed by using X-ray diffractometer. A voltage of 40 kV and current of 35 mA was used. The angular range (2θ) covered was between 5° and 60. The relative degree of crystallinity (RDC) was assessed by relating some illustrative peak heights in the diffraction designs of the binary or dualistic systems with a standard (Shah *et al*., 2017).

Differential Scanning Calorimetry (DSC) and Thermo Gravimetric Analysis (TGA)

Nanogel, miconazole and gelatin were analyzed for their thermal stability by thermogravimetric analysis. Nitrogen atmosphere was used for analyzing the material. The flow rate was 20ml/min and scan was completed at rate of 9ºC min^{-1} from the room temperature and raised upto 800ºC. Differential Scanning Calorimetry was done with scanning up to 400°C at a heating range of 10 °C/min (Zhou *et al*., 2017).

In vivo studies

In vivo skin deposition study

Miconazole loaded gelatin based nanogel formulations (2%w/w) were used to carry out the *in vivo* skin deposition study against a blank nanogel. Female rats having weight between 250-300 g were anesthetized with the help of IP of cocktail anesthesia which contained xylazine (10 mg/kg) and ketamine HCL (90 mg/kg). The skin from the dorsal region of the rat was shaved with the help of razor. Razor was run in direction from tail towards head and hairs were visualized to ensure no removal of skin. Then, properly weighed nanogel containing miconazole (equivalent to 2 mg of miconazole) and blank were applied on the skin of rat. The rats were divided into two group having 5 rats in each group and then samples were collected at different time intervals 1 h, 2 h, 6 h, 12 h and 24 h. Then after specified interval of time animals were sacrificed and their skin was excised carefully from dorsal region to check deposition of miconazole in the skin. Skin was prepared to extract the miconazole as have been studied (elnaggar *et al*., 2016) and miconazole was subsequently analyzed.

In vivo antifungal activity of the miconazole loaded nanogel

Antifungal activity of prepared miconazole loaded gelatin based nanogel was performed by the method described in (Babaei *et al*., 2008) with slight modifications.

Preparation of the animals

Female albino mice having weighed 110-140 grams were kept in individual cabins and food along with water was served. Animal paw was cleared from all sorts of hair. Hairs were completely removed with the help of hair removing cream 48 prior to administration of *Candidiasis* strain. (Elnaggar *et al*., 2016)

Preparation of candida and mice infection

Candidiasis albicans strain was taken from the University of Veterinary and animal sciences (UVAS), Lahore and then it was grown in YPD broth which had the composition (peptones 20g/L, dextrose 25g/L and yeast extract 12 g/L). Next day this culture was transferred to freshly prepared YPD broth and shaken continuously for 2 to 3 hours. These steps helped in the conversion of *Candidiasis* into pseudo hyphae which could induce cutaneous infection. *Candidiasis albicans* was pasted on a butter paper and the butter paper was previously pasted on an aluminum foil. It was then wrapped on the right paw of the mice. The left paw served as the reference to check either infection developed or not. It remained wrapped on the paw for 7 days (elnaggar *et al*., 2016).

Treatment of fungal infection

Treatment of the infected paw started after 48 hours of removing the aluminium foil containing *Candidiasis. albicans* strain from paw of animal. Mice were divided into two groups which had 4 mice in each group. The first group of mice was treated with nanogel containing miconazole (2% w/w) and the second group served as a control group. All the animals were sacrificed 24 hours

after the last treatment dose was received. Infected sites of skin were excised and kept in sterile saline. Skin samples were allowed to homogenize to release *Candidiasis. albicans* cells from skin into sterile saline solution. Then it had been placed in diluted saline and plated onto YPD agar. Plates were incubated at 35°C±1°C for 24 h and then CFU values were counted (Elnaggar *et al*., 2016).

STATISTICAL ANALYSIS

Result for rheological studies of nanogel, effect of concentration of gelatin on viscosity and particle size of nanogel were expressed as mean ± standard deviation (SD) by using SPSS version 19.0. During the *in vivo* skin deposition studies results were expressed as mean ± standard error of mean (SEM) by using SPSS version 19.0.

RESULTS

Evaluation of nanogel

Physical characterization of nanogel

The prepared formulations showed a clear nanogel having good consistency, spreadability, transparency and flow property. It was observed that there was a uniform distribution of particles. It was also observed that dispersion was uniform with polymer and drug.

Rheology

All the prepared formulations of miconazole nanogel were investigated for their rheological behaviour. Contact time of the formulations and spreadability of the formulations are closely related to the rheological behavior. The effect of different percentage of gelatin was determined by the viscosity profile of the nanogel as given in the table 2. The viscosities of the nanogel comprising 0.5%, 1.0% and 1.5% w/v gelatin were 0.12, 1.16 and 13.43 cP respectively. Particle size of the miconazole loaded nanogel was ranged from 210 nm to 252nm. This increasing size was due to increased cross linking of the polymeric network of gelatin nanogel. A plot had been plotted between sheer stress and the shear rate of the nanogel and depicted in the fig. 1 which demonstrated the thixotropic behavior of pseudoplastic system.

When the shear rate of the nanogel was increased i.e. upcurve, shear stress was also increased with yield value which indicated its pseudo plastic behavior as shown in fig. 1. Further when the shear rate of the nanogel was decreased (upcurve), shear stress also decreased proportionality, demonstrating thixotropic properties of nanogel.

In vitro drug release

Prepared nanogel was observed for the miconazole release. Miconazole nanogel formulations F1 showed the maximum release i.e. 78 % approximately while the formulations F7 has shown the minimum release i.e. 45% approximately after 24 hours. During *in vitro* drug release studies it was observed that all the formulations followed the 1st order release kinetics. Drug release from all the seven formulations has been shown in fig. 2.

Differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA)

TGA analysis was performed on raw materials and nanogel formulation. Miconazole showed complete decomposition at 350°C and gelatin at 320°C fig. 7a and 7b respecively. However, the decomposition of the crosslinked polymer was observed above 470°C shown in fig. 7c. The DSC thermogram of miconazole-co-gelatin nanogel showed a large endothermic-peak at about 340°C as shown in fig. 7d.

Stability Studies

Miconazole nanogel mean particle sizes were assessed of all prepared seven formulations during the stability as per ICH guideline. Clarity and appearance was not changed at all. It was almost as similar as it was at zero time. The

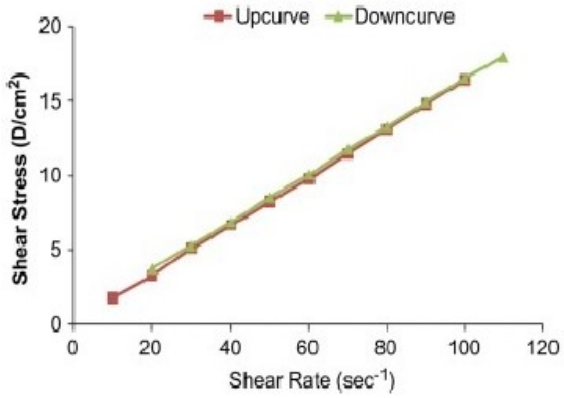

Fig. 1: Rheology of miconazole nanogel showing correlation between shear stress and shear rate.

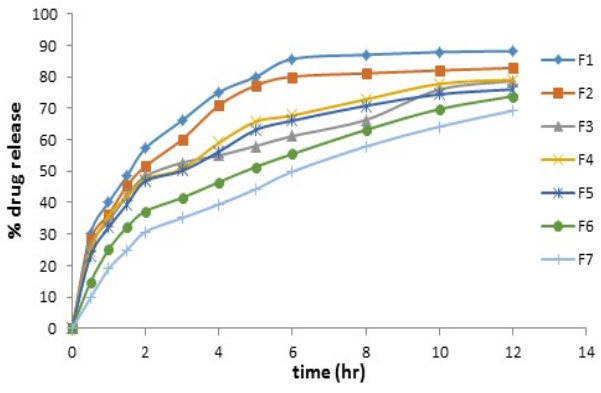

Fig. 2: *In vitro* drug release from all 7 formulations of miconazole loaded nanogel in phosphate buffer pH 7.4.

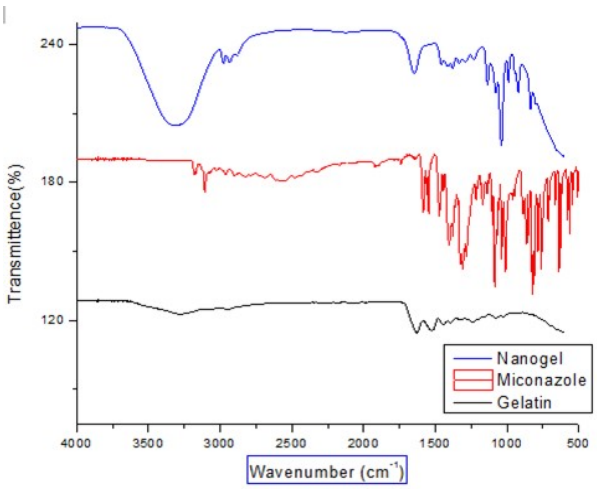

Fig. 3: FTIR Spectra of gelatin, miconazole and nanogel

particle size of the nanogel observed unchanged during the ambient storage period. But particle size of the nanogel was increased from 210 nm to 305, 380 and 520 nm at the end of 1st, 2nd and 3rd months respectively at the accelerated storage conditions. This showed that particle size increased as the storage temperature was increased.

In vitro antifungal activity

Candidiasis albicans have been reported to cause many skin diseases. During research *Candidiasis albicans* had been used as reference standard to assess the *in vitro* antifungal activity of miconazole loaded nanogel. Mean inhibition zone (MIZ) of the plates was calculated by measuring the mean diameter of MIZ after application of miconazole nanogel (2% w/w) in the wells. miconazole loaded nanogel (2% w/w) showed quite high antifungal activity (34.33± 0.79) as compared to placebo where no miconazole was applied (0.41± 0.04). Higher *in vitro* antifungal activity of miconazole loaded nanogel as compared to blank i.e. having no antifungal drug confirmed the enhancement of physicochemical characteristics of miconazole after its incorporation in nanogel system.

Fourier transforms infra-red spectroscopy (FT-IR) of nanogel

FTIR spectrum of pure gelatin displayed a broad peak at 3450 cm^{-1} and 3423cm^{-1} which were due to stretching vibrations of secondary amine (-NH) as shown in fig. 3. Low intensity peaks at 2922 cm^{-1} and 2850 cm^{-1} can be attributed to C-H stretching. Strong peaks at 1680 cm^{-1} and 1640 cm^{-1} were associated to stretching due to carbonyl groups (C=O). Lastly, amine group (-NH) exhibited its bending between 1550 cm^{-1} and 1500 cm^{-1}.

FTIR spectrum of pure miconazole showed characteristic peaks at 3180 cm^{-1} due to Imidazole ring structure (C-N stretching) as shown in fig.. 3. Strong peak at 3107 cm^{-1}

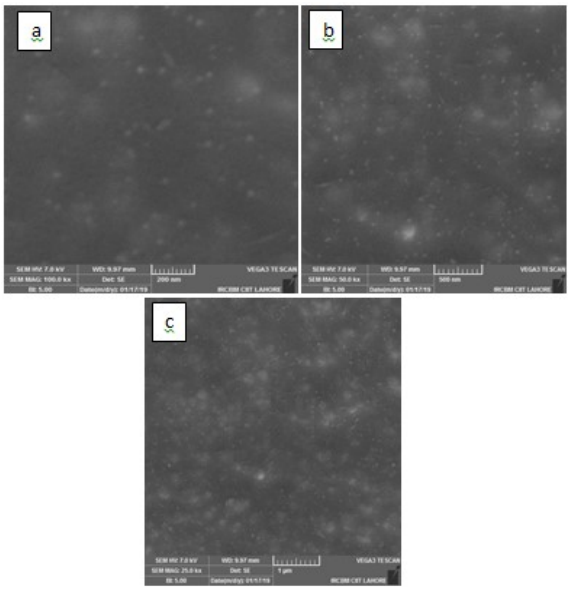

Fig. 4a, b, c: SEM image of Nanogel F1 at 200 nm scale, at 500 nm scale and at 1 µm scale

was presented by stretching of aromatic CH; broad peak at 2960 cm^{-1} was due to stretching vibrations of aliphatic CH2 group and peaks in the range of 1500 to 1600 cm^{-1} can be ascribed to stretching of aromatic C=C. Strong peaks at 1385 cm^{-1} and 1330 cm^{-1} revealed to C-H bending vibrations and C-N stretching vibrations, respectively. Additionally, peak in the range of 1000 to 1100 cm^{-1} corresponds to C-C stretching vibrations. The spectra of polymers and drug loaded nanogel have been shown in fig. 3.

FTIR spectrum of developed Nanogel showed a broad valley in the range of 3600-3000 cm^{-1} which may be due to stretching vibrations of free –OH of gelatin, –NH of Amide groups of gelatin and amidazole group of miconazole. –OH stretching vibrations are overlapped by –NH stretching vibrations forming a broad valley. Characteristics peaks of gelatin and miconazole were present at 2922 cm^{-1} and 2850 cm^{-1} (C-H stretching). Similarly, bands were also observed at 1680 cm^{-1}, 1640 cm^{-1}, 1550 cm^{-1} and 1500 cm^{-1} (stretching due to carbonyl groups and amine group (-NH) bending vibrations); 1385 cm^{-1} (C-H bending vibrations); 1330 (C-N stretching vibrations) and 1100 cm^{-1} (C-C stretching vibrations). These characteristic bands of miconazole confirmed the entrapment of drug in the nanogel structure. Similar findings were observed by (Ali *et al*., 2014) who loaded poly vinyl alcohol hydrogels with venlafaxine and found no interaction between drug and polymeric system (Ali *et al*., 2014).

Scanning electron microscopy (SEM) of nanogel

Surface morphology and shape of the prepared nanogel was observed with the help of scanning electron microscopy. As the SEM images reveal that prepared

Table 1: Composition of different nanogel formulations

Sample No	Gelatin/100 g solution	Gelatin/Chitosan (Wt %)	Polyacrylic acid/100 g of Ge/PVP (Wt. %)	MAA/100 g solution
F1	14.5	82.85/17.15	3.8	2.660
F2	16.5	84.61/15.39	3.8	2.964
F3	19.0	86.36/13.46	3.8	3.344
F4	16.5	82.50/17.50	3.8	3.040
F5	16.5	80.48/19.52	3.8	3.116
F6	16.5	78.57/21.43	3.8	3.192
F7	16.5	84.61/15.39	3.8	2.730

Table 2: Effect of percent of gelatin on viscosity and particle size of nanogel

Gelatin w/v	Viscosity (cP)), 25 ºC ±SD	Particle size (nm) ±SD
0.5 %	0.12 ±0.02	210±14
1.0 %	1.16 ±0.03	226±11
1.5 %	13.43±0.02	252±16

Table 3: *In vivo* skin deposition study representing amount of miconazole deposited in the skin at various time intervals after single topical application of miconazole loaded nanogel

Time (hour)	Formulations (µg deposited)
1 h	69.1±1.8
2 h	61.2±2.7
6 h	50.4±1.7
12 h	24.4±4.6
24 h	19.7±1.4

nanogel was moderately spherical and oval in shape. The SEM images were taken at different resolutions i.e. having scale of 200 nm, 500nm and 1 µm as shown in fig. 4a, 4b and 4c respectively. The surface of the nanogel is smooth somewhat. Particle size range is also in nanometers as depicted by SEM. Some of the prepared nanogel were observed in the form of clusters and mostly uniform dispersion of the miconazole all over the nanogel have been shown in images by SEM. SEM spectra at different scales have been shown in fig. 4a, b & c.

Average particle size, PDI

Nanogel prepared had the size in nm ranges. The size ranges of prepared nanogel as observed was between 207 nm to 2601 nm suggesting that there was different particle size distribution in the formulations as shown in fig. 5, poly dispersibility index had been seen in the result with value 0.337.

X-Ray Diffraction (XRD)

Nanogel diffraction pattern of miconazole was distinctive from the superimposition of each of the component if the nanogel loaded with miconazole formed. X-ray diffractctometry can be useful for investigation of differences between the solid state and gel formed. Crystallinity had been interpreted by comparing the different peak heights in the diffraction pattern of the binary system with the reference. The X-ray diffraction pattern of miconazole, gelatin and nanogel prepared have been shown in fig.. 6 a, 6b and 6c respectively. As seen in X-ray diffractogram of miconazole the several sharp peaks were observed at following diffraction angle (2θ) of 13.0°, 14.8°, 22.4°, 25.4°, 28.5°, 30.5°, 31.5°, and 32.6° suggesting that the drug is present in a crystalline form. A decrease in peak intensity nanogel suggested that the crystallinity of the miconazole had been lost. Probably it was due to presence of gelatin used which was amorphous in nature.

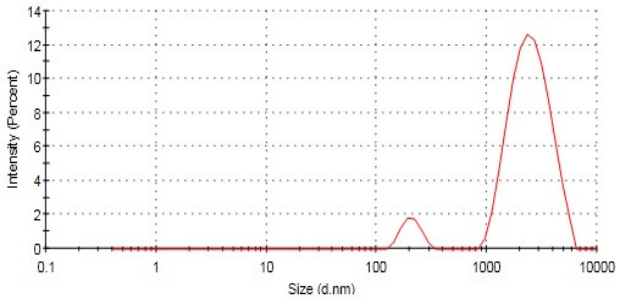

Fig.. 5: Size range of nanogel

***In vivo* Studies**

In vivo skin deposition studies

Miconazole loaded gelatin based nanogel formulations were investigated to determine the amount of miconazole deposited in the skin at different time intervals. It was good for all the skin targeted preparation that they exhibited rapid penetration into skin as well. The result

obtained for the *in vivo* skin deposition of miconazole loaded nanogel (mean µg ± SEM) shown in the table 3.

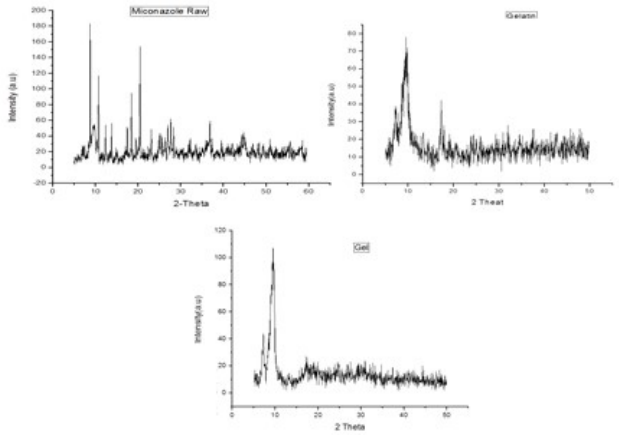

Fig.. 6a: X-ray diffraction Spectra of Miconazole(upper Left), **6b** X-ray diffraction Spectra of Gelatin(upper right) and **6c** X-ray diffraction Spectra of prepared nanogel(bottom)

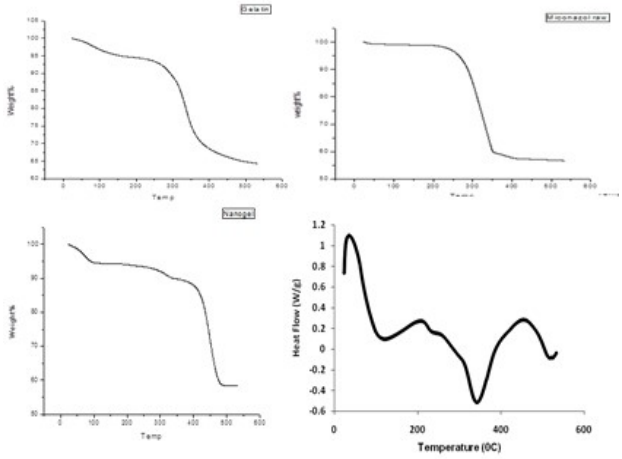

Fig.. 7a: TGA of Gelatin, **7b**: TGA of Miconazole raw, **7c**: TGA of nanogel and 7d: DSC curve of the nanogel

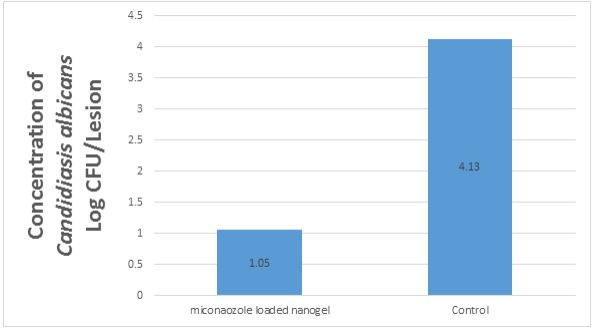

Fig. 8: *In vivo* antifungal efficacy of miconazole loaded nanogel as compared to untreated group (control) after three successive days of single application per day

This table depicted the rapid deposition of miconazole loaded nanogel into the skin i.e. after 1 h, 69.1±1.8 µg of miconazole from the gelatin based nanogel.

In vivo antifungal activity

Gelatin based nanogel containing 2% w/w miconazole was assessed for its anti-fungal activity against *Canddiasis albicans* using female albino mice in comparison to the other controlled group of animals. Fungal infection was induced on the paw of mice and then antifungal activity of miconazole loaded nanogel was assessed through a quantitative analysis of the fungal infection in terms of CFUs found in the lesion of infected paw after the completion of treatment. *In vivo* microbiological activity of miconazole loaded nanogel was compared to negative control receiving no treatment. After the 3 days of treatment, the mice were sacrificed and their skin was removed and homogenized in sterile saline. After homogenization the CFUs of *Candidiasis albicans* were determined (Elnaggar *et al*., 2016). *In vivo* anti-fungal activity depicted that miconazole loaded nanogel showed log CFU of 1.05±0.34 while untreated group showed log CFU 4.13±0.04 as shown in fig. 8.

DISCUSSION

Here we reported a modified emulsification-diffusion method for the preparation of miconazole loaded nanogel made up of biodegradeable polymers. Gelatin in larger quantity was used along with chitosan which had been used in smaller quantities. MAA was added in the aqueous phase on a high speed homogenizer at 8000 rpm approximately. The organic solution was introduced in the aqueous phase containing MAA. The resultant dispersion was stirred continuously at 10000 rpm for 15 minutes and followed by the sonication of the dispersion for 15-20 minutes. This resulted in the formation of nanodispersion which was converted into nanogel by the incorporation of lecithin, a gelling agent (Wu *et al*., 2010).

The prepared formulations showed clear nanogel having good consistency, spreadability, transparency and flow properties.

From the rheological studies it was clearly revealed that as the concentration of the gelatin increased viscosity also increased and vice versa. So viscosity of the nanogel formulations was found to be directly proportional to amount of gelatin present in the formulations as shown in fig. 1.

In vitro antifungal activity was tested by plate diffusion method in which fungal cultures were grown and then miconazole loaded nanogel introduced in the plate. Nanogel retard the growth of fungus. It was calculated by measuring the mean diameter of MIZ (Elnaggar *et al*., 2016).

In vitro drug release studies have depicted that formulations F1 had given maximum release at pH 7.4 in which higher amount of chitosan was incorporated as

compared to other formulations which had lesser chitosan and more amount of gelatin polymer (Indulekha *et al*., 2016).

Stability studies had revealed that the prepared nanogel were stable under normal storage conditions but they formed clusters when the temperature was increased (Indulekha *et al*., 2016).

FTIR spectrum of developed nanogel showed a broad valley in the range of 3600-3000 cm^{-1} which might be due to stretching vibrations of free –OH of gelatin, –NH of Amide groups of gelatin and amidazole group of miconazole. –OH stretching vibrations are overlapped by –NH stretching vibrations forming a broad valley Characteristics peaks of gelatin and miconazole were present at 2922 cm^{-1} and 2850 cm^{-1} (C-H stretching)as shown in fig. 3 (Ali *et al*., 2014).

From SEM it was revealed that some of the prepared nanogel was in the form of clusters and mostly uniform dispersion of the miconazole all over the nanogel had been found (Kuckling *et al*., 2002).

Average particle size ranges of prepared nanogel as observed was 207 nm and poly dispersibility index (PDI) result had value 0.337(Sanap *et al*., 2013).

XRD results depicted that the drug was present in a crystalline form. A decrease in peak intensity nanogel suggested that the crystallinity of the miconazole had been lost. Probably it was due to presence of gelatin which amorphous in nature (Shah *et al*., 2017).

TGA and DSC characterization confirmed the formation of a new co-graft polymer. Crosslinked nanogel revealed high thermal stability. The DSC peaks indicated the water loss from the nanogel followed by decomposition at approximately 340^{o}C. Clearly our method of nanogel fabrication yields thermally stable cross-linked nanogel (Zhou *et al*., 2017).

From the skin deposition studies it was revealed that amount of miconazole gradually decreased regardless the amount of nanogel used at the start of treatment. The rapid deposition of the miconazole into skin could be an influence of nanostructure of the gels and semi-solid to liquid consistency of the nanogel. The rapid drug excretion/elimination from the site of application could be due to the rapid supply of blood into the skin of rats. It has been vital to report that the current research work has focused mainly on subcutaneous targeting of miconazole into the skin and not on its percutaneous permeation (Elnaggar *et al*., 2016).

In vivo anti-fungal activity depicted that miconazole loaded nanogel showed log CFU of 1.05±0.34 while untreated group showed log CFU 4.13±0.04 as depicted in fig. 8. This showed that *in vivo* anti-fungal results were in settlement with the result of *in vivo* deposition studies which confirmed the improvement of physicochemical activities and skin targeting of miconazole after its incorporation into the nanogel. Nanosize of the nanogel also helped in improving the contact and adhesion to the skin (Elnaggar *et al*., 2016).

CONCLUSION

Topical nanogel loaded with miconazole was successfully prepared using modified emulsification-diffusion method. Scanning electron microscopic studies revealed that the prepared nanogel particles were spherical in shape while the DSC studies have revealed that there was no crystalline structure of drug present in the final nanogel formulation. FTIR studies had revealed that both the drug and polymer had been incorporated in the prepared nanogel. Nanogel physicochemical properties revealed that it was effective for topical delivery of the nanogel. *In vitro* and *in vivo* antifungal activity had confirmed the prepared nanogel retarded the growth of *Candidia albicans.*

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REFERENCES

Ali L, Ahmad M, Usman M and Yousuf M (2014). Controlled release of highly water-soluble antidepressant from hybrid copolymer poly vinyl alcohol hydrogels. *Polym. bull.,* **71**(1): 31-46.

Badri W, Eddabra R, Fessi H and Elaissari A (2014). Biodegradable polymer based nanoparticles: Dermal and transdermal drug delivery. *J. Colloid Sci. Biotechnol.,* **3**(2): 141-149.

Babaei Z, Jahanshahi M and Sanati MH (2008). Fabrication and evaluation of gelatin nanoparticles fordelivering of anti-cancer drug. *Int. J. NanoSci. Nanotechnol.,* **4**(1): 23-30.

Balthasar S, Michaelis K, Dinauer N, Briesen Hv, Kreuter J and Langera K (2005). Preparation and characterisation of antibody modified gelatin nanoparticles as drug carrier system for uptake in lymphocytes. *Biomaterials,* **26**(15): 2723-2732.

Bartosova L and Bajgar J (2012). Transdermal drug delivery *in vitro* using diffusion cells. *Curr med. Chem.,* **19**(27): 4671-4677.

Bhuptani RS and Patravale VB (2019). Starch microsponges for enhanced retention and efficacy of topical sunscreen. *Mater. Sci. Eng., C*: 109882.

Cascone MG, Lazzeri L, Carmignani C and Zhu Z (2002). Gelatin nanoparticles produced by a simple W/O emulsion as delivery system for methotrexate. *J. Mat. Sci.: Mat. Med.,* **13**(5): 523-526.

Elnaggar YS, Talaat SM, Bahey-el-Din M and Abdallah OY (2016). Novel lecithin-integrated liquid crystalline nanogels for enhanced cutaneous targeting of terconazole: development, *in vitro* and *in vivo* studies. *Int. J. nanomedicine.,* **11**(1): 5531.

Erk N and Altun ML (2001). Spectrophotometric resolution of metronidazole and miconazole nitrate in ovules using ratio spectra derivative spectrophotometry and RP-LC. *J. Pharm. Biomed. Anal.,* **25**(1): 115-122.

Indulekha S, Arunkumar P, Bahadur D and Srivastava R (2016). Thermoresponsive polymeric gel as an on-demand transdermal drug delivery system for pain management. *Mat. Sci. Eng.,: C* **62**(5): 113-122.

Kang MG, Lee MY, Cha JM, Lee JK, Lee SC, Kim J, Hwang YS and Bae H (2019). Nanogels Derived from Fish Gelatin: Application to Drug Delivery System. *Mar. Drugs.,* **17**(246): 1-11.

Kuckling D, Vo CD and Wohlrab SE (2002). Preparation of nanogels with temperature-responsive core and pH-responsive arms by photo-cross-linking. *Langmuir* **18**(11): 4263-4269.

Li JK, Wang N and Wu XS (1998). Gelatin nanoencapsulation of protein/peptide drugs using an emulsifier-free emulsion method. *J. microencapsul.,* **15**(2): 163-172.

Lu Z, Yeh TK, Tasi M, Au JLS and Wienjes MG (2004). Paclitaxel-loaded gelatin nanoparticles for intravesical bladder cancer therapy. *Clin. Cancer. Res.,* **10**(22): 7677-7684.

Mukherjee S, Ray S andThakur RS (2009). Solid lipid nanoparticles: A modern formulations approach in drug delivery system. *Ind. J. Pharm. Sci.,* **71**(4): 349.

Özcan İ, Abaci O, Uztan AH, Aksu B, Boyacioğlu H, Güneri T and Ozer O (2009). Enhanced topical delivery of terbinafine hydrochloride with chitosan hydrogels. *AAPS PharmSciTech.,* **10**(3): 1024.

Raemdonck K, Demeester J and Smedt SD (2009). Advanced nanogel engineering for drug delivery. *Soft Matter.,* **5**(4): 707-715.

Sanap GS and Mohanta GP (2013). Design and evaluation of miconazole nitrate loaded nanostructured lipid carriers (NLC) for improving the antifungal therapy. *J. App. Pharm Sci.,* **3**(1): 46.

Shah RM, Eldrige DS, Palombo EA and Harding IH (2017). Microwave-assisted microemulsion technique for production of miconazole nitrate-and econazole nitrate-loaded solid lipid nanoparticles. *Eur. J. Pharm. Biopharm.,* **117**(4): 141-150.

Singh R and Lillard Jr JR (2009). Nanoparticle-based targeted drug delivery. *Exp. mol. Pathol.,* **86**(3): 215-223.

Tan Q, Liu W, Guo C and Zhai G (2011). Preparation and evaluation of quercetin-loaded lecithin-chitosan nanoparticles for topical delivery. *Int. J. nanomedicine.,* **6**(2): 1621.

Wang Q, Xu H, Yang X and Yang Y (2009). Rheological study of aqueous dispersions of in situ gelable thermosensitive polymer nanogels. *Polymer Eng. Sci.,* **49**(1): 177-181.

Wu W, Aiello M, Zhou T, Berliner A, Banerjee P and Zhou S (2010). In-situ immobilization of quantum dots in polysaccharide-based nanogels for integration of optical pH-sensing, tumor cell imaging, and drug delivery. *Biomaterials.,* **31**(11): 3023-3031.

Zhou L, Zhang P, Chen Z, Cai S, Jing T, Fan H, Mo F, Zhang J and Lin R (2017). Preparation, characterization and evaluation of amphotericin B-loaded MPEG-PCL-g-PEI micelles for local treatment of oral Candida albicans. *Int. J. Nanomedicine.,* **12**(6): 4269.