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Formulation and evaluation of antifungal agent in a hydrogel containing nanoparticle of low molecular weight chitosan

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ABSTRACT

The research aimed to formulate and prepare hydrogel of low molecular weight chitosan nanoparticles with the help of antifungal agents such as clotrimazole and Nystatin for the topical application. Using the ionic gelation process, placebo and nanoparticles containing drugs were prepared. By cationic interaction is suitable for pH, and gel sodium tripolyphosphate is used as a crosslinker which cross-links the chitosan particles to form the gel, interaction between drug and polymer was observed during the formulation development. Formulated nanoparticle with chitosan and nystatin and clotrimazole showed the mean diameter 273.7nm, 983nm, and 501nm. Clotrimazole and Nystatin had encapsulation capacity was about 74.6% and 63.0%, respectively. *In vitro* drug release at pH 4.7 chitosan molecules are examined for the version of clotrimazole and nystatin.it has noticed that both the formulation shows sustained drug release about 12 hours. Using SEM, nanoparticles are directed for surface morphology character; it shows that nanoparticles are smooth and spherical in structure. Chitosan itself has the antimicrobial property; it was carried out for the antifungal activity against the fungus *Candida Albicans* along with the prepared formulations drugs for the comparative study. This inhibitory effect was due to the parameter such as zeta potential and particle size of chitosan nanoparticles. Hence CTZ and Chitosan were formulated and applied as a natural antifungal agent into nanoparticle to increase the fungal activity. According to the ICH Q1A (R2) guidelines, stability studies are conducted to prove no degradation of the drug. The prepared formulation of chitosan nanoparticles based hydrogel incorporated with clotrimazole and nystatin are promising and significant properties for the efficient treatment of topical fungal infections.

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INTRODUCTION

In recent years there has been very popular interest in the change in the application of medical and health fields. Chitosan of nanoparticle containing hydrogel is very useful drug delivery system because of the non-toxic and biodegradable properties it has many advantages hence it is a material of choice for the preparation of nanoparticles, chitosan is easily soluble in acidic solution also its polymeric chain having free ions will protonate and donate to its positive charges (Ing, 2012; Bachhav and Patravale, 2009).

The formation of chitosan nanoparticles is based on the spontaneous addition of Sodium tripolyphosphate(STPP) polymer, which is polyanionic by nature with a vigorous stirring process in the chitosan solution. Chitosan nanoparticles are utilized and cultivated in the pharmaceutical drug delivery system and gene molecular therapy (Rampino, 2013). The presence of Chitosan shows the least solubility above the pH range of 6.5; hence, many different derivatives of chitosan having increased solubility in water by incorporating various techniques (Elsabee *et al.*, 2009).

Chitosan polymer under free condition has been reported and proved to have maximum antifungal activity against different fungus which includes Aspergillus Niger, Candida albicans, Rhizopus oryzae and Phomopsis asparagi (Abd-Elsalam *et al.*, 2019). According to this information it could finalize that depending on the molecular weight, degree of substitution, various concentration, different types of fungus and many functional groups present in the chitosan molecule will affect the antifungal nature, Chitosan nanoparticle (Ramos *et al.*, 2003) having, polycationic nature of chitosan enhances the antifungal activity therefore without using any chemical changes or modification in the chemical process chitosan exhibit the natural antifungal activity (Croisier and Jérôme, 2013).

Based on the inhibition rate of chitosan, mainly, three mechanisms are advanced. Firstly chitosan Permeabilization of the fungal plasma membrane and its uptake into fungal cells was indicated as energy-dependent. Secondly, the positive charge present on the chitosan molecule permits the connection of fungi membrane containing negatively charged phospholipid. This results in which there will be an increase in the membrane permeability and contents present in the cellular contents that will cause the leakage, which ultimately leads to the death of the cell (Royo and Mate, 2009). In the same way, the second mechanism shows that by connecting with trace elements causing the unavailability of essential nutrients for the growth of fungus chitosan acts as a chelating agent (Corbo *et al.*, 2009). The third mechanism involving chitosan can perforate the cell wall of the fungi and bind to the DNA, due to this prohibition of the synthesis of mRNA hence changes in the formation of proteins and enzymes (Xing *et al.*, 2016).

In pharmaceutical fields, researchers have gained more importance on the antifungal activity in this present work, criteria of study are to explore the antifungal activity of chitosan nanoparticles. Chitosan concentration at which size and the surface charge was determined by using *Candida Albicans.* The aim is to explore the antifungal activity of solution containing chitosan nanoparticle adding on the Minimum inhibitory concentration of loaded chitosan nanoparticles was assessed in antifungal activity (Pillai *et al.*, 2009).

MATERIALS AND METHODS

Chitosan with low molecular weight ($C_8H_{15}NO_6$) (which should be powder by nature having 80-85% of deacetylation and 80% of deacetylated high molecular weight chitosan) were purchased from the Sigma Aldrich (Germany). Try polyphosphate is purchased from the Heppe Medical chitosan GmbH (Germany). The phosphate buffer used in the preparations is From the R&M chemicals. All excipients used were laboratory grade.

Methods

Formulation of hydrogel for the topical application

Chitosan solution

1.3%w/v concentration of the solution is prepared with dissolving low molecular weight chitosan containing 3%v/v acetic acid to make sure about antifungal activity would not be altered with the acidic medium so that by adding the sodium hydroxide solution pH is modified to 5.9 and by adding 0.07g TPP was prepared by adding 0.07gm in 6ml water (Oluwaseun, 2018).

Preparation of chitosan nanoparticles

In untrapped water at a concentration of 2% w/v of TPP was dissolved, at stirring 700rpm. Cross-linking of chitosan with TPP to the ratio of 6:1 has carried out to the room temperature. At a flow rate of 5.0ml/min. TPP was added to the chitosan in a dropwise price from this; a multi-colored solution was obtained. This process is known as the ionic gelation method Figure 1. This confirms how nanoparticles are synthesized. Visually indicated, the stirring of the solution is continued for 20 more min and sonicated in pulse probe for 10 min at 90% amplitude (Clogston and Patri, 2011). The obtained chitosan solution was further centrifuged at 12000rpm for 10 min to obtain hydrogel-based nanoparticle in the form of placebo suspension. The supernatant liquid was removed, and the remaining precipitate was redissolved in water by continuous stirring. The obtained solution was later sonicated for 5 min and kept for the centrifugation process the obtained nanoparticles are collected as Hydrogel. Loading of clotrimazole and nystatin were dissolved to obtained chitosan solution at different concentrations. TPP stirred at room temperature acted

as a crosslinking agent.

Characterization of Nanoparticles

Fourier transformed infrared spectroscopy

The Prepared nanoparticles were evaluated for the FT-IR process In which the sample powder was added and dispersed in the KBr powder. The pellets were prepared by applying the pressure of 6000 kg/cm^2, the FTIR spectra were obtained from the diffusion of dust reflectance on the spectrophotometer 8400S, and Shimadzu. The FTIR spectra of the formulation prepared with the clotrimazole and nystatin were compared to the FTIR spectrum of clotrimazole and nystatin the resulting disappearance of drugs peaks or shifting of peaks in different spectra was observed (Bhosale *et al.*, 2016).

Differential scanning calorimetry

Differential scanning calorimetry was carried out by mixing pure drugs with polymer using the Shimadzu spectroscopy, DSC 60 apparatus; by making empty cells as a reference, calorimetric measurements are observed, with the help of high purity indium metal as standard instruments were calibrated. With the heating rate of 10c min^{-1}using nitrogen atmosphere, the dynamic scanning was observed the temperature is set to be 180-195c (Zhang and Feng, 2006).

Particle size analysis and zeta potential measurements

The obtained nanoparticle size was subjected for the average particle size distribution with the help of particle size analyzer (nano track wave 11 Q,) further experiments were carried out using clear disposal zeta cell, and water is used as dispersant having refractive index 1.32 and viscosity in the range of CP-0.73 temperature is kept as constant at 25c (Kumar *et al.*, 2002).

Determination of surface morphology by SEM

Scanning electron microscope(Zeiss EVO LS 15, Smart 5.05, Germany) using two-sided adhesive tape the sample is placed on the sample holder of the instrument, and it is further coated with the gold of 160 A for 2 min using sputter coater in a vacuum of 3*10^{-1}atm of argon gas (Ning *et al.*, 2005).

Efficiency of Encapsulation

Nanoparticle dispersion prepared are centrifuged at 5,000 rpm for 30min to observe the drug entrapped in the nanoparticle powder, untrapped drug present in the supernatant liquid is to be eliminated. The formation of nanoparticle sedimentation is observed further washed with the water to remove any untrapped particles. Spectrophotometric analysis was performed at 256nm (clotrimazole) and 290nm (nystatin) (Tavman, 2009).

Encapsulation Effiency =

$$\frac{Total\ drug - Untrapped\ drug}{Total\ drug}$$

Loading of drug content

The exact amount of weighed nanoparticles about 50mg was dispersed in a little amount of methanol and subjected for the sonication about 20min to remove the extracted drug completely. about 2ml was diluted using a phosphate buffer solution and analyzed UV-spectra at 265nm (clotrimazole) and 290nm(nystatin) solution to observed drug content (Shivhare *et al.*, 2009).

Drug loading content has to be calculated with below formula

Drug loading =

$$\frac{wt.of\ drug\ incorporated\ in\ NPs * 100}{Total\ weight\ of\ nanoparticles}$$

Characterization of gel for viscosity and determination of spreadability

Prepared gel was observed for various factors such as colour, consistency, spreadability. With the help of Brookfield viscometer model DV-111 having spindle no. 40 viscosity of the gel incorporated nanoparticles was checked out at a temperature range 25c.in a beaker sample was subjected for the equilibration for 5 min before reading the spindle no40 at 0.5,1,2.5rpm during every cycle each dial reading has been noted, gradually spindle speed has to low down and note the difference this is done for triple times, direct dial readings with factor in viscometer gives the viscosity in centipoises (Bachhav and Patravale, 2009).

With respect to the spreadability, about 1gm of a hydrogel is sandwiched within the two glass plates; to remove the air, 1kg of the weight was to keep on the glass plate. The top plate was subjected to a pull of 100g with string. And the time is noted to cover the 10cm from the top plate then the spreadability was calculated, which is expressed in gms.cm sec (Mei *et al.*, 2011).

$$S = \frac{m*}{T}$$

where, S= spreadability (gm.cm.sec^{-1})

m=mass of the weight applied (gm.)

T=time taken (sec)

Measurement of pH

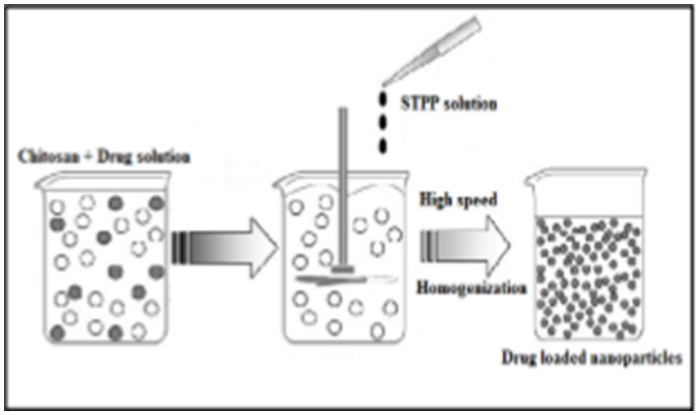

Figure 1: Schematic representationshows the ionic gelation method

Using pH meter, the pH of the 10 %(w/w) gel was determined, which is previously calibrated with 5.5 and 7.0 standard buffers. The pH of the was observed with the direct immersion of the electrode of the ph meter in the system at 25+2c (Poly, 1992).

In vitro antifungal activity

In vitro fungal activity of formulated hydrogel was tested by using the fungal species such as *Candida Albicans* involving the agar diffusion method by the cup-plate method. *Candida albicans* (MTCC183) subculture was procured from the microbial culture collection, and in sabouraud dextrose media the strains were inoculated and incubated for 35c for 24h. using the culture 0.9%NACL to $5*10^{15}$CFU/ml. Sterile Petri plates having chloramphenicol and seeded with inoculums (0.2ml) were added aseptically, keep it for solidify about 4mm a negative control such as placebo gel, nystatin gel, and clotrimazole were added to the cups and these plates incubated at 37°C and the zone of inhibition was calculated with the help of antibiotic zone. DMSO was used as a negative control. Activity set-up was established in aseptic condition (Tonglairoum *et al.*, 2017).

In-vitro drug release

Franz diffusion cell was used to perform *an In-vitro* diffusion study. The receptor compartment was filled with 20ml of phosphate buffer pH 5.5 and a magnetic stirrer rotating at 25 RPM. Dialysis membrane with pore size 0.65μm was clamped between the receptor and donor compartment and was in contact with the receptor compartment media. A sample of 1gm was directly weighed onto the membrane in the donor compartment. The entire system was maintained at 37$\pm$ 1°C by temperature regulating the water jacket. The test was carried out for 12 hrs. 3ml of the sample was withdrawn at specific time intervals from the receiver compartment and was replenished with fresh PBS to maintain sink condition. The samples were analyzed using a UV-visible spectrophotometer at 289, 200, and 286nm, respectively (Dyer, 2002).

Stability studies

Stability studies were performed for all formulations according to ICH Q1A R2 guidelines by storing at 25°C $\pm$ 2°C/60% RH $\pm$ 5% RH and the accelerated stability at 40°C $\pm$ 2°C/75% RH $\pm$ 5% RH 3 months. The formulations were also tested for stability in the refrigerator at 5°C $\pm$ 3°C for 3months to determine the stability of the prepared formulations. The formulations were kept in test tubes and covered with cotton and wrapped with aluminum foil. Samples were tested periodically on 0th, 15^{th} 30th, 60th, 90th day and checked for cracking, pH, appearance, homogeneity, and viscosity (Liu and Yao, 2002).

RESULTS AND DISCUSSION

Nanoparticle Characterization

FT-IR analysis

A mixture of drugs, polymer, and pure drugs was exposed to FT-IR analysis for studying the compatibility and to make sure if there was any interaction within the drug and polymer being used (Brabander *et al.*, 2002). The clotrimazole spectrum shows all characteristic peaks every peak related to basic functional group of CTZ were found to be matched with the spectrum of peaks and CHT physical mixture Figure 7.

The degree of deacetylation of chitosan is 85%, and the absorption bond of an amino group of 2- amino glucose unit and the carbonyl group of 2-amino glucose unit is found to be 1589 and 1656 cm^{-1} respectively. Because of systemic stretching of carboxylate anion, weak bonds are present at 1415$cm^{-1}$and bands such as 1230 and 1160cm^{-1}assigned to the C-O stretching.

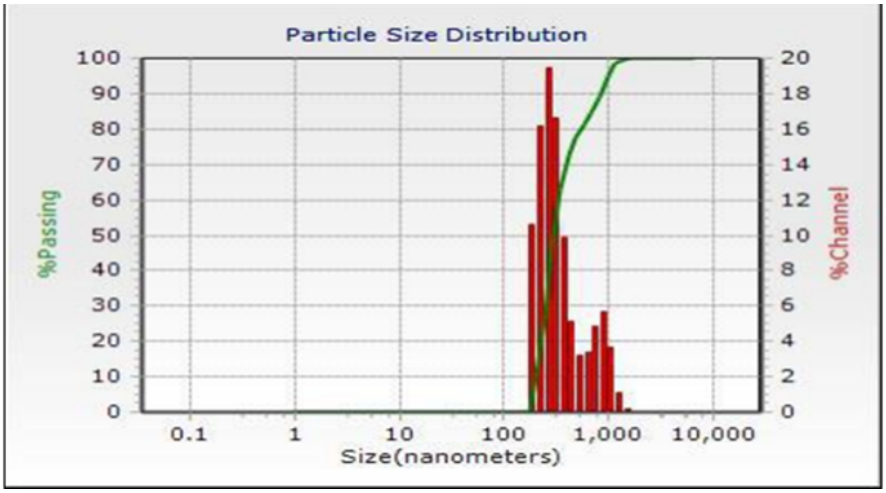

Figure 2: particle size distribution of CHT nanoparticles(placebo)

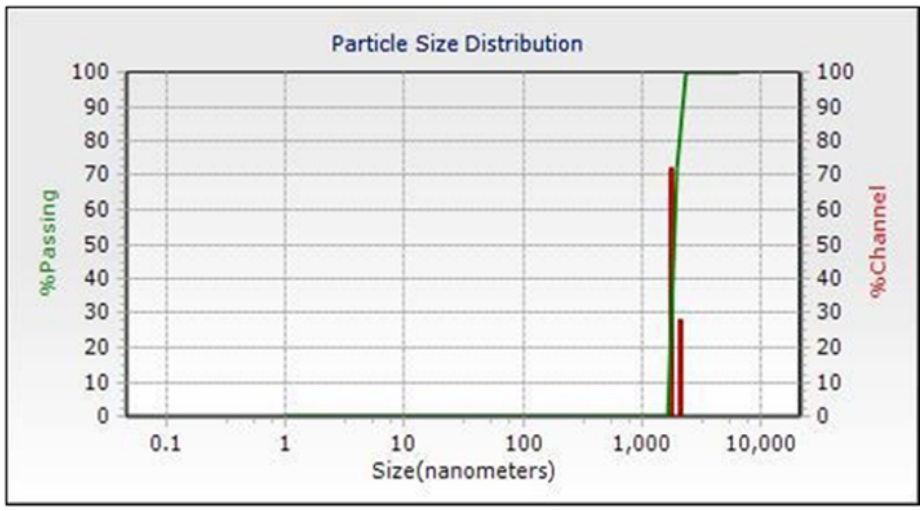

Figure 3: Particle size distribution of Clotrimazoleloaded Nanoparticles

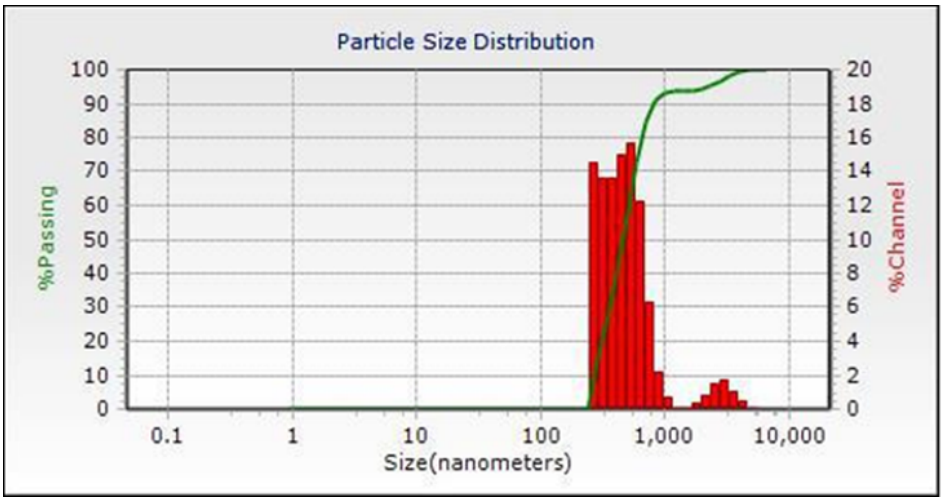

Figure 4: Particle size distribution of Nystatin loadedNanoparticles (NYS)

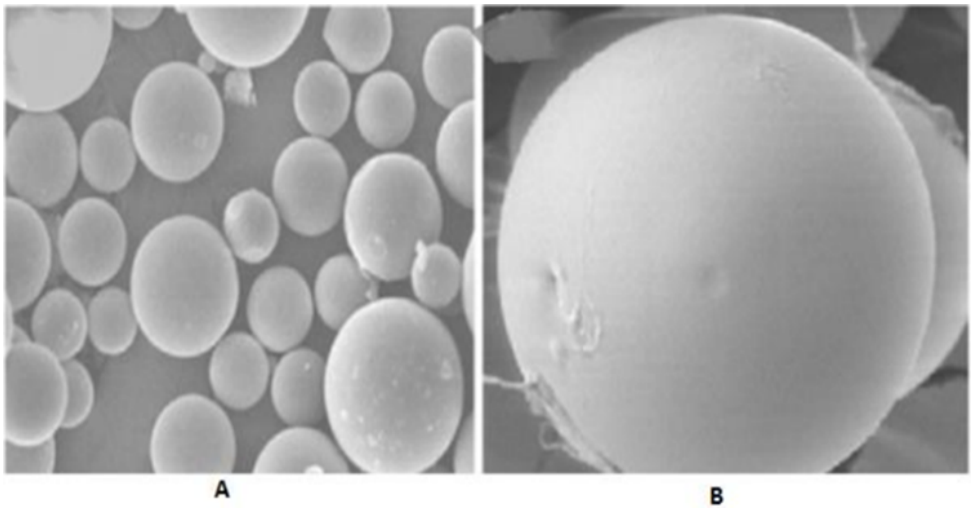

Figure 5: SEM images of Nanoparticles containingclotrimazole (a) 1μm and (b) 0.1μm

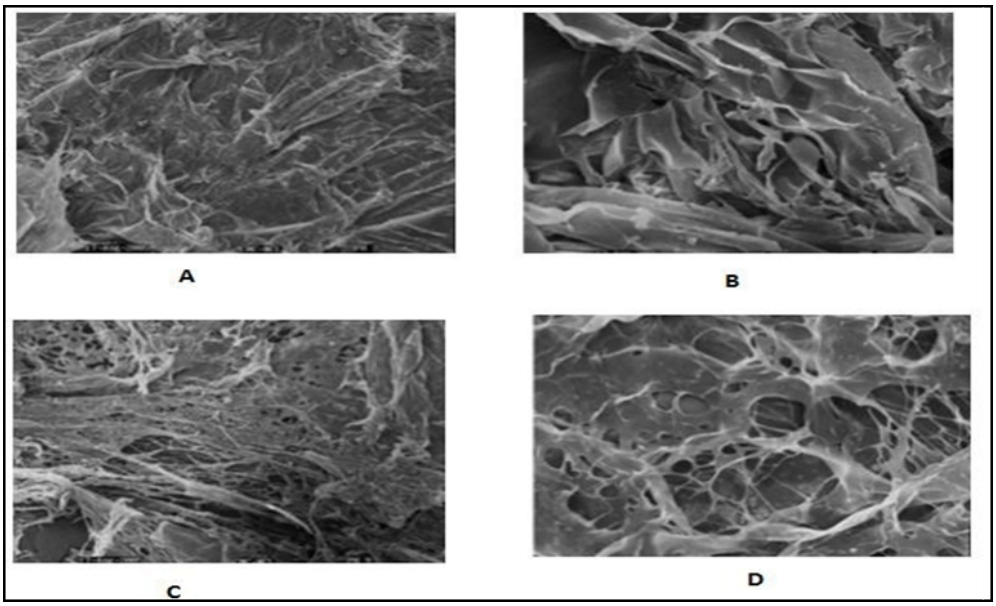

Figure 6: SEMimages of chitosan cross-linked with TPP of CTZ and NYS

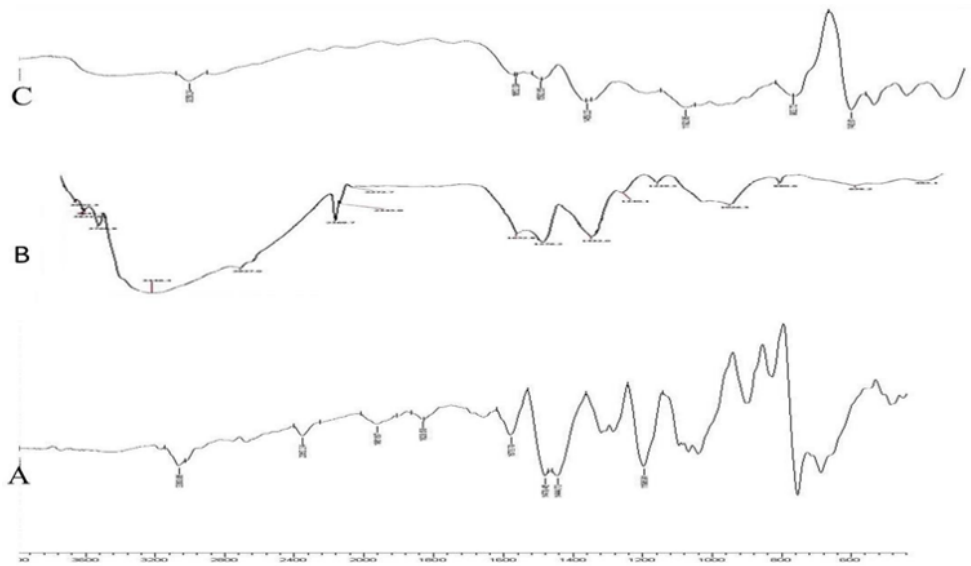

Figure 7: Overlain FT-IR spectraof (A) CTZ, (B) CHT, and (C) physical mixture

The FT-IR absorbance bands in the pure nystatin shows broad intense absorption bands with 2922.42cm^{-1}maxima due to the stretching vibration of bonded hydrogen, at 2927cm^{-1}because of asymmetric and symmetric stretching vibration of CH_2 group, and 1741cm^{-1}due to the stretching vibration of carbonyl from ester and carboxylic acids, 1574cm^{-1}due to the bonding vibration (C-H) and at 2922.42cm^{-1}because of hydroxyl groups from nystatin.

Hence it was found that there was no disappearance shift in the drug peak position in any spectra of drug mixture, which gives that polymer and drug used for the study were compatible (Damian, 2000).

Differential scanning calorimetry (DSC)

Clotrimazole exhibited a single peak at 146.9°C related to its melting point, and polymer at the peak of 147.9°C. And one broad endothermic peak about 90°C connected with the evaporation of bound was also identified in the physical mixture. In the same manner, NYS displayed a single peak at 105.5°C, which was also found in the mixture at 105.7°C Figure 9. As correlation with endothermic peaks of drug and also polymer were optimally employed in their physical mixture. Interaction of drug and polymer at the binding site seen in endothermic peaks Figure 8. DSC Thermogram depicted there was no interaction between the drug and the polymer (Stefanescu *et al.*, 2009).

Particle size before Centrifugation

The average size of the particle obtained from chitosan and TPP loaded nanoparticles showed a marked increase in the presence of chitosan and different concentration with increased molecular weight. TPP produced the nanoparticles as across linker, which produced particle size less than 1 for 1mg/ml (Gan and Wang, 2007). Nanoparticles of all types exhibited a narrow size distribution, and the PDI values were found to be low in the range of 0.10 to 0.60. However, formulations with a concentration of 2, 3 mg/ml of HMW chitosan were found to be an exception. Apart from this, the chitosan nanoparticles were proven to be correlated with the chitosan molecular weight statistically in terms of the particle size and also that on using an increased molecular weight, there was a rise in chitosan nanoparticle size (Harde *et al.*, 2015).

Low molecular weight chitosan resulted in higher zeta potential values, as shown in Figure 2. It can also be interpreted that the particle size of the placebo NPS was 273.7nm, whereas particle size of NPs loaded with clotrimazole and nystatin were found to be 1847 & 501 as shown inTable 1 .

It is noted that an increase in the particle size was obtained in clotrimazole optimal formulation than NYS Figure 3andFigure 4. Increased particle size obtained because of the nature of drug and physical form of the drug used in formulation (Osmani *et al.*, 2018).

Zeta potential and particle size after Centrifugation

The zeta potential showed a high degree of repulsion due to the same charged particles in the medium. For the molecule having a smaller particle size, a maximum zeta potential will confirm stability, based on the nature of the polymer, pH of the medium. Zeta potential of prepared hydrogel nanoparticle is dependent (Alexis, 2008).

Zeta potential was found when an increased quantity of chitosan was used. Hence during the formulation of nanoparticle of the hydrogel by using acrylamide derivatives and non- ionic polymers gives the negative zeta –potential values because of the presence of carboxylic polymer in the terminal group. It was found that formulated nanoparticles of hydrogel showed the zeta potential have enough charge to inhibit aggregation (Agnihotri *et al.*, 2004).

The average particle size distribution of the resulting nanoparticles were determined using particle size analyzer (Nanotrac Wave II Q, Microtrac, USA) The experiment was performed using clear disposable zeta cell, water as a dispersant which has a refractive index (RI)1.330 and viscosity CP -0.73 and the temperature was kept constant at 25°C.

Scanning electron microscopy

SEM images depicted no particulate aggregation in hydrogel nanoparticles, and results were well matching with particle size analysis results, intact crystals of clotrimazole and nystatin. Adding on the Nanoparticle surface residual was observed, provides the fact that NPS was formed from CTZ, and NYS was stable and within the particle size range scale Figure 5 and Figure 6, indicates the result of scanning electron microphotographs of cross-linked chitosan with TPP. Chitosan cross-linked was porous, for its least cross-linking density loose and open structures with maximum pores could be assigned. SEM picture also shows that nanoparticles prepared are having smooth surface and globule in shape. This result founds that distinctive internal structure composed of shell assembly of drug-polymer and inside containing many spaces, and surface appearance of the particle was smooth (Woodruff and Hutmacher, 2010).

Nystatin containing nanoparticles showed homogeneous pores structure similar to a sponge. Drug

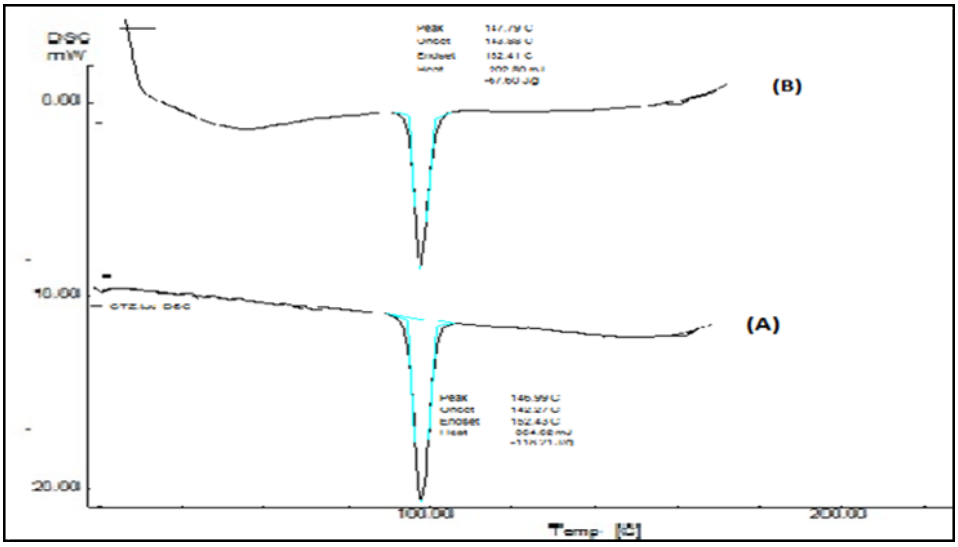

Figure 8: OverlainDSC thermogram of (A) CTZ and (B) Physical mixture

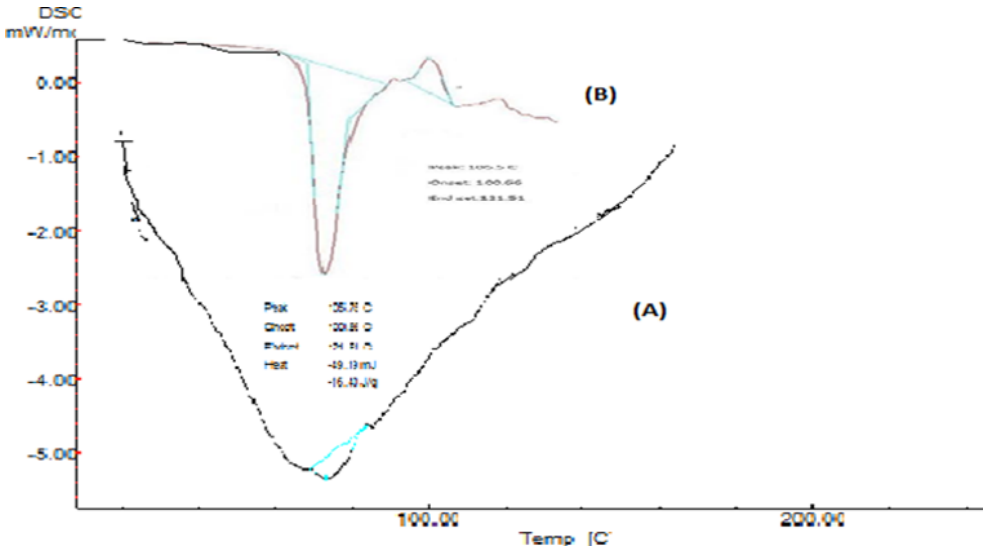

Figure 9: OverlainDSC thermogram of (A) Physical mixture (B) NYS

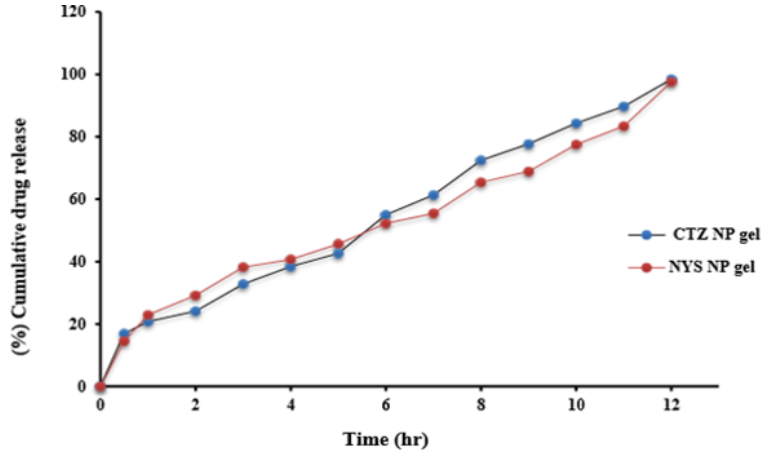

Figure 10: In-vitro drug release of hydrogels in phosphatebuffer solution

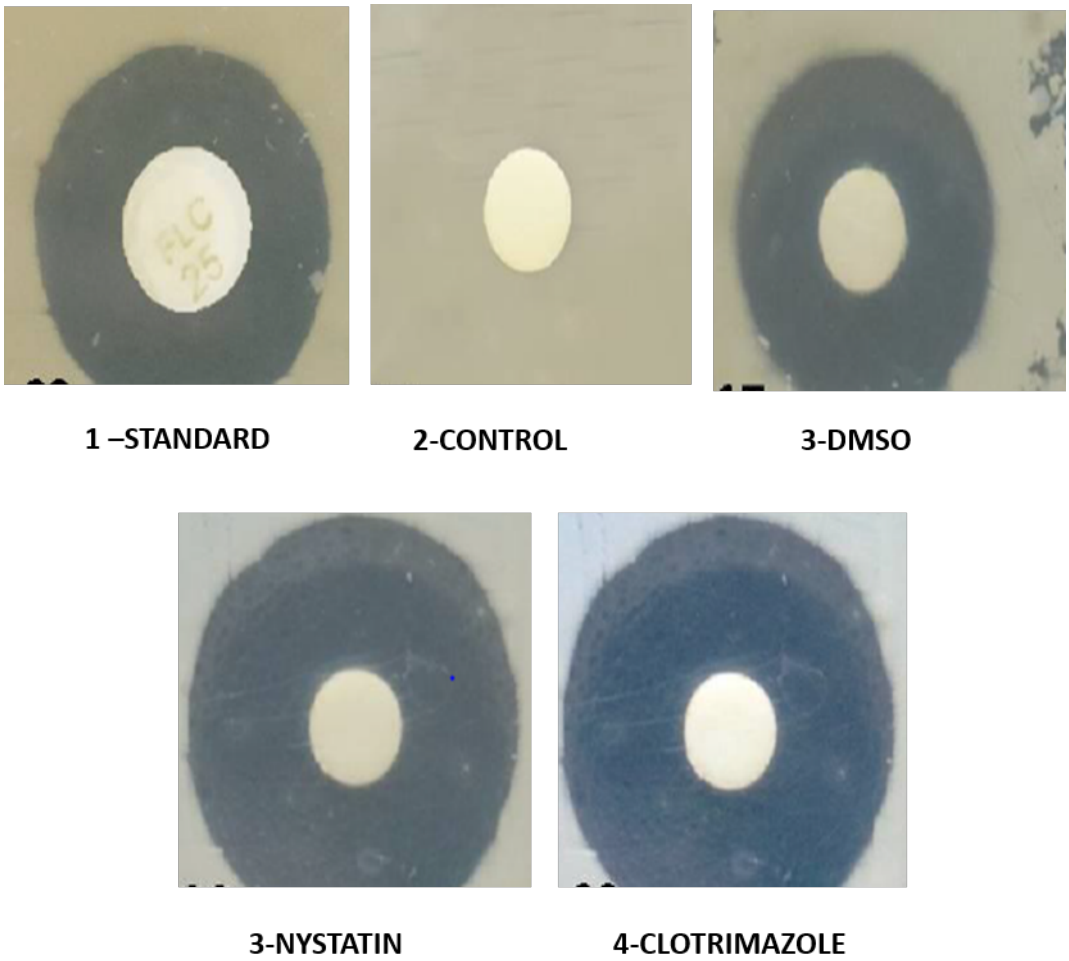

Figure 11: Antifungal effect on and C. Albicans

Table 1: Results of formulated hydrogel nanoparticle

Formulation	Particle size (nm)	Zeta-Potential (mv)	Viscosity (cp)	Drug loading	Encapsulation Efficiency (%)
CTZ	273.7	129.8	4219±0.69	64.34±0.65	74±0.57
CHT	983	-23.6	3748±0.28	-	-
NYS	501	-28.3	2935±0.66	5148±0.44	63.0±0.78

*Mean ± SD, n=3 (Encapsulation Efficiency); *Mean ±SD,n=3 (Viscosity of Hydrogel); * CHT=ChitosanNanoparticles
*CTZ= Clotrimazole; * NYS=Nystatin
**Clotrimazole nanoparticle displayed goodEncapsulation Efficiency compared to Nystatin loaded nanoparticle
**Viscosity ofClotrimazole loaded gel indicates significantly more Viscosity when compared tothe Nystatin load gel

loaded NPs remained intact, which was proven by SEM images. The result of a porous structure for hydrogel formulation reaches to the maximum internal surface area with the least diffusional resistance (Buschmann, 2013).

Drug loading and encapsulation efficiency

Clotrimazole nanoparticles showed better encapsulation efficiency compared to Nystatin loaded nanoparticles. The encapsulation efficiency of CTZ and NYS was found to be 74.6±0.57, 63.0±0.78 51, Respectively. The drug loading of CTZ was found to be 34±0.65, and NYS is found to be 48±0.44.

Viscosity studies

Prepared formulations were measured for the viscosity using Brookfield viscometer 10-15 rpm with the help of less volume adaptor having a thermos stated water jacket and SC4-18 spindle, it was observed that optimized formulations were a shear-thinning system. Hydrogel, which is the placebo of a drug, represented maximum viscosity with the interaction between the polymer and entanglement (Berger, 2004). Comparing with CTZ and NYS,

Table 2: In vitro release data for prepared hydrogel nanoparticlesformulation

Time (hrs)	Cumulative % Drug Release at pH 7.4	
	Clotrimazole (CTZ) ± SD	Nystatin (NYS) ± SD
0.5	16.90±0.31	18.54±0.43
1	20.81±0.19	25.93±0.22
2	24.13±0.27	30.24±0.57
3	32.84±0.24	38.24±0.11
4	38.13±0.34	40.69±0.23
5	42.65±0.22	45.65±0.33
6	55.02±0.29	52.25±0.34
7	61.38±0.15	55.48±0.46
8	72.47±0.32	63.43±0.64
9	77.69±0.39	68.93±0.92
10	84.32±0.43	77.52±0.42
11	89.75±0.36	83.41±0.19
12	98.46±0.29	97.62±0.22

Table 3: Stabilitystudy data of formulations

Storage condition	Sampling intervals (months)	pH			Drug content (%)	
		LMW CHT gel	CTZ NP gel	NYS NP gel	CTZ NP gel	CTZ NP gel
40 ± 2°C and 70 ± 5% RH for 90 Days	0	5.33±0.06	4.87±0.03	4.96±0.06	97.68±0.21	96.92±0.17
	1	5.33±0.04	4.86±0.01	4.96±0.05	97.51±0.27	96.73±0.19
	2	5.33±0.05	4.86±0.02	4.95±0.04	97.34±0.28	96.57±0.37
	3	5.32±0.13	4.86±0.06	4.95±0.09	97.19±0.15	96.36±0.34

*Mean ±SD, n=3; ** LMW CHT= Lowmolecular weight chitosan nanoparticle; ***CTZ NPS= Clotrimazole Nanoparticles
***NYSNP= Nystatin Nanoparticles

loaded gel found low viscosity, and viscosity of CTZ loaded gel shows significantly more thickness when compared to the NYS loaded gel (Cristina, 2014).

pH determination

Determination of pH is very prominent for a topical dosage form; it causes skin irritation if it varied from the normal pH of skin condition (Lodén, 2003) chitosan polymer showed consistency in pH within the range of 4-5, so every preparation was evaluated for pH. mao (Mao, 2001) . The pH of Clotrimazole, Nystatin, and placebo loaded gel were 4.87, 4.96, 5.33, respectively, which is correlates with the normal skin pH. Thereof, the prepared hydrogel shows no irritation as per the p^H is concerned.

Antifungal effect on and *C. Albicans*

The inhibitory effect of the LMW chitosan nanoparticles was found to be more prominent on C. *Albicans*. Least HMW chitosan nanoparticle count resulted in a profound impact (a 1mg/ml of chitosan concentration) (Kong, 2010). For concentrations of 2 and 3 mg/ml, LMW and HMW chitosan nanoparticles exhibited similar antifungal effects when tested microbiologically through cup plate method, No zone of inhibition was observed by DMSO solvent which is used as a negative control and very clear zone of inhibition were found with the placebo (CHT), Clotrimazole and Nystatin loaded gel Figure 11, (Shi, 2006).

***In vitro* drug release study**

The in vitro release test gives the important observation for the release of explaining the performance of the formulation *in vivo*. The observation made in *the in-vitro* test marked that drug was release (Dumortier, 2006).The prepared formulations represented extended-release of Clotrimazole and Nystatin in burst release. From the Release of the drug from the Nanoparticle was because of moderate erosion of the Nanoparticle and concomitant diffusion of the drug, the extended-release form Nanoparticle gels are of more interest for increasing

the topical drug delivery and keeping the required concentration for all the treatment of fungal infection (Desbrieres, 2019). The release rate from the Nanoparticle-based hydrogel was observed into different models of kineticsFigure 10. The obtained values of r^2 were found to be more for the zero-order models reported in Table 2 which show that the test product followed concentration-independent release and polymeric diffusion dependent release kinetic (Khurana *et al.*, 2013).

Stability studies

A stability study was to predict the shelf life of the product by accelerating the rate of decomposition, with increasing the temperature. By keeping at 80 days, stability studies were carried out for keeping at 40±2°C and 75±5% RH (George *et al.*, 2011) and it was observed that there were no changes in the pH, drug content, and physical appearance of the preparation's while study time Table 3, which shows that during study period optimized development had shown better stabilityTable 3 (Wilson, 2010).

CONCLUSIONS

Different methods are used to produce chitosan nanoparticles. Some of the common techniques used are complex coacervation, spray drying, ionic gelation, and cross-linking of emulsions. One of the methods which are quick and straightforward to be implemented is the ionic gelation method, and the same was used for this study. In this process, an amino group with a positive charge is made to interact with polyanions having a negative charge electrostatically. A constant stirring below the ambient temperature generates intermolecular and intermolecular cross-linkages, consequently leading to an uninhibited production of nanoparticles. Also, the high controllability of the ionic gelation process helps to easily change the process variables, including the ratio of chitosan weight to polyanions weight, pH level of the solution, chitosan concentration, etc. and thereby allows to easily alter properties such as surface charge and particle size of the nanoparticles.

When the concentration was increased, it was found that the particle size also increased, and the variation was found to be linear. A not too dissimilar result was observed for the impact of the molecular weight of chitosan on the particle size of the nanoparticles.

Standard drugs such as clotrimazole and Nystatin are compatible with chitosan molecules as informed by FT-IR and DSC analyze. These Hydrogels were more viscous and showed acceptable spreadability. Hence the pH of the preparation was observed in the range value of 4.5-5.5, which is correlated with the normal skin pH and ruled out from skin irritation; hence, the pH is standard for topical preparations.

Antifungal activity of the nanoparticles is mainly dependent on the zeta potential and the particle size, and therefore, the inhibitory effect on different fungi species if different for nanoparticles with varying zeta potential and particle sizes. It is for this reason that this study was conducted; to analyze the impact of particle size and zeta potential on the antifungal action of nanoparticles. C. Albicans was chosen for the study, and the antifungal effect of different nanoparticles was analyzed on these species, and it exhibited excellent antifungal activity. Loading efficiency and drug loading are more to present its antifungal activity.

Prepared formulations indicated sustained release with the drug for a period of 12hr; preparation was stable for a study period of 80 days under accelerated condition.

Based on all the above-stated results, it can be concluded that the prepared chitosan nanoparticles based hydrogel of clotrimazole and nystatin are promisingly formulated Hydrogel is efficient in treating topical fungal infection.

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