**Formulation Of Econazole Nitrate Nanocrystals Loaded Hydrogel And Fungicidal Activity**

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**ABSTRACT**

Fungal diseases caused by *Candida albicans* were commonly treated with antifungal econazole nitrate as a standard regimen. According to the poorly water soluble of Econazole nitrate, the nanocrystal formulation was prepared. The aim of this study was to investigate nanocrystals produced by precipitation homogenization method on the Superficial fungal infection in immunocompromised patients can lead to many disorders and complications. Currently, new topical treatment options are critically needed to treat these fungal infections. Econazole nitrate (EN) is a topical antifungal medicine used for fungal infection treatment. The purpose of this paper was to develop a new topical econazole nitrate nanocrystal (ENC) incorporated hydrogel. This study suggested the potential benefits of ENC embedded in a gel as a drug delivery system for topical antifungal treatments. Preliminary experiments were therefore carried out to characterize the ENC in comparison with raw drug. Prepared gel was homogeneous for human use with non-irritant and safe. Nano-systems showe higher skin retention and better antifungal activity. Drugs retained from LNC hydrogel (N-GEL) in different skin layers within 8 h were the highest. Therefore, it was observed that ENC loaded hydrogel was more effective in killing the fungus. Consequently, hydrogel incorporated with ENC could be a new approach with improved activity and increased dermal delivery for drugs with poor aqueous solubility rather than coarse drug containing gel.
INTRODUCTION:
Nanocrystals are promising not only for the oral route but also for an improved skin delivery of poorly soluble drugs(1). The nanoprecipitation technique (or solvent displacement method) for nanoparticle manufacture was first developed. This technique presents numerous advantages, in that the nanoparticle formation is instantaneous and the entire procedure is carried out in only one step. Briefly, it requires two solvents that are miscible. Ideally, both the polymer and the drug must dissolve in the first one (the solvent), but not in the second system (the non-solvent). Nanoprecipitation occurs by a rapid desolvation of the polymer when the polymer solution is added to the non-solvent. Indeed, as soon as the polymer-containing solvent has diffused into the dispersing medium, the polymer precipitates, involving immediate drug entrapment. The rapid nanoparticle formation is governed by the so-called Marangoni effect, which is due to interfacial turbulences that take place at the interface of the solvent and the non-solvent and result from complex and cumulated phenomena such as flow, diffusion and surface tension variations(2).

Econazole, 1-{2-[4-Chlorophenyl]methoxy}-2-(2,4-dichlorophenyl)ethyl}-1H-imidazole nitrate was developed in 1969 by Janssen Pharmaceutica and has efficacy against Candida spp., Coccidioides immitis, Cryptococcus neoformans, Dermatophytes, and Actinomycetes. It is also active against some Gram(þ) bacteria. Indications for its topical use include cutaneous and mucous membrane mycoses as well as onychomycoses and seborrheic dermatitis. It can be applied as a cream, ointment, vaginal tablet or shampoo. Several nano-formulation strategies have been studied for delivering econazole through targeted skin sites(3). The stratum corneum (SC) is commonly known as the principal physical barrier to most substances that come in contact with the skin(4). The success of dermatological products depends on the ability of the drug to overcome the barrier properties and to penetrate through skin in sufficient quantities to achieve its desired therapeutic effect (5). Thus, significant effort has been devoted to developing strategies in order to promote the transport of drugs across the (6). These approaches encompass both particulate carrier systems and penetration enhancers (7). Hydrogels have been settled in pharmaceutical applications and become very popular due to their distinctive features such as high water content, soft consistency, flexibility and biocompatibility (8). Natural and synthetic hydrophilic polymers can be cross-linked and modified in physical or chemical manner to obtain hydrogels. Their resemblance offers opportunities for a variety biomedical applications closely simulating natural living tissue. Hydrogels may be chemically stable or they may degrade and eventually disintegrate and dissolve (9). The elastic nature of swollen or hydrated hydrogels after being applied shows non-irritation to the surrounding tissues (10). Hydrogels might protect drugs from the potentially harsh environment in the vicinity of the release site(11). Hydrogels are three dimensional, hydrophilic, polymeric networks with potential absorbing of large water amounts biological fluids. Currently, hydrogels are used for manufacturing contact lenses, hygiene products, tissue engineering scaffolds, drug delivery systems and wound dressings and topical treatment (12). Two types of hydrogels were distinguished namely reversible or physical gels, if molecular entanglements and/or secondary forces such as ionic, H-bonding or hydrophobic forces play the main role in forming the network, and ‘permanent’ or ‘chemical’ gels, where the network of covalent bonds is achieved by cross-linking polymers in the dry state or in solution (13). Permanent hydrogels containing polymers are divided in charged or noncharged ones relying on the functional groups present in their nature (14). Advantages of chitosan hydrogel are seen in chitosan’s biocompatibility, biodegradability and low toxicity (15).

MATERIAL AND METHOD
Materials
Econazole nitrate salt bought from online Vit. E TPGS received as a gift from Antares Health Products Inc., Bhiwandi, India; HPMC K100 and Carbopol 934P
acquired from SIP Laboratories; Candida albicans (MTCC No 183) bought from Microbial Culture Collection Bank, Microbiology department, Prayagraj Agriculture Institute of Deemed University, Prayagraj.

**Preformulation studies**

**Determination of Solubility**

The solubility of Econazole nitrate were tested in various solvent such as distilled water, methanol, and ethanol. (17)

**Compatibility study**

FTIR analysis for Econazolet nitrate salt, Vit. E TPGS, Physical Mixture (Econazole nitrate salt :Vit. E TPGS in 1:1) and ENC was performed by FTIR NICOLET 6700. Each sample was mixed in 1:100 with potassium bromide and later compressed into pellets observed from 4000 to 400cm⁻¹.

**Preparation of ENC**

The modified method of nanoprecipitation used in this study was adopted from studies conducted Vit. E TPGS (0.1–1%w/v) was employed as a dispersion stabilizer. Initially, 10ml organic solution (0.05%w/v of drug in methanol) was added to 50ml of aqueous Vit. E TPGS solution using a 22 gauze syringe while continuously stirring at 2000rpm with a mechanical stirrer (Remi Electrotechnik Limited). The aqueous solution was maintained at 2°C using ice bath while stirring for immediate precipitation, preventing crystal growth and acquiring a uniform size distribution. Rapid addition of organic phase to aqueous phase maintained at 2°C resulted in immediate precipitation from antisolvent. Formed dispersion was stirred at 2000rpm for additional half hour and probe sonicated (Sonics Vibro Cell, 20 kHz, 1500 Watt) for 30 min a t pulse rate 30/30 while maintaining the dispersion a t 25°C. ENC was collected by centrifuging for 10 min at 4°C and 10000rpm using a high speed cooling centrifuge (Remi C-24 BL). For modifying the ENC surface. (18)

**Evaluation of drug loaded nanocrystals**

**Scanning electron microscopy (SEM)**

The morphology of ENC was examined by SEM (LEO, model no. 435 VP, England). A small quantity of drug nanocrystals was placed on the surface of metal stubs by aid of adhesive tape and was gold coated with a sputter coater for preparation of sample. (19)

**Preparation of gel**

Firstly gel base was prepared by dispersing the Carbopol 934P into known quantity of water while continuously stirring at 600rpm followed by addition of methyl paraben sodium (0.02%w/v) and propyl paraben sodium (0.1%w/v) and was stirred for additional half hour. Prepared gel base was kept aside for a period of 24h. Secondly Econazole nitrate salt (0.5%w/w) and ENC (0.5%w/w) was dispersed in appropriate quantity of propylene glycol (5%w/w) and 1% ethanol (20%w/w) later added to carbopol gel bases while stirring at 1000rpm followed by further agitation for 30min to obtain EN gel (D-GEL) and ENC gel (N-GEL) respectively. Tri-ethanol amine (TEA) was added to maintain pH of 5.5–6.5 for maximum efficiency and stirred thoroughly to get clear homogeneous gel. (18).

**Evaluation of gel**

**Determination of pH**

Determination of gels’ pH was done with the help of Digital pH meter (Systronics). The glass electrode of pH meter was dipped in prepared gel and rotated to measure the pH of gel.

**Determination of viscosity**

. Brookfield digital viscometer (model no. INST.R./04) was used to measure the viscosity (in cps) of the prepared gel formulations. The spindle number 4(spindle code S 64) was rotated at 1.5 rpm for the viscosity measurement.

**Determination of spreadability**

The spreadability of hydrogel was determined using the following technique: hydrogel (100g) was placed within a circle of 1cm diameter pre-marked on glass plate over which a second glass Plate was placed. A weight of 20g was allowed to rest on the upper glass plate for 5min. The increase in the diameter due to spreading of the hydrogel was noted. S=M×L/T

Where, S is the spreadability, M is the weight tied on the upper slide, L is the length of glass slides and T is the time taken by the slides to get separated.
**Skin irritation study**

Skin irritation study of gel was assessed on male albino Wistar rats 110–125g after receiving an approval from Control and Supervision of Experiments on Animals, CPCSEA Committee (SIP/IAEC/011/10/19). The formulation was applied on 1cm² region of skin and left exposed for 48h. The application site was wiped with water and examined for the dermal reaction (edema and erythema) after predetermined interval.

**Antifungal study**

Using Candida albicans (MTCC No 183), an in vitro inhibition zone assay was conducted. Filling 25mL of Sabgroud Dextrose Agar into a petri dish prepared the agar plates. Two wells with volumes of 100μL were punched into the agar plate after solidification. To avoid the growth of other undesirable microorganisms except for Candida albicans, all plates were sterilized at 121°C for 15min before use, followed by a uniform spread of 100μL Candida albicans (4×10⁶ cfu/ml) across the entire agar surface using a sterilized spreader rod. After 1 h of rest, 100μL of Econazole nitrate salt suspension (1 mg/ml) as control and 100μL of ENC nanosuspension (ENC equivalent to 1 mg Econazole nitrate salt /1ml) as test were filled into two separate wells in a plate and 100mg each of D-Gel and N-GEL into two separate wells of other plate were filled. All plates were incubated for 72 h at 30°C. The inhibition zone assay samples were examined in triplicate . (17,18).

**RESULTS**

**Preformulation studies**

**Determination of solubility**

Solubility in different solvents was determined and tabulated (Table 1).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Propylene</th>
<th>Acetone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Econazole nitrate</td>
<td>Very slightly soluble</td>
<td>Slightly soluble</td>
<td>Soluble</td>
<td>Slightly soluble</td>
<td>Slightly soluble</td>
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(Fig. 1: FTIR spectra of pure econazole nitrate)

(Fig. 2: FTIR spectra of econazole nitrate: vitamin E TPGS physical mixture at 1:1)
FTIR analysis for EN, Vitamin E TPGS, physical mixture and ENC was successfully conducted. The FTIR analysis of EN showed absorption at 3036.39, 30456 & 3219cm\(^{-1}\) for Aromatic C–H Stretching, 3941 for C–H aliphatic stretching, 2427 & 2514cm\(^{-1}\) for S–H Stretching, 2179.08 for C≡N stretching, 1668cm\(^{-1}\), 1727, 1716, 1692 for C=C Alkene stretching, 1434cm\(^{-1}\) for C=N stretching, 155.96cm\(^{-1}\) for C=C Aromatic stretching and 7561 & 1100cm\(^{-1}\) for C–Cl stretching. Spectra of Vit E TPGS showed absorption at 2416.5 for C–H aliphatic stretching, 1565, 1564.6, 1543 C=C for aromatic stretching, 722.7 for C–Cl stretch and 3454 for O–H stretching. Physical mixture showed band at 3139, 3024 for C–H Aromatic stretching, 2825 C–H aliphatic stretching, 2301, 2340 C\(_\equiv\)N stretching, 1542 C=N- stretching, 1371, 1657 C=C aromatic stretching, 1461, 1576 Aromatic C=C for Chlorobenzene, 769 C–Cl stretch and 3340 for O–H stretching. No difference was observed in the absorption band for chemical bonding of pure drug and vitamin E TPGS.

**Evaluation of drug loaded nanocrystals**

**Scanning electron microscopy (SEM)**

From SEM studies it was found that the samples had crystal and almost crystalline nature. The crystal was induced by the diffusion of the solvent from the surface of the nanocrystals. The average size of the prepared nano-crystals was found to be in the range of 10-50 nm. The shape of the nano-crystals is of crystalline in nature.

(Fig.3: SEM image of econazole nitrate nanocrystal)

**Formulation of topical carbopol hydrogel of econazole nitrate nanocrystals**

Formulations of Econazole nitrate and Econazole nitrate Nanocrystals was developed using carbopol 934P, ethanol, methyl paraben sodium, propyl paraben sodium, propylene glycol, Triethanolamine, and water. Carbopol 934 was used as polymer; ethanol as a penetration enhancer; methylparaben and propylparaben as preservatives; Triethanolamine as Ph balancer and water as a vehicle.

**pH**

The pH value of developed formulation of Carbopol (N-gel) was found to be 6.4

**Viscosity measurement**

The viscosity of formulated Econazole nitrate gels was measured using Digital Brookfield viscometer. The rheological behavior of formulated gel was studied. In-gel system, consistency depends on the ratio of solid fraction, which produces the structure to a liquid fraction.

<table>
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<tr>
<th>Table:2: Evaluation parameter of gel formulation</th>
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<tbody>
<tr>
<td><strong>Formulation</strong></td>
</tr>
<tr>
<td>Pure econazole nitrate gel (D-Gel)</td>
</tr>
<tr>
<td>Econazole nitrate nanocrystal gel (N-Gel)</td>
</tr>
</tbody>
</table>
Skin irritation study
In order to confirm the safety of formulated gel, skin irritation test was conducted. Even after 48h, the formulation did not show any erythemal and edemal scores. All excipients used in the formulation were therefore found to be non-irritating and safe for topical application.

![Skin irritation study before and after](image1)

(Fig. 4: (A) Before skin irritation study (B) After skin irritation study)

Antifungal study
In vitro antifungal activities were encouraging where ENC systems. Possibly LNC showed wider diffusion and improved efficiency against Candida albicans providing enhanced antifungal action. Thus, formulating EN as nanocrystals could be an effective way to improve antifungal potency.

![Antifungal study results](image2)

(Fig. 5: A. Hydrogel apply on 1st day, B. Hydrogel apply on 2nd day, C. Hydrogel apply on 3rd day, D. Hydrogel apply on 4th day, E. Hydrogel apply on 5th day, F. Hydrogel apply on 6th day treated disease)

CONCLUSION
Econazole Nitrate is a topical antifungal drug with lower bioavailability problem due to its poor aqueous solubility. Improving the solubility could increase the dermal bioavailability and thus ENC were prepared using Vit E TPGS. For preparation of Econazole Nitrate Nanocrystal (ENC), a modified nanoprecipitation method was used involving several optimization parameters such as the stirring speed, solvent system, stabilizer concentration, and temperature. ENC revealed several benefits over parent drug (EN), including increase in skin retention and a better antifungal activity. Here, ENC loaded topical hydrogel was prepared successfully using carbopol as gelling agent which could potentially be considered as a new antifungal treatment. The results obtained from this study revealed that prepared ENC loaded hydrogel has a great potential to improve the topical delivery of EN as compared with conventional formulations. In vitro skin permeation and skin retention studies revealed that ENC loaded hydrogel could obviously increases skin permeation of EN in skin and amount of drug retention in skin layers was significantly enhanced as compared to conventional...
formulation (coarse suspension). The skin irritation studies in rat was furthermore revealed that irritation potential of ENC hydrogel was minimal. Therefore, it was concluded that ENC loaded hydrogel formulation has a great potential for topical delivery with better drug penetration and retention as well as were safe for treatment of various skin diseases. However, further studies are needed to fully explore these formulations such as extensive pharmacokinetic studies, histopathological studies and toxicity studies. In conclusion, ENC hydrogel could be a new approach which can be applied in future to improve the dermal delivery of drugs with poor aqueous solubility.

REFERENCES
7. V. Sanna, G. Caria, A. Mariani, Effect of lipid nanoparticles containing fatty alcohols having different chain length on the ex vivo skin permeability of Econazole nitrate, Power Technology201(2010)32-36.

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