Cosmeceutical and Therapeutic Potential of Isotretinoin Nanoparticulate Gel in Management of Acne

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ABSTRACT
This study intended to develop and evaluate isotretinoin (ITR) loaded nanoparticles taking chitosan as a polymer of interest. Ionic crosslinking method was utilized to prepare nanoparticles. Nanoparticles were formulated using varying ratios (chitosan: tripolyphosphate) and evaluated for its size, distribution of size, zeta potential, percentage of ITR entrapped within nanoparticles, in vitro drug release and its stability under accelerated conditions. The prepared nanoparticles (NPs) were spherical, white in color and free flowing, 321± 4.5 nm was the average size of optimized chitosan nanoparticles and it was able to entrap 88.76 ± 3.5%. The outcomes assured vast promise of the CNs of ITR (optimized) in management of acne and also increasing the therapeutic efficacy, thus establish to be a promising, effective and patient compliant formulation.

INTRODUCTION
Acne, a cutaneous pleomorphic condition of the pilosebaceous unit involving sebum production rate anomaly and described by inflammatory (pustules, nodules and papules) as well as non-inflammatory comedones (closed and/or open) (Knutson, 1974). Regular pus-forming microbes Propionibacterium acnes and Staphylococcus epidermidis are responsible for development of many forms of acne vulgaris (Rawat, Tripathi et al., 2015). It is a pleomorphic disorder and can manifest at any time during life but it most commonly occurs between ages of 12-24 years, accounting effective 85% of population (Cordain, Lindeberg et al., 2002). *P. acnes* is an anaerobic microorganism existing in acne lesionsthat promotes inflammation through a variety of mechanisms and involved in producing pro-inflammatory mediators that diffuses through the follicle wall (Itoh, Tsuchida et al., 2014).

Prominent in adolescence and puberty, acne is definitely associated with function of sebaceous gland, which stimulate higher secretion of sebum androgenically (Singh, Gangwar et al., 2016). However, the consequential sebaceous glands abnormality due to hormonal changes modify the composition of sebum and decrease content of linoleic acid (Kanlayavattanakul and Lourith, 2011). The primary pathogenic mediator responsible for acne vulgaris on skin and hair follicles is *P. acnes* (Cunliffe, Holland et al., 2004). Thus, *P. acnes* has become a major pathogen of interest for researching the treatment against acne vulgaris (Meraj Anjum, Kanoujia et al., 2016).

Whether drug or cosmetic, a nanoparticle formulation has more advantages for their delivery to the skin over conventional formulations (Ghosh, 2000). Incorporated within nanoparticles, active ingredients are protected against chemical degradation and drug release can be modulated. Only well tolerated excipients are utilized and in cases of large scale production (Jafarinejad, Gilani et al., 2012, Meraj Anjum, Kanoujia et al., 2016). Chitosan (CN) is an interesting polymer used extensively in pharmaceuticals (Wang, Zeng et al., 2011). CN based nanoparticles are used world wide for different applications owing to their non-toxicity, biodegradability, high permeability and cost effectiveness (Wang, Zeng et al., 2011). Being smaller in size, they are capable of passing through the biological barriers and deliver drugs to the lesion with improved efficacy (Saharan, Mehrotra et al., 2013).
Topical retinoids are the therapeutic agent of choice for treating acne by formulation scientists because of their safety profile, non-toxicity and biocompatibility, and isotretinoin (ITR) (one of the form of topical retinoid) is the first choice of drug for treating all acne types (Thiellitz and Gollnick, 2008). Severe side effects (skin dryness, psychological disorders and teratogenicity) are reported with oral administration of ITR (Layton, 2009). So it is worth to formulate it as topical dosage which could prove to be safer than its oral administration.

Thus, the purpose of the present work was to fabricate chitosan nanoparticles loaded with ITR by ionic gelation technique and to access for size of particles, particle homogeneity, morphology, zeta potential, drug (ITR) entrapped, stability aspects and ITR release from the formulation in-vitro. ITR CN nanoparticles have been prepared for skin delivery aspects (skin permeation) and its potential to treat acne induced by testosterone in albino wistar was studied.

MATERIALS AND METHODS

ITR (13-cis retinoic acid) was received from M/s Ranbaxy laboratories Ltd Gurgaon (India) as a gift sample. CN was purchased from M/s Himedia laboratories Pvt ltd Mumbai (India). Sodium tripolyphosphate (TPP) and glacial acetic acid (GAA) were acquired from Merck Specialties Private Limited, Mumbai, India. Analytical grade chemicals were utilized for this study.

Preparation of ITR-loaded CN Nanoparticles

Ionic cross linking method was opted to fabricate CN nanoparticles (Ghosh, 2000, Ajun, Yan et al., 2009). CN was first dissolved in 1% v/v GAA and then, the pH of mixture was made up to pH 4.5 by using 0.1N NaOH. Tween80 (2% w/v) was added in the polymeric solution. ITR was dissolved in small volume of acetone and this ITR solution was mixed uniformly with the chitosan solution by continuous stirring. Aqueous solution of TPP (concentration: 2 mg mL$^{-1}$) was added drop wise to the CN solution and it was allowed to stir for 30 minutes followed by sonication (Labsonic-M®, Sartorious Stedim) for 15 minutes at 6 cycle and 80% amplitude. Nine formulations were prepared by varying the concentration of CN and CN/TPP ratio (2-5%w/w) (Nesalin and Smith, 2013).

Characterization of the Nanoparticles

Assessment of size and homogeneity (via polydispersity index)

The size and the polydispersity index of ITR loaded CN nanoparticles was measured by a Zetasizer NS 3000 (Malvern Instruments, UK) using dynamic light scattering. All the experiments were done in triplicate.

Zeta Potential Determination

The Malvern zetasizer equipped with zeta potential analyzer (Zetasizer NS 3000; Malvern Instruments, UK) was used for measurement of zeta potential of all samples in distilled water. The principle for measuring zeta potential is phase analysis light scattering (M3-PALS) technique, which employs to measure the electrophoretic mobility of particle in thermostatic chamber. The experiments were done in triplicate (Sonker, Gupta et al. 2017).

Drug entrapment efficiency (% DEE)

Drug entrapment was determined indirectly by centrifuging the resuspended nanoparticles suspension (Remi CPR-24) at 15,000 rpm for 15 min at 20°C so that the free drug can be seperated from the nanoformulations. Concentration of drug in the supernatant is expected to be unentrapped drug, which was estimated spectrophotometrically using UV -Visible spectrophotometer (Labtronics LT-2910) at 333 nm. The ITR entrapped within CN nanoparticles was thus determined using the relationship given below:

\[
\text{Entrapment efficiency (％DEE)} = \left(\frac{\text{Experimental ITR content}}{\text{Theoretical ITR content}}\right) \times 100
\]

The experiment was carried out by means of dissolution apparatus (Veego, India). Dialysis membrane with molecular weight cut off range 12,000–14,000 Dalton (Hi-media, Mumbai, India) was soaked in phosphate buffer (pH 7.2) for 12 hour. The cylinders were filled with 250ml of freshly prepared dissolution medium (phosphate buffer; pH 7.2). 3 mL of formulation was taken and filled into the dialysis membrane sac; this sac was fixed to the shaft. Stirring speed (50 rpm) and temperature of the set-up (37 ± 5°C) were kept constant during the entire experiment. The experiment was preceded for 24 hour and aliquots of 5 mL were withdrawn at pre-determined time interval and the sink condition was maintained by replenishing with fresh phosphate buffer (pH 7.2). Samples were analyzed by UV visible spectrophotometer (Labtronics LT-2910) at a wavelength of 333 nm. Data obtained from the experiment were fitted to various kinetic equations for finding out the mechanism by which ITR is released from CN nanoparticles.

Transmission Electron Microscopy of ITR Loaded CN Nanoparticles

ITR loaded CN nanoparticles were evaluated for the surface morphology using transmission electron microscopy (TEM, EM208S, Philips, Netherlands). Briefly,
diluted sample of ITR loaded CN nanoparticles was taken and a drop from this solution was laid on a carbon coated copper grid, excess fluid on the surface was removed by the help of a filter paper and the remaining fluid was allowed to dry in air before TEM examination (Jafarinejad, Gilani et al., 2012).

Skin Permeation Study
Permeation of ITR from CNitr5 through the skin was studied through modified Franz diffusion cells. After animal sacrifice and receiving the mice skin, the dorsal hairs and fat layers were all removed from the skin. Franz diffusion cell (cross-sectional area: 3.14 cm$^2$) was filled with phosphate buffer pH 7.2 (receptor volume: 30.0 ml) and the mice skin was mounted over it. By the help of outer thermo-regulated water jacket, 37±1°C was maintained and the magnetic stirrer kept the diffusion medium continuously stirred. Known amount of CNitr5 was applied onto the surface of mice skin facing the donor compartment and 1 ml aliquots of samples were withdrawn from the sampling port of receptor compartment at pre-decided intervals of time and each time, an equal quantity of fresh diffusion medium was added back to the receptor compartment to carry on with the sink condition. Amount of drug in the samples was accessed by UV visible spectros copy at 333 nm. A graph constructed between the amount released per unit surface area and time gave permeation flux. The Slope of obtained regressed line corresponds to permeation flux (Raza, Singh et al., 2013).

In-vivo anti acne study
Before the commencement of experiment, the albino wistar rats (100-150 g) were permitted to acclimatize for one week under standard temperature conditions (25 ± 1°C) in polypropylene cages with 12 h light and dark periods with access to standard diet (commercial pellets and water ad libitum). Animals were arbitrarily divided into total 4 groups (group 1: testosterone treated; group 2: testosterone and ITR commercial gel treated; group 3: testrosterone and CNitr5 treated; group 4: no treatment, served as control) with 6 animals in each group. Hairs were removed from the dorsal side of the albino wistar rats; testosterone was applied to each group daily till the termination of experiment. The treatment with ITR commercial gel or CNitr5 was started following the induction of acne. The applications were made one time in a day repeatedly for 4 weeks. Skin was observed for the presence of visible acne papules and/or other visible change(s). Reduction in the papule density (4 cm$^2$ skin area), after the 4th week of treatment w.r.t. that of Group 1 show that the formulations possess anti-acne activity.

Stability Study
To carry out the stability study of CN-Itr-5, the formulation was divided in to 3 sample sets. One was stored at 4°C in refrigerator; one at room temperature and the remaining sample set at 40 + 2°C, 75% R.H. in humidity control ovens. Drug content of all samples were determined by UV-Vis Spectroscopy at 333nm at specific time period(s).

RESULTS AND DISCUSSION
Size distribution and polydispersity index measurements
The particle size of ITR-loaded CN nanoformulations ranged from 347± 3.21 to 276.5 ±10.43 nm. It was observed that an equal amount of CN and TPP contribute to smaller particle size as shown in table 1. Particle size distribution is portrayed in terms of polydispersity index (PDI). The range for the PDI was found to be from 0.129 to 1.00 for all the formulations. The low PDI value ensures a narrow distribution of particle size and a homogenous system (Waghmare, Waghmare et al., 2011). The particle size of optimized formulation (CN-Itr5) was found to be 347±3.21 nm (which also had low PDI of 0.208 reflecting NPs in the narrow size range and stable dispersion).

Zeta Potential Measurement
Zeta potential reveals the stability of nanoparticulate system and a higher positive surface potential of the optimized CN-Itr5 indicates that the prepared nanoparticles was stable in nature(Singh, Parashar et al., 2017). The optimized formulation displayed a zeta value of 34±2.32 mV as shown in (Fig. 1)

ITR entrapment efficiency
The entrapment efficiency of ITR in CN nanoparticles was found to be in the range of 86.96 ± 2.87% to 95.77 ± 2.33%. It was observed (Table 1) that increase in CN concentration increased the entrapment efficiency as it provided enough space for loading of drug followed by TPP crosslinking.

Surface morphology study
Shape and surface morphology of optimized sample CN-Itr-5 was studied by transmission electron microscopy indicating that the particles were spherical shaped having 200 nm as a mean size of particles.

In vitro release studies
Figure 3 represents percent cumulative release of ITR from CNs in phosphate buffer 7.2. The NPs displayed sustained
release of ITR from CNs due to slow degradation of chitosan in dissolution medium. The drug release was slow and controlled with increase in chitosan concentration. It was clear that the optimized batch (CN-Itr-5) showed sustained release (18.48±1.23%) at 8 hrs and (26.8±1.89%) at 24 hr. The Higuchi model was found to be best fitted with a R2 value of 0.9589 revealing release to be diffusion control (Singh, Parashar et al., 2017). The possible mechanism could be the slow entry of dissolution media through the porous matrix reasing the drug into dissolution media.

Skin Permeation Study
Permeability parameter included steady state flux (Jss = 0.012 ± 0.009 mg/cm²h) and permeability coefficient (Kp = 0.008 ± 0.004 cm²/h). The flux result revealed lower penetration of drug into deep skin layers and higher retention of drug in the upper layer of skin. This is desirable property of a dermal formulation to act suitably on the affected area (Nesalin and Smith, 2013, Raza, Singh et al., 2013).

Anti-acne study (in vivo)
The data of anti-acne in vivo study revealed a significantly higher (p <0.05) anti acne potential of ITR nanoformulation when compared with free ITR and ITR CN gel (Fig. 4). This proves supremacy of nanoformulations over conventional treatment. The higher therapeutic efficacy could be due enhanced local effect over affected area by the nanoformulation (Verma, Kanoujia et al., 2017). The nanoformulation displayed a significant drop in acne (p <0.01) in CN-Itr-5 treated group as compared to other groups.

Stability study
As revealed from Table 3, the optimized formulation displayed slight change in drug content at higher temperature (45°C ± 20°C). However, an insignificant change in drug content was found at 4 ± 10°C and room temperature. This proves the method selected for formulation nanoformulation was accurate and precise.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>CN (mg/mL)</th>
<th>TPP (mg/mL)</th>
<th>Particle size (nm)</th>
<th>PDI</th>
<th>EE (%)</th>
<th>% drug release in 8hrs</th>
<th>% drug release in 24 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN-Itr1</td>
<td>2</td>
<td>2</td>
<td>361 ± 3.21</td>
<td>0.244</td>
<td>87.93 ± 1.43</td>
<td>13.3 ± 2.11</td>
<td>26.4 ± 1.33</td>
</tr>
<tr>
<td>CN-Itr2</td>
<td>5</td>
<td>1</td>
<td>979 ± 1.29</td>
<td>0.175</td>
<td>89.02 ± 3.12</td>
<td>14.85 ± 2.03</td>
<td>18.8 ± 1.89</td>
</tr>
<tr>
<td>CN-Itr3</td>
<td>2</td>
<td>3</td>
<td>537 ± 4.32</td>
<td>0.128</td>
<td>90.16 ± 1.65</td>
<td>16.97 ± 1.11</td>
<td>18.8 ± 2.91</td>
</tr>
<tr>
<td>CN-Itr4</td>
<td>5</td>
<td>3</td>
<td>511.7 ± 1.11</td>
<td>0.149</td>
<td>95.77 ± 2.33</td>
<td>12.73 ± 1.23</td>
<td>18 ± 1.74</td>
</tr>
<tr>
<td>CN-Itr5</td>
<td>2</td>
<td>2</td>
<td>347 ± 2.79</td>
<td>0.208</td>
<td>88.76 ± 1.09</td>
<td>18.48 ± 1.23</td>
<td>26.8 ± 1.89</td>
</tr>
<tr>
<td>CN-Itr6</td>
<td>5</td>
<td>2</td>
<td>388.3 ± 2.23</td>
<td>0.449</td>
<td>90.8 ± 3.02</td>
<td>11.82 ± 2.10</td>
<td>18 ± 1.11</td>
</tr>
<tr>
<td>CN-Itr7</td>
<td>3.5</td>
<td>1</td>
<td>273.4 ± 3.23</td>
<td>1.00</td>
<td>90.44 ± 3.21</td>
<td>11.6 ± 1.09</td>
<td>18.8 ± 1.23</td>
</tr>
<tr>
<td>CN-Itr8</td>
<td>3.5</td>
<td>3</td>
<td>785 ± 2.75</td>
<td>0.826</td>
<td>89.98 ± 1.66</td>
<td>9.8 ± 1.21</td>
<td>16 ± 1.87</td>
</tr>
<tr>
<td>CN-Itr9</td>
<td>3.5</td>
<td>2</td>
<td>276.5 ± 10.43</td>
<td>1.00</td>
<td>86.96 ± 2.87</td>
<td>10.5 ± 1.11</td>
<td>19 ± 1.05</td>
</tr>
</tbody>
</table>

Fig. 1: Particle size and zeta potential of optimized formulation CN-Itr-5
CONCLUSION

ITR loaded chitosan nanoparticles (CN-Itr) were successfully developed. The CN-Itr nanoparticles proved their potential in impeding acne and the capability to act locally. The positive results were attained from the experiment as a drop in acne occurrence was observed. Hereby, we conclude that the developed ITR-loaded CN nanoformulation hold a promising formulation as topical dermal formulation for treating acne. These can be utilized for commercialization in future for therapeutic and cosmeceutical administration, as the methods used for the preparation is easy and economical.

REFERENCES