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Quality by Design (QbD)-enabled development of aceclofenac loaded-nano structured lipid carriers (NLCs): An improved dermatokinetic profile for inflammatory disorder(s)

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ABSTRACT

Present study was designed to prepare and characterize aceclofenac loaded nanostructured lipid carriers (NLCs) employing Quality by Design (QbD)-oriented approach. The NLCs were evaluated for their transdermal penetration potential and stability. Aceclofenac loaded nanostructured lipid carriers (NLCs) were prepared & characterized, by employing Quality by Design (QbD)-oriented approach and further evaluated for transdermal penetration potential and stability. Different lipids and surfactants were chosen to prepare NLCs using microemulsion method as critical material attributes (CMAs). A 3^3 factorial design was used for optimization of NLCs, and evaluating them for different critical quality attributes (CQAs), *viz.* particle size, polydispersity index (PDI), zeta potential, *in vitro* drug release, entrapment efficiency. The effect of CMAs such as lipids, oil: lipid ratio and concentration of surfactants on CQAs viz. drug entrapment efficiency and particle size were systematically evaluated to optimize NLCs. The optimized NLCs were further incorporated into carbopol gel and characterized for texture and rheology profile followed by *in vitro* and *in vivo* evaluations. The optimized ACE-NLCs were found to be spherical, nanometric in size with higher drug loading and entrapment efficiency. Results of the *in vitro* drug release study showed that the developed formulation followed Korsmeyer-Peppas model showing Fickian diffusion. The release was biphasic *i.e.*, initial burst release followed by sustained drug release upto 48 h. The optimized NLCs-based gel formulation showed superior texture, rheological profile and showed better cell uptake efficiency on hyperkeratinocytic cells (HaCaT cell lines) with higher ex vivo skin permeability efficiency vis-à-vis marketed formulation. In conclusion, dermatokinetic modeling and pharmacodynamic study using carrageenan induced edema mice suggests that aceclofenac loaded NLCs hydrogel may provide a better delivery alternative to target various skin layers.

Abbreviations: NLCs, Nanostructured lipid carriers; SLNs, Solid lipid nanoparticles; CA, Cetyl alcohol; QbD, Quality by Design; FbD, Formulation by Design; CQA, Critical Quality Attributes; CMA, Critical Material Attributes; CFA, Critical Formulation Attributes; Opt, Optimized; ACE, Aceclofenac; NSAIDs, Non steroidal antiinflammatory drugs; TNF-α, Tumour necrosis factor α; GIT, Gastrointestinal tract; MKT, -Marketed; QTPP, Quality Target Product Profile; ME, -Microemulsion; PDE, Percentage drug entrapment; BBD, Box Behnken Design; RSA, Response Surface Analysis; RAM, Risk Assessment Matrix; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy; CLSM, Confocal laser scanning microscope; HBSS, Hank's balanced salt solution; PL, Phospholipid; S_{mix}, Surfactant mix; C-6, Coumarine 6.

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1. Introduction

Aceclofenac [2-[(2, 6-Dichlorophenyl)-amino]-acetyl]-oxy]-acetic acid (ACE) is a non-steroidal anti-inflammatory drug (NSAID), used for reducing pain, subsiding inflammation in the pathologies such as rheumatoid arthritis, osteoarthritis and ankylosing spondylitis (Brogden and Wiseman, 1996). ACE has inhibitory actions against a variety of inflammatory mediators including interleukins (IL-1β, IL-6 and tumor necrosis factor (TNF-α)). ACE acts as preferential inhibitor of COX-2 (Hinz and Brune, 2004) due to sustained but limited biotransformation of ACE into diclofenac. The long term oral administration of ACE is associated with adverse effects such as gastrointestinal ulcers and bleeding. Thus, prolonged drug therapy used for treating inflammatory disorder, arthritis, inevitably requires alternative to oral delivery having minimum gastric complications. The transdermal route allows controlled and sustained release of active ingredient with improved patient compliance (Cullander and Guy, 1992). The topical route eliminates GIT associated side effects, increases patient compliance, and avoids first-pass metabolism. Therefore, development of topical delivery vehicles are aimed at modifying drug permeation through the skin (Modi and Patel, 2011).

Several topical products of ACE are available in the market (*i.e.*, Hifenac, Intas Pharma; Acemiz gel, Lupin Ltd; Dolowin-gel, Micro Labs Ltd.; Prestiflam-gel, Interphar Healthcare Pvt. Ltd. etc.), and are efficacious as compared to oral dosage forms. However, the stability and frequent dosing (3–4 times a day) schedule of drugs when delivered through vehicles has been major challenges, during the manufacturing process and storage. This leads to withdrawal of market formulations. Therefore, there is need to develop a novel dosage form which might be useful for targeted and controlled delivery of drug that adopts the recent development in the area of Novel Drug Delivery System (NDDS). Hence, among the pool of carrier systems (polymeric nanoparticles, liposomes, niosomes), nano-lipid carrier systems are chosen to address the above-stated issues (Fadda et al., 2013; Garg et al., 2016a; Jain et al., 2011; Shukla et al., 2015).

Recently, nanostructured lipid carriers (NLCs) have been shown as emerging pharmaceutical dosage forms derived from solid lipid nanoparticles (SLNs) (Müller et al., 2007). NLCs, novel generation colloidal lipid carrier systems, are promising in transdermal drug delivery with numerous features in transdermal applications (Müller et al., 2007). NLCs overcome few limitations associated with SLNs; lower drug loading efficiency, leakage of drug during storage caused by lipid polymorphism (Müller et al., 2002). NLCs are made up of a mixture of physiological & biodegradable solid and liquid lipids in a ratio from 70:30 to 99.9:0.1. The resulting amalgamates of lipids have a lower melting point than the original solid lipid, but matrix remains solid at body temperature. The increased drug loading capacity minimizes the potential expulsion of active compounds during storage, and may prevent a reduction in the water content of particle suspension (Pardeike et al., 2009). Their significance in the enhanced permeation of many drug substances has been proved (Souto and Müller, 2005; Souto et al., 2004). NLCs render an occlusive promoting adhesiveness effect which leads to an increased skin hydration, and thereby enhance skin bioavailability of active compounds. Besides, nano-sized lipid particles ensure close proximity with stratum corneum and increase the amount of drug penetrating the skin. All these effects facilitate drug permeation deeper into the skin layers.

Formulation by Design (FbD) approach is based on the principles of Quality by Design (QbD) and is useful in obtaining the "best possible" formulation composition and provides holistic understanding of the process and product behaviors (Singh et al., 2005b, 2011). Present study was aimed to develop the optimized ACE-NLCs by investigating various NLCs-specific attributes for topical application employing the QbD strategy. Also, this work focuses on drug permeation and retention potential of developed NLCs. In the end, dermatokinetic modelling was also studied to ascertain the accumulation of drug in different skin layers viz., epidermis and dermis followed by *in vivo* pharmacodynamic evaluation.

2. Materials and methods

2.1. Materials

ACE as a gift sample, supplied from IPCA, Laboratories, Mumbai. Lipids such as glyceryl monostearate (GMS), stearic acid (SA) and cetyl alcohol (Vitorino et al., 2013) were purchased from Loba Chemie Pvt. Ltd., Mumbai, India. Marketed gel (MKT-gel) (Hifenc-gel, Intas Pharmaceutical Ltd., 1.5% w/w) of ACE was procured from local medical store. Liquid lipid Transcutol® P, Labrafac®, and labrasol® were supplied by Gatefosse, France. Phospolipon S 100 was supplied as a gift sample by Sasol, Germany. Poloxamer (Pluronic F-68) was obtained as a gift sample from BASF, Mumbai, India. Tween 80 was purchased from Fischer Scientific Pvt Ltd, Mumbai, India. All other chemicals and reagents are of analytical grade.

2.2. Methods

2.2.1. Risk assessment

The probability of risk(s) or failure(s), at the same times, leads to the potential product failure, which was identified by carrying out the risk assessment. Ishikawa fish-bone diagram was made by employing the software, Minitab 17.0.4 (M/s Minitab Inc., Philadelphia, PA) to assume a cause-effect correlation among the probable critical material attributes (CMAs) that affecting the critical quality attributes (CQAs) of the formulations (Lionberger et al., 2008). The resultant fish-bone diagram depicted the effect of process parameters or material attributes for development of ACE-loaded NLCs (ACE-NLCs). Additional, risk assessment matrix (RAM) was carried out for prioritization of selecting the high risk factor, which showed the potential risk(s) related with each of the CMAs or CPPs of ACE-NLCs formulations.

2.2.2. Defining *the QTPP and CQAs*

Quality target product profile (QTPP) is a primary step towards QbD-oriented development of ACE-NLCs, which is defined as a potential summary of the quality features of the drug product that will be attained to confirm the desired quality, are taking into account for safety and efficacy of the drug product. Various CQAs were allocated such as particle size, permeation flux (indicator of the drug permeation through), release (essential to ensure sustained and controlled release), and high entrapment, in order to meet the QTPP. These elements of QTPP are essential for the effective development of NLCs. The key QAs for the product/process affecting the performance of ACE-NLCs are enlisted in Table 1.

2.2.3. Screening of components for formulation of NLCs

For NLCs, Taguchi orthogonal array design that utilizes seven factors at 2 levels (L8-array; resulting in eight formulations) (Negi et al., 2014; Sharma et al., 2015) was used to screen the critical formulation attributes (CFAs) out of type of liquid lipid, type of solid lipid, type of surfactant, stirring speed, % surfactant, temperature, ratio of lipid mixture and liquid lipid. A total of 8 formulations were made according to the design and evaluated for CQAs including percent drug entrapment (PDE), particle size and PDI. CFAs were decided, after feeding the observations of the CQAs in the Design Expert® (v 10.0) software, and analyzing the results.