STUDY ON NANO FORMULATION RESEARCH AND DEVELOPMENT FOR TOPICAL SKIN INFECTION THERAPY

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ABSTRACT

This study develops, optimizes, and tests AZA-loaded lipid nanocarriers to increase skin targeting and retention. Pure AZA tested antibacterial and pure. AZA-loaded nano-preparation was improved using 33 full factorial design after melt emulsification and ultra-sonication.S14 (SLNs) and N23 (NLCS) were optimum with 200 nm in 27 Design runs. During physiochemical evaluation, SLNs and NLCs had zeta potential values of -13.2+1.02 and -14.30.95 mV, indicating physical stability. Nanoparticles were spherical in TEM. SLNs failed stability and particle properties tests. Therefore, N23 was tested in-vitro and in-vivo. NLCs (N23) were added to aloe-carbopol gel for administration and investigated for physiochemical and anti-acne properties. The gel had optimum wetness, spreadability, and occlusivity and was non-irritating. In-vitro permeation investigations showed Nano gel increased pharmaceutical skin retention and reduced cross-skin permeability. NLC skin distribution studies showed deep fluorescence into the skin's deeper layers, verifying the carriers' drug delivery ability. In rabbit and mouse skin irritation tests, nano-gels did not cause oedema, dryness, or redness. In NLC gel, Saccharomyces cerevisiae cytotoxicity tests indicated less than 10% cell mortality. In-vivo mice ear tests showed that nano gels were more anti-inflammatory and anti-microbial than commercial treatments. Thus, NLCS gel increased tissue targeting and retention without compromising medication safety or effectiveness.

INTRODUCTION

Bacterial, fungal, viral, and parasitic skin illnesses ensue. Depending on the pathogenic agent, bioburden, and exposure period, skin infections may range from mild to severe discomfort. Bacterial infections are the most frequent, affecting 155 million people annually (Vos et al., 2015). Acne vulgaris is the most common bacterial infection.

Acne, the seventh most common skin disease, affects 650 million people globally, according to statistics (Garcines et al., 2016; Garg, 2016). Acne, especially in adolescents, has a severe impact on social and psychological well-being. Affected people feel despondent about their appearance and experience extreme pain and deformity (Frank, 1971). Acne vulgaris, a multifactorial skin disorder that affects people of all ages, is centered in the pilosebaceous unit of the face, neck, chest, and upper back, according to the WHO (Bowe and Shalita, 2011; Raza et al., 2012; Hay et al., 2014; Vos et al., 2016). Men are more likely to experience acne throughout adolescence (15 years or older) and into adulthood (Bershad, 2001; Adebamowo et al., 2008). Based on severity, acne is divided into four grades (I–IV) (Tutakne and Chari, 2003; Vyas et al., 2014). Whiteheads and blackheads with occasional papules; Papules, comedones, and pustules.

Grade III: mainly pustules, abscesses, and nodules

Grade IV: Widespread scarring, inflammatory cyst, and abscess.

Azimi et al. (2012) and Raza et al. (2013) identified four pathogenic factors that cause acne vulgaris:

(v) Hyperkeratinization of the follicles (vi) Pilosebaceous unit microbial colonization (vii) Follicle inflammation (viii) Excessive sebum secretion and production

Acne is caused by microbial growth (Propionibacterium acnes, Staphylococcus epidermidis, and Pityrosporum ovale). P.acnes, a naturally occurring acne-free strain, dominates the skin's surface and follicular site (Bojar et al., 1993). Epidermal P.acnes numbers may reach 107 per sebaceous unit (Leyden et al., 1975; Raza et al., 2013a). As they form in the pilosebaceous unit infundibulum, they feed on sebum, cell debris, and metabolic wastes. P. acnes release inflammatory mediators and chemotactic factors to create severe nodulocystic acne (Charnock et al., 2004; Vijayan et al., 2013). Sebocyte proliferation and sebum generation by 5-reductase 1-produced dihydrotestosterone (DHT) induce acne in adolescence. Abnormal keratinization in the top hair follicle infundibulum blocks sebum excretion to the skin, causing comedones. Colonizing anaerobic bacteria (P. acnes) may cause inflammatory lesions (Stecova et al., 2007).

Acne has historically been considered a self-limiting, incurable medical condition that is part of puberty (Cook-Bolden, 2015). However, as people grow more aware of the social shame and long-term implications of acne, such as scars, psychological pain, etc., they are becoming more flexible and receptive of the treatments they previously hated. Even medical therapies and equipment for acne have improved confidence and reliability in treating early acne.

Keratolytic, antibacterial, anti-inflammatory, and anti-androgenic oral, topical, and systemic acne medications are available. Disease kind and severity determine calculations. Topical antibiotics (Clindamycin, Tetracycline, Doxycycline, Azithromycin, etc.), retinoids (Tretinoin, Adapalene, Tazoterene), benzoyl peroxides, azelaic acid, etc. are only used for mild to moderate cases, while oral and systemic antibiotics, retinoids, contraceptives, steroids, etc. are only used for moderate to severe cases (acne resistant topical medication, truncal acne, etc.). They may be administered alone or in combinations depending on the patient's needs and condition (Rathi, 2011; Veltri, 2013). Tetracycline and erythromycin are first-line antibiotics for moderate to severe illnesses (Kathani et al., 2007). Prolonged usage increases the risk of bacterial resistance, gastrointestinal discomfort, oral mucosa hyperpigmentation, vertigo, skin dryness, and other side effects. These disadvantages restrict their acne therapy. Thus, researchers examined different drugs including spironolactone, steroids, and contraceptives. These drugs may cause thrombosis, renal failure, and adrenal suppression, hence they should only be administered in rare or severe cases (Thiboutot et al., 2009; Bowe and Shalita, 2011). The disadvantages necessitated a safe, effective, and low-risk localized therapy. The subclinical skin condition requires topical medications to be treated topically over a vast epidermis. Topical retinoids with multiple target activities and therapeutic efficacy were excellent acne

maintenance treatments. Financial considerations and side effects including skin irritation, erythema, peeling, photosensitivity, etc. restrict its usage (Ghali, 2009; Raza et al., 2013b). No cosmeceutical acne treatment has worked. Traditional remedies have also been banned or discontinued due to negative effects. According to current trends, organic acid equivalents such benzoic acid, capric acid, azelaic acid, and others may be used topically to treat acne (Benitez, 1996). These biocompatible, biodegradable compounds have no history of microbial resistance or photosensitivity. Azelaic acid (AZA) is anti-inflammatory, anti-microbial, and stabilizing. AZA kills bacteria like P. acnes and S. epidermidis. AZA also stabilizes follicular infundibulum keratinocytes, preventing cell shedding, duct obstruction, and comedone development (Sieber and Hegel, 2013). By modifying the sebaceous gland's follicular milieu and establishing an unfavourable aerobic environment for P. acnes, the sickness is prevented from worsening and causing inflammation.

However, higher doses of AZA are needed to achieve the desired results, which might cause skin irritation, erythema, dryness, peeling, and scaling. AZA is water- and skin-insoluble (Gasco et al., 1991; Sieber and Hegel, 2013). Unfortunately, dose impacts API efficacy and adverse effects. Reduced dosages to reduce side effects reduce the medicine's therapeutic efficacy. In order to acquire a stable and secure AZA acne therapy, unique formulation strategies (liposomes, ethosomes, noisomes, fullerenes, SLNs, etc.) must be added to the arsenal. These technologies also improve acne treatment by targeting follicles and reducing concentrations. Physical/chemical instability, drug leakage, inadequate drug loading capacities, and scale-up issues restrict the previous systems' topical drug delivery capabilities.

SLNs and NLCs were found to overcome the limits without losing their therapeutic benefits. SLNs/NLCs, the next generation of lipid carriers, are biodegradable, stable, and can carry substantial bioactive loads. These provide controlled medicine administration and long-term drug supply. These new carriers gradually distribute medicine with enhanced skin penetration and decreased skin permeation, reducing systemic side effects.

METHODOLOGY

1. **Preliminary Evaluations of Azelaic Acid**

II. Spectrophotometric Analysis of Azelaic Acid

III. Development and Validation Of Hplc Method For The Determination of Aza

IV. In-Vitro Antimicrobial Activity of Azelaic Acid

2. Preparation of Nano Formulation (Slns/Nlcs)

3. Characterization and Evaluations of Lipid Carrier

PRELIMINARY EVALUATIONS OF AZELAIC ACID

PHYSIOCHEMICAL CHARACTERIZATION OF AZELAIC ACID

Melting point

Digital auto melting point equipment CE ISO 9001, Labtronics were used to measure it**.**

Differential Scanning Calorimeter (DCS)

DSC was used to assess the Azelaic acid (AZAthermal)'s characteristics (EVO 131,

SETARAM Instrumentations France). Aluminum crucibles were hermetically sealed/crimped and filled with 5-7 mg of the medication, with empty crucibles serving as a standard. The sample was heated in the range of 30–250 °C at a heating and cooling rate of 10°C min−1 and 20°C min−1, respectively**.**

FTIR-ATR

The frequency range of 4000-500 cm-1 was used to record the spectra of AZA powder using an ATR spectrometer (Alpha, Bruker, Berlin, Germany)..

Solubility AZA's solubility in several solvents, including ethanol, methanol, acetonitrile, and water, was determined.

SPECTROPHOTOMETRIC ANALYSIS OF AZELAIC ACID

Absorption spectra

On a Hitachi U-2900 double-beam UV-Visible spectrophotometer (Tokyo, Japan), a solution of AZA (1 mg/mL) was scanned across the wavelength range of 200-800 nm, and the wavelength corresponding to the peak maxima was recorded.

3.1.2.2 Preparation of calibration plot of AZA using UV spectrophotometer

Standard solution

In a volumetric flask, 100 mg of AZA were dissolved in 50 mL of methanol to create a stock solution containing 2 mg of AZA per mL. After that, methanol was used to dilute the AZA stock solution to create a series of dilutions ranging from 0.4 to 2 mg/mL. The spectrophotometer was used to measure the absorbance of these solutions, and a standard curve was drawn between absorbance and medication concentration.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined by the calibration curve method using the following equations:

$$LOD = 3.3\times\sigma/S \quad (1)$$

$$LOQ = 10\times\sigma/S \quad (2)$$

Where,

σ - Standard deviation of y-intercepts of regression line S - Slope of calibration curve.

The calibration curves were obtained by least square linear regression analysis.

DEVELOPMENT AND VALIDATION OF HPLC METHOD FOR THE DETERMINATION OF AZA

Instrumentation

The research used a Waters HPLC system with a 515 binary pump, a 2487 dual wavelength UV detector, and a rheodyne manual injector (20 l injection volume). Using a Kromacil 100-5C18 column (250mm x 4.6mm), AZA was separated by chromatography, and eluents were seen using a UV-VIS detector. Software called Empower 2 (Build 2154) was used for all analyses.

3.1.4.2 Stock solution

To create a solution containing 1 mg of AZA per mL, 50 mg of AZA were dissolved in 50 mL of mobile phase in a volumetric flask. To generate a range of dilutions ranging from 5-400 g/mL, this stock solution was further diluted using mobile phase. Table 3.1 shows the several factors that were taken into consideration and changed throughout HPLC testing.

CONCLUSION

Even though Acne Vulgaris (AV) is not life threatening, it nevertheless has an impact on the patient's social and psychological wellbeing. It is the eighth most prevalent skin condition, affecting approximately 650 million individuals

globally, according to reports. There are several pharmacological and cosmetic treatments for AV control, but none of them have been able to effectively treat or cure the disease. The problems with conventional preparations like antibiotics, retinoids, steroids, and peroxides (as well as those associated with bacterial resistance, formulation stability, and the occurrence of undesirable side effects (burning, stinging, photosensitivity, itching, etc.)) have either caused their removal from the market or led to their discontinuation as prescriptions.

Azelaic acid (AZA), in particular, is being researched recently as an option for the topical therapy of AV. With no past indication of microbial resistance or photosensitivity difficulties, AZA's biocompatibility, antibacterial, anti-inflammatory, and anti-keratinizing capabilities have promoted its use in the majority of impending commercial formulations, either as an active moiety or as an excipient. The therapeutic application of AZA has been limited, nevertheless, by low water solubility, skin penetrability (stratum corneum), and the incidence of side reactions as itching, burning, dryness, redness, etc.

Therefore, innovative approaches (such as liposomes, ethosomes, noisomes, fullerenes, etc.) are required to address the aforementioned problems and enable improved follicular targeting with regulated drug administration. In addition to serving as a stable and secure delivery vehicle, nano-emulsions, liposomal, and other new systems would improve medication targeting and effectiveness. However, the previous techniques' suitability as a topical drug delivery vehicle is limited by difficulties such physical/chemical instability, drug leakage, poor drug loading capacities, and scale-up issues.

Alternative next generation stable lipid nano carriers like SLNs and NLCs may carry greater drug payloads and have better skin penetration with less permeability than standard preparations. It is thus possible to assume that adding AZA to such nano-colloids would result in the development of a stable system with improved tissue targeting and anti-acne effectiveness.

The goal of the current inquiry was to construct and assess the anti-acne potentials of SLNs/NLCs that had AZA loaded upon them. The study's goal was to obtain regulated distribution of AZA with improved skin targeting and retention and fewer drug-related adverse effects. The work started with the creation of an analytical technique for the quantification of AZA. The Kromasil 100-5C18 column (250 4.6 mm; 5 m particle size) and high-performance liquid chromatography equipment from Water were used to create an isocratic technique. With the use of a 2487 dual wavelength ultra violet detector, the effluents were seen at 206 nm. A clear resolution peak was produced in an experiment that used mobile phase containing 75 volumes of sodium di-hydrogen orthophosphate (pH 3.5; 50 mM) and 25 volumes of acetonitrile, which varied in mobile phase ratios, pH, flow rate, and other factors. Within a concentration range of 5-400 g/mL, the calibration plot showed a linear relationship (correlation coefficient, r2 = 0.998). The approach was further evaluated for stability in accordance with ICH and CDER requirements and was found to be accurate (>96% recovery),

exact (%RSD 2), and robust (10% content difference). Under a variety of processing settings, the drug and stock solution shown high stability (>96% recovery) with no obvious signs of AZA degradation or instability. The simplicity, ease of sample preparation, and lack of a pre-derivatization step were all major benefits of the established HPLC approach. Additionally, when this technique was tested for the analysis of AZA in topical application, it produced a resolution peak (recovery of >97%) without any excipient interference, demonstrating its suitability for regular AZA analysis in pharmaceutical preparations.

Several in-vitro methods were used to assess AZA's physiochemical and antibacterial qualities. Melting and DSC investigations discovered that the meeting peaks occurred approximately 108°C, which was found to be inconsistent with earlier results confirming the purity of the sample used for the research. AZA (200 mg/mL) demonstrated greater inhibitory zones (ZOI) in contrast to standards (benzoyl peroxide; Benzac AC® and azelaic acid; Aziderm®), indicating a better antibacterial action of AZA. This was verified by agar diffusion study against P. acnes. AZA's MIC value was discovered to be 4 mg/mL using the spread plate technique.

Lipid nanoparticles (SLNs/NLCs) were prepared using a variety of nano-preparation techniques, including solvent injection, melt emulsification, low temperature solidification, melt emulsification, and ultra-sonication, using solid lipid, liquid lipid, and surfactants that had been pre-selected based on their solubility for the drug. Melt emulsification and ultra-sonication approach were discovered to be the most effective preparation procedures for SLNs and NLCs. In contrast to glyceryl monostearate, which produced a hazy/clear solution in the nano-metric range, stearic acid produced an unstable flaky preparation.

The approach was then pre-screened for several formulatory factors, and it was discovered that the lipid:drug ratio, the concentration of the surfactant, and the duration of the sonication had a substantial impact on the particle properties. Particle size (PS) and entrapment efficiency (EE) were used as dependent variables in a 33 complete factorial design (Design Expert 10) to further improve the process and identify the individual and combinatorial effects of these variables on formulation properties. Different models, including linear, 2FI, quadratic, and cubic, were applied to the data responses, and analysis of variance was used to assess the models' viability (ANOVA). The quadratic model showed the greatest match for the particle size (PS) and the percent entrapment efficiency in the mathematical equations created for the analyzed response variables of SLNs/NLCs (EE). The statistical study showed that the model had low PRESS values and closely linked predicted and adjusted R2 (0.2) values, which indicated the response surface model's stability and quality of fit. The Design program produced a set of 27 tests that showed PS to be between 177 and 535 nm and 41 and 105 nm, respectively, and entrapment efficiencies to be between 49 and 79% and 52 and 83.4% for SLNs and NLCs.

The formulations (SD and ND) proposed by the Design were discovered to have theoretically predicted particle properties that closely matched experimental data,

therefore proving the accuracy of the nano-preparation procedure. PS values were determined to be 189.23.37 nm and 49.61.24 nm, respectively, for formulations S14 (lipid:drug 7.5:1, surfactant 1.5% w/w, and sonication time 15.0 min), and N23 (lipid:drug 10:1, surfactant 1.5% w/w, and sonication time 15.0 min), while the percent EE values for each formulation were 78.42.63% and 8 These formulations were chosen for further research since it was determined that they were superior to the formulations (SD and ND) indicated by the Design.

The zeta potential values for SLNs and NLCs, respectively, were shown by physiochemical characterization of optimal preparations to be -13.21.02mV and -14.30.95mV, showing high physical stability. DSC thermo grams confirmed the sample purity used for the investigation. The lack of AZA endotherms and the decrease in enthalpy values that were seen in DSC thermo grams of nano-carriers (SLNs/NLCs) as opposed to excipients and their physical mixes further supported the shrinkage and creation of AZA encapsulated nanoparticles. The development of uniformly dispersed, spherical nanoparticles (SLNs 200 nm; NLCs 100 nm) was shown by TEM investigation. However, stability experiments showed that SLNs were unstable after 2 weeks at room temperature and 5 weeks in a refrigerator, but NLCs remained stable for 12 months in both environments. Therefore, only NLCs formulation was used in further research (in-vitro and in-vivo study).

The secondary vehicle (aloe-vera based carbopol gel) was combined with the optimized NLC preparation (N23) to investigate the therapeutic potentials. It was then tested for its physiological properties, drug content, in-vitro release, skin distribution (fluorescent microscopy), and anti-acne potential using various in-vitro and in-vivo models. According to the physiological characterization of nanogels, these gels are non-irritating, homogeneous, and have the ideal amount of moisture for spreading and occlusivity. The nano gel was further revealed by rheological study to exhibit shear thinning behavior, suggesting its simplicity in application and spreadability across skin surfaces. Studies on in vitro skin permeation revealed that NLC preparation had greater skin retentiveness compared to medication suspended in gel and commercial preparation with low skin penetration (10%). Rhodamine 6G was used in the skin dispersion investigations (Fluorescent and Confocal microscopy), which highlighted the target ability of NLCs to deeper skin layers.

The modest toxicity (10%) of NLCs with a safety profile comparable to that of commercial preparations and positive controls was further supported by cytotoxic experiments against S. crevasse. This demonstrated the gel's biocompatibility and better safety as well as therapeutic application on human skin. Histological analyses and in-vivo skin compatibility testing, such as the Draize patch test, further supported the findings that AZA-loaded NLC gels were safe, nontoxic, and well-tolerated in comparison to the marketed product.

When compared to commercially available products, in-vitro and in-vivo antimicrobial and anti-acne models showed a considerable reduction in inflammatory and microbiological activity from NLCs. The effectiveness of nano gels

in lowering inflammation and infiltrating inflammatory cells count with restoration of impaired skin physiology was also confirmed by histological analysis (acne skin). The NLCs gels seemed to be a viable delivery method for AZA with greater skin retention and safety in the treatment of acne vulgaris, according to all testing results.

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