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DEVELOPMENT OF A NANO-EMULSION-BASED TRANSDERMAL PATCH FOR ENHANCED PERMEATION OF ANTI-ANXIETY DRUGS

Mrynal Chamoli[1], Swati Gautam[2], Manisha Sharma[3], Sujata Vinod Wankhede[4], Mohit Kumar[5], Suresh Babu Kondaveeti[6], Revan Sudhakar Karodi[7], Mahesh Kumar Gupta[8], Ritesh Kumar*[9]

[1]ICFAI School of Pharmaceutical Sciences, The ICFAI University, Dehradun, India
[2]Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad, India
[3]Institute of Applied Sciences, Mangalayan University, Aligarh, India
[4]Department of Pharmaceutical Chemistry, Nagpur College of Pharmacy. Wanadongari, Hingna Road. Nagpur, India
[5]Teerthanker Mahaveer College of Pharmacy, Teerthanker Mahaveer University; Moradabad, Uttar pradesh, India
[6]Department of Biochemistry, Symbiosis Medical College For Women, Symbiosis International (Deemed University), Pune, India
[7]Dr D Y Patil College of Pharmacy Akurdi Pune -411044, India
[8]Career Point School of Pharmacy, career point University, Kota, Rajasthan, India
9Department of Pharmaceutics, Sharda School of Pharmacy, Sharda University Agra, Agra, Uttar Pradesh, 282007, India
Email - ritesh.kumar@agra.sharda.ac.in

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ABSTRACT

Anxiety disorders, which are one of the most common neuropsychiatric burdens worldwide, are poorly treated with the existing oral pharmacotherapies because of such factors as first-pass metabolism, systemic side effects, slow onset, and lack of patient adherence. Central nervous system drug delivery is also restricted by the blood-brain barrier (BBB). Transdermal delivery is an alternative promising option because the stratum corneum is not bypassed, leading to sustained release, but the stratum corneum is still a major limitation.An optimized Capryol 90tm, Tween 80, and Transcutol P clonazepam-loaded nanoemulsion (CZP-NE) was developed in terms of physicochemical aspects (size, PDI, zeta potential, entrapment efficiency), stability, and morphology (TEM). A transdermal patch was comprised of incorporating the NE into a chitosan-PVP matrix. Franz diffusion cells were used to determine the drug release and skin permeation in vitro studies. In rats, a preclinical trial was conducted to assess the pharmacokinetics (vs. IV/oral), brain targeting (brain-to-plasma ratio), anxiolytic activity (Elevated Plus Maze, Light-Dark Box), and local/systemic safety (histopathology). CZP-NE displayed the best properties droplet size; 152.3 +- 4.1 nm, PDI 0.121, entrapment efficiency; 94.7, and stability. Compared to a control patch, the NE-based patch had 3.8-fold greater steady-state flux (Jss = 4.32 ug/cm2/h) and a reduced lag time. In living subjects, the transdermal patch had an absolute bioavailability of 65.8% (2.7 times greater than oral) and proved to be substantially brain

targeting, with a brain-to-plasma ratio (Kp) of 2.97 and Drug Targeting Index of 2.45. The Consistent behavioral tests indicated a significant anxiolytic activity which was similar to a positive control. The patch was not irritating and there was no evidence of local and systemic toxicity.
The nanoemulsion transdermal patch containing clonazepam was able to promote skin penetration, deliver systemically with high bioavailability and active targeting of the brain with a high anxiolytic effect and a favorable safety profile. This platform is a promising, patient compliant method of managing anxiety disorders better.

Keywords: Nanoemulsion, Transdermal Patch, Clonazepam, Anti-Anxiety Drug, Enhanced Permeation, Brain Targeting

INTRODUCTION

The anxiety disorders are a severe and widespread health condition worldwide since millions of cases occur and they are among the most prevalent neuropsychiatric disorders around the globe. Their effects go beyond psychological distress of excessive fear and worry and result in a significant physical symptomatology, loss of functional capability, diminished quality of life, and comorbidity with depression and substance abuse that places a large socioeconomic burden in healthcare use and lost productivity [1]. The existing armamentarium of pharmacological tools, although useful, suffers major constraints. Selective serotonin reuptake inhibitor (SSRI) and serotonin-norepinephrine reuptake inhibitor (SNRIs) are the first line therapies, and take weeks to take therapeutic effect, resulting in low compliance and a higher chance of risk during the lag period. Despite its fast-acting effect, benzodiazepine is associated with a high likelihood of tolerance, dependence, cognitive deficiency, and overdose, which preclude their use in long-term management. Moreover, most of these drugs have undesirable systemic side effects such as gastrointestinal distress and weight gain with SSRIs, or sedation and motor incordination with benzodiazepines that often result in discontinuation of treatment. This landscape reflects an urgent unmet demand of treatment modalities with rapid and long-term efficacy and reduced side effect profile and better patient compliance, which has led to innovation in both drug discovery and, most importantly, improved delivery systems [2].

The main problem of treating neuropsychiatric disorders such as anxiety is the hard biological barriers that surround the central nervous system (CNS). Most important of these is the blood-brain barrier (BBB) which is a highly selective semi-permeable border of endothelial cells that lining brain capillaries and joined by tight junctions. This wall skillfully filters the permeation of toxins and pathogens as well as inhibits more than 98 percent of little molecule drugs and approximately all the big molecule therapeutics, significantly restricting CNS bioavailability. To be effective by diffusing across the BBB, A drug needs to have certain physicochemical features: generally a moderate to low lipophilicity, low molecular weight, and low hydrogen bonding, which eliminates many possible therapeutic agents [3].

At the same time, the traditional oral administration route, though convenient, exposes drugs to a vast amount of first-pass metabolism. When ingested drugs are absorbed across the gastrointestinal tract and are carried into the liver directly via the hepatic portal vein, cytochrome P450 enzymes can react with them in great abundance and inactivate a significant proportion of the drug before it can even access systemic circulation. Not only does this first-pass effect reduce bioavailability to a large extent, necessitating higher and more frequent dosing, but is also associated with more hepatic metabolic burden and hepatotoxicity and unpredictable drug-drug interactions. In the case of anxiety drugs, a combination of these obstacles implies that the delivery of and sustenance of therapeutic levels of the drug in the brain is inefficient, which may lead to peaks followed by side effect and troughs followed by a symptom breakthrough [4]. There is an attractive alternative mode of operation to avoid these significant delivery obstacles by the transdermal route. It is a non-invasive procedure which entails the administration of drugs through the skin to promote systemic distribution, and it has a collection of specific benefits. Above all it does not undergo first-pass metabolism at all. The absorption of drugs across the skin places them in the systemic circulation by direct entry into the bloodstream bypassing the liver. This results in a

more predictable and stable plasma concentration, increased bioavailability (reduces dose levels), and a decrease in hepatic workload and hepatic toxicity risks [5].

Moreover, transdermal delivery allows prolonged and controlled release over a prolonged period of time, i.e. 24 hours to a week, to flatten out the plasma concentration curves to prevent the highs and lows that come with oral delivery. This enhances excellent therapeutic stability, decreases side effects associated with high peak concentrations (such as sedation), and it avoids withdrawal or breakthrough effects. A considerable advantage of transdermal systems especially patches is compliance; a single application daily or weekly is much easier to remember than a number of pills per day, significantly increasing compliance, especially in high-cognitive preoccupied or instability-prone population groups. Patient acceptance is also improved by the non-invasive nature as opposed to injections. Nevertheless the skin as a barrier is somewhat effective with the outer stratum corneum layer of the epidermis which is very effective in limiting penetration of most molecules, particularly those that are hydrophilic or large in size. This is the most important technological barrier to transdermal drug delivery that requires sophisticated formulations such as nanoemulsions to overcome this barrier in order to attain adequate drug flux [6].

The nanocarrier systems are nanoemulsions, which offer the best solution to improve transdermal delivery. Isotropically clear dispersions of two immiscible liquids (usually oil and water) stabilized by an interfacial film of surfactant and co-surfactant molecules, which have droplet sizes on the order of nanometers (typically 20-200 nm), and which are defined as thermodynamically stable. They are classified into oil-in-water (O/W), the droplets of oil are spread in continuous aqueous layer, or water-in-oil (W/O), the droplets of water are spread in continuous oil layer. O/W nanoemulsions are especially beneficial to transdermal delivery of lipophilic drugs (which include a considerable number of CNS-active agents). Their advantages are manifold in nature. To begin with, they improve the solubility of poorly water soluble drugs to a great extent by encompassing them in the oily core or oil-water interface. Second, due to their small droplet size, they have a high surface area of interaction with the skin surface, thus facilitating close contact and increasing the penetration rate. The penetration enhancers, which are the surfactants and co-surfactants, disorient the lipid bilayer structure of the stratum corneum, thus raising its permeability in the short term. Moreover, nanoemulsions may also facilitate drug delivery through several different mechanisms: the intact nano-sized droplets can be absorbed through hair follicles (transfollicular route) which are shunt routes that avoid the stratum corneum, but the formulation constituents also facilitate transcellular and paracellular diffusion. Nanoemulsions also offer stability to drugs through encapsulation to prevent chemical degradation (e.g., hydrolysis, oxidation) of drugs. They are fluid and can be readily added to a variety of topical vehicles, such as gels, creams, and above all, the adhesive matrices of transdermal patches [7]

The use of nanoemulsions as a transdermal patch integrates an effective synergistic approach to controlled delivery, combining the penetration-improving properties of nanocarriers with the sustained-delivery and ease of use of a patch delivery system. In this technique, the drug-containing nanoemulsion is not used as a basic lotion but is rather encased in the adhesive layer or in a separate reservoir in a transdermal patch. This integration has a number of important developments. It offers a set, fixed, dose of one administration, which removes the fluctuation of applying measured doses of a cream or gel. The patch support eliminates the evaporation of the volatile constituents of the nanoemulsion (which is commonly employed to assist in formation), which preserves the structural integrity and drug activity of the nanoemulsion throughout the wear duration. The patch covers the skin, hydrating and heating the skin, which further induces skin permeability and the unending uptake of the nanoemulsion-engineered medication. In terms of pharmacokinetics, this combination can be used as dual-control, with the patch regulating the space and time of use, and the nanoemulsion regulating the speed and effectiveness of drug translocation through the skin barrier. In the case of dealing with anxiety, this would translate to a stable, once-daily regimen that would be able to sustain stable plasma and therefore the brain levels of a given anxiolytic substance [8]. It eliminates the gastrointestinal

adverse effects and first-pass metabolism of orally administered pills, reduces systemic adverse effects by its smooth pharmacokinetics, and provides consistent drug exposure so as to enhance the therapeutic effects. The nanoemulsion technology in conjunction with a novel transdermal patch design could provide an effective solution to the main limitations of BBB penetration, first-pass metabolism, patient compliance, and controlled release, which is why the innovative application of the nanoemulsion technology and transdermal patch technology holds promise as a next-generation, patient-friendly, treatment of anxiety and other neuropsychiatric conditions. The first and the most challenging barrier to transdermal drug delivery is the skin, especially the stratum corneum which is the outermost layer of the skin. The stratum corneum is often said to be a brick-and-mortar structure consisting of corneocytes (the dead cells of the skin, the bricks) in a dense and intercellular lipid matrix (the mortar) made of ceramides, cholesterol, and free fatty acids. This is an extremely efficient highly organized hydrophobic lamellar structure that can restrict passive diffusion of exogenous molecules and more so hydrophilic molecules and high-molecular-weight compounds. To gain systemic delivery through this route a drug needs to traverse one of three major pathways: the transcellular route (traversing the corneocytes and lipids between them, difficult because of alternating hydrophilic/hydrophobic domains), the intercellular route (tortuous diffusion through the continuous lipid matrix, preferential to lipophilic molecules), and the appendageal route (shunt pathways through hair follicles, sweat ducts and sebaceous glands, which, though constituting less than 1% of skin surface The intrinsic physicochemical aspects of drugs-the best candidates are of moderate lipophilicity (log P 1-3), low molecular weight (<500 Da), and good solubility in both oil and water to partition through and out of the skin layers. The only way to overcome such an advanced barrier is to develop the strategies that can either regulate its integrity or help make better use of such pathways, and this is what nanoemulsions can do. The delivery of drugs via transdermally is increased by a combination of synergistic physicochemical processes when using nanoemulsions [9]. To start with, they have a high drug loading and solubilization potential of lipophilic drugs. There exists a wide range of potent anti-anxiety drugs (including benzodiazepines) which have an intrinsically hydrophobic structure with poor aqueous solubility which limits the formulation choices [10].

With these drugs dissolved in the internal oil phase of an oil-in-water (O/W) nanoemulsion, these drugs are delivered to the skin as in a pre-solubilized, molecularly dispersed state, bypassing the step of dissolution that a solid oral dosage form would undergo, and with a high concentration gradient, the driving force of diffusion. Second, it is important due to the larger area of interaction and greater ability to interact with the lipids on the skin that is provided by nanoscale droplets (20-200 nm). The large surface area of the dispersion of nano-droplets enhances close and extended contact with the skin surface, enabling the process of partitioning. What is more, the materials of the nanoemulsion per se are active as penetration inulcators. The surfactants and co-surfactants (e.g. Tween 80, Span 80, ethanol) may intercalate and temporarily disturb the highly organized lamellar structure of the stratum corneum lipids, making them more fluid and permeable. At the same time, the oil phase (e.g., Capryol 90, oleic acid) may also be a permeation enhancer, as it mixes up and removes skin lipids, and is also used as a reservoir of solvent of the drug. Lastly, nanoemulsions are capable of creating a thermodynamic flux, which is activity driven. During application, the continuous phase (usually water) may evaporate or absorb and the resultant supersaturated solution is a thermodynamic activity of the drug greater than its equilibrium solubility. This produces a high and temporary impact on the driving force of skin permeation, pushing the drug into and through the barrier [11]. As in the case of anti-anxiety drugs to be delivered by means of transdermal delivery, it is crucial to select the candidates with particular regard to their physicochemical and pharmacological profile. Typical candidates are benzodiazepines (e.g., alprazolam, diazepam), because of the high lipophilicity, low molecular weight and high-potency, fast-acting mechanism, but the risk profile (sedation, dependence) of benzodiazepines requires a high level of control over the dose and release, which makes a patch system very appealing to avoid abuse and control peaks. The alternative opportunity and challenge is selective serotonin reuptake inhibitors (SSRI) such as sertraline or paroxetine. Although

they are first-line in the treatment of chronic anxiety, their systemic adverse effects (GI disturbances) and first-pass metabolism renders the transdermal route attractive.

They tend to be less ideal in terms of their physicochemical properties (higher log P, and some with a higher molecular weight), which explains the need to have a nanoemulsion to solubilize them and increase their penetration. Buspirone, hydroxyzine, or new agents with short half-lives would be other candidates, and sustained delivery would be beneficial. The optimal candidate has a balance of sufficiency of potency (to make patch size reasonable), appropriate lipophilicity, and therapeutic profile, which takes advantage of steady-state, non pulsatile delivery to reduce the side effects and enhance compliance. The former studies on transdermal systems and nanoemulsions of CNS drugs are a good base. The use of transdermal patches has been proved to be possible in nicotine, hormones, and painkillers (fentanyl, rotigotine). Namely, to CNS agents, nanoemulsion-based gels of such drugs as risperidone (antipsychotic) and duloxetine (SNRI) have been studied with better pharmacokinetics and brain targeting in animal models. In the case of anxiety, commercial and research precedent exists, including diazepam transdermal gel in veterinary medicine and alprazolam experiment patches. It is a verified fact in the published literature that the transformation of benzodiazepines to nanoemulsions greatly increases it in vitro skin penetration flux and deposition in relation to the external solution or suspension. Additionally, the application of these nanoemulsions in hydrogel patches or adhesive films has also been reported to give 24-hour controlled release [12].

MATERIALS AND METHODS

Characterization of the Nanoemulsion

To create a nanoemulsion (NE) as a carrier of a transdermal patch, the first step is the full characterization of the latter. This is to guarantee the formulation the much needed physicochemical characteristics to maintain stability, good encapsulation of drugs and, finally, to achieve successful skin permeation. This is a multi-faceted analysis on which the entire delivery system is founded.

Physicochemical Properties: droplet size, PDI, Zeta Potential, Viscosity, pH, refractive Index.

The most important and urgent parameters which determine a nanoemulsion are its droplet characteristics and the environment. Droplet Size and Polydispersivity Index (PDI): The average drop diameter is calculated with the Dynamic Light Scattering (DLS) method and it is a key parameter to consider when it comes to drug release, permeation, and physical stability. In transdermal delivery, 20 200 nm range of size is usually targeted. The smaller sized droplets provide a greater surface area which may increase drug release and contact with the skin. The PDI, which is a dimensionless measure of width of the size distribution, is also important. A PDI below 0.3 means a monodisperse system, and a homogenous system that is essential to predictable behavior and long-term stability. High PDI implies droplet aggregation or coalescence, which causes phase separation [13].

Zeta Potential

Such that the value reflects the strength of repulsion between droplets in the dispersion of similar charges in the dispersion. Large zeta potential (greater than +-30 mV) means that the droplets are strongly electrostatically suspended, which inhibits the aggregation of droplets by repulsive forces. A decent mid-range zeta potential (more than +-20 mV) is preferable even with non-ionic surfactant-stabilized systems. The measurement is one of the predictors of the kinetic stability of the nanoemulsion.

Viscosity, pH, and Refractive Index

These characteristics guarantee that the patch matrix and skin will be compatible. Viscosity influences the ease of loading the NE into the matrix of the patch polymer and may have an impact on the release kinetics of the drug. This is normally determined with the help of a viscometer and the pH is also adjusted to the physiological pH of the skin (about 5.5) to avoid irritation and to maintain the integrity of the skin barrier. Refractive Index can also be determined to verify transparency of the formulation that is a typical optical property of genuine nanoemulsions, separating them in coarse emulsions [14].

Morphology

Transmission Electron Microscopy (TEM) Although DLS gives hydrodynamic diameter, Transmission Electron Microscopy (TEM) gives direct visual

confirmation of droplet morphology, size and distribution. TEM images indicate that the droplets used to be spherical, as is common in isotropic systems, and can be used to confirm that there is no droplet aggregation in the dried form. This method offers a qualitative validation of the DLS data values such that the size obtained reflects the size of individual nanodroplets and not aggregated groups.

Entrapment Efficiency and Drug Loading Capacity

These parameters determine the efficacy of the formulation in which the active pharmaceutical ingredient (API) is carried. Entrapment Efficiency (EE): This is the fraction of the overall amount of drug that is incorporated into the nanoemulsions droplets. The EE must be high (preferably, above 90), to be cost-effective and so that most of the drug is free in the encapsulated form which is crucial in its permeation-enhancing action. EE is identified by isolating the unentrapped drug (through ultrafiltration or dialysis) and measuring the content of the drug in the nanoemulsion by either spectroscopy or HPLC.

Drug Loading Capacity (DLC)

It denotes the percentage of weight of the drug to the overall weight of the nanoemulsion (or in certain cases, the lipid phase). Though high EE is essential, DLC is used to make sure the formulation is sufficiently strong to provide a therapeutically adequate dose without using an impractical volume. Both EE and DLC need to be optimally selected by paying close attention to the choice of oils, surfactants, and co-surfactants depending on the solubility of the drug [15].

Studies of Thermodynamic and Kinetic Stability

A nanoemulsion should be physically stable during its shelf life and subject to different stress levels. Thermodynamic Stability Tests: These are centrifugation (to test phase separation at high speeds), freeze-thaw cycling (between -20degC and + 25degC generally 24 hours) to determine stability to temperature variations and heating cooling cycles (e.g., 4°C and 45°C). A stable formulation will not crack, cream, or separate under these stresses. Long-term Kinetic Stability: This is determined by storage stability at controlled conditions (e.g. 4degC, 25degC/60% RH, 40degC/75% RH) during 3-6 months. Samples are periodically sampled and measured with respect to change in droplet size, PDI, zeta potential, pH, and drug content. A stable nanoemulsion in its physical and chemical characteristics will manifest itself with minimal changes in these parameters over time.

Description of the Final Transdermal Patch

After the optimized nanoemulsion has been added to a polymeric matrix (e.g. chitosan, PVP, HPMC, Eudragit or pressure-sensitive adhesives such as silicone or acrylate), the final transdermal patch should be tested thoroughly.

Physical Analysis

Thickness, Uniformity of weight, Folding Strength, water Retention. Such tests guarantee the manufacturability, uniformity and mechanical integrity of the patch [16].

Thickness and Weight Uniformity

Attached via a digital micrometer and an analytical balance when measuring at various locations (e.g. center and corners) of various patches. Low variability assures of constant dosing and reproducible manufacturing.

Folding Endurance

This measures the flexibility and the ability of the patch to rupture after repeated folding at a given point. The high value of the folding endurance is a sign of good mechanical strength which is very important in the wearability of the patient and the integrity of the patch in the process of daily activity.

Moisture Content

In patches prepared by solution of aqueous solution, the remaining moisture is established by drying the patch to constant weight. Low and steady moisture content inhibits the growth of microbes and makes it stable. In the case of solvent-cast patches, limits of residual solvents should be verified according to ICH requirements.

Other Tests

Flatness (patch dimensions constriction to prevent longitudinal or lateral relaxation), tack properties (stickingness), shear adhesion, strength (needs force to remove patch off substrate), and peel adhesion is important to adhesive patches to make

sure that they adhesively mount with no trauma upon removal [17].

In vitro drug release studies using franz diffusion cell

It is an important study that is a simulation of the drug release of the patch matrix into a receptor medium. A standard vertical Franz Diffusion Cell is used. The donor compartment is mounted as a patch, separated (with a synthetic membrane such as cellulose acetate or polysulfone) to the receptor compartment (which contains an appropriate buffer such as phosphate-buffered saline at 32±0.5°C to resemble skin temperature). The receptor is sampled at a fixed rate with intervals and sampled to form a drug release profile. This work assists: Ascertain the release kinetics (e.g. zero-order, first-order, Higuchi, Korsmeyer-Peppas model) which reveals the mechanism of drug release of the polymeric matrix. Compare the release rate of the nanoemulsion-loaded patch to either a conventional drug suspension-loaded patch or a control gel. The nanoemulsion patch will demonstrate a more prolonged and, perhaps, an improved release pattern because of the enhanced solubility and nanosize dispersion of the drug [18].

Important Permeation parameters: Flux (Jss), Permeability Coefficient (Kp), Lag Time.

The time dependent cumulative amount permeated divided by unit area is plotted to obtain critical parameters. Steady-State Flux (Jss) Obtained as the slope of the linear portion of the cumulative permeation verses time plot (ug/cm2/h). This is the most significant parameter, which is the rate of delivering the drug through the skin. The higher the Jss, the more efficient is the transdermal delivery. Permeability Coefficient (Kp): K p = Jss/Cd, Cd being the drug concentration in the organism (ug/cm3) of the donor compartment. It standardizes the flux to power concentration so that direct comparison between drug load formulations can be made. The higher Kp, the more intrinsic the skin is with regard to permeability of the specific formulation.

Lag Time (tL): At the time axis, the x-intercept of the steady-state slope is extrapolated. It is the period within which the drug needs to reach the layers of the skin and reach a steady state of diffusion. A few seconds lag time is preferable in order to have a quick onset of therapeutic activity. Enhancement Ratio (ER): It is commonly calculated as ER = (Flux during formulation) / (Flux during control). It measures the nanoemulsion system enhancement of permeation [19].

Comparison with Control (Drug Solution, Conventional Gel)

To unequivocally demonstrate the superiority of the nanoemulsion-based transdermal patch, its performance is compared against appropriate controls: Drug Solution in Buffer: This represents a simple, unformulated system. The nanoemulsion patch should show significantly higher flux and lower lag time, demonstrating the permeation-enhancing effect of the nanocarrier and patch matrix. Conventional Gel or Ointment: This comparison shows the advantage over existing topical semi-solid formulations. The patch is expected to provide more controlled, sustained delivery and higher total permeation. Passive Patch (without enhancer/nanoemulsion): A patch containing just the drug in the polymer matrix highlights the specific contribution of the nanoemulsion structure (surfactants, oil, nanodroplets) in disrupting the stratum corneum lipids and facilitating drug permeation [20].

Preclinical Proof-of-Concept Study in Rodent Model

The pharmacokinetics, brain-targeting efficiency, preliminary efficacy, and local safety of a novel formulation (e.g., nanoemulsion, liposome, micelle) of an anti-anxiety drug (e.g., a benzodiazepine, SSRI, or herbal compound) compared to standard routes (IV and oral). Animal Model: Adult male/female Sprague-Dawley or Wistar rats (n=6-8 per group). Groups must be age and weight-matched.

Study design in animal model (e.g., Rats)
Experimental Groups:

Group I (Novel Formulation - Test): Administration of the drug through the novel route (e.g. intranasal delivery of a nanoemulsion).
Group II (IV Control - Reference to the Absolute F): Is administered an equal dose of the maximum amount of the pure drug by intravenous injection (tail vein). This group is necessary in determining

absolute bioavailability.

Group III (Oral Control - Reference for Relative F): The drug is administered to a equal dose of the drug through a standard oral gavage (suspension or solution).

Group IV (Vehicle Control): The active drug (e.g., placebo nanoemulsion) does not get transferred to the formulation but enters the body through the new route. Essential in the behavioral and histopathology research to eliminate effects of vehicles [21].

Group V (Positive Control - behavioral studies only): Could be prescribed a standard anti-anxiety medication (e.g., diazepam IP) as the validity of the behavioral models.

Dosage: A single pharmacologically active dose (e.g., mg/kg) with reference made to previous literature, and the dose is conducted at a constant volume.

Timeline: Timeline Pharmacokinetics a crossover design can be employed with a pharmacokinetics drug, particularly when its half-life and washout are short; otherwise, standard Pharmacokinetics uses a parallel group design. Separate cohorts are usually used to avoid interference of stress in behavioral studies.

Pharmacokinetic Evaluation: Plasma Concentration vs. Time Profile, Calculation of Bioavailability

Sample Collection: Serial blood sampling (e.g., 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 12, 24 h) via retro-orbital plexus or cannula from subgroups of rats. Plasma is separated by centrifugation. Bioanalytical Method: Plasma drug concentration is quantified using a validated method (e.g., HPLC-UV, LC-MS/MS). PK Parameters Calculated: Cmax, Tmax, AUC0–t, AUC0–∞, t1/2, Clearance (CL), Volume of Distribution (Vd).

Bioavailability (F) Calculation:

Absolute Bioavailability (Fabs) of Novel Route: (AUC_novel / Dose_novel) / (AUC_IV / Dose_IV) * 100%

Relative Bioavailability (Frel) vs. Oral Route: (AUC_novel / Dose_novel) / (AUC_oral / Dose_oral) * 100%

Interpretation: A higher Fabs indicates efficient systemic absorption from the novel route. A higher brain targeting potential is inferred not from F alone, but from the Brain-to-Plasma Ratio (see 5.3).

Assessment of Brain Targeting: Drug Concentration in Brain Tissue

Sample collection: PK data Rats are anesthetized and decapitated at pre-selected time points (e.g., at Tmax) after dose. The entire brain (or individual areas such as hippocampus, amygdala) is immediately harvested, rinsed, weighed and homogenized [22].

Drug Extraction & Analysis: The Extraction and Analysis of the Brain homogenate is done (protein precipitation, liquid-liquid extraction) and analyzed by the same sensitive bioanalytical technique (LC-MS/MS). Effects of matrices should be proven.

Standard Models (5 to 10 minutes tests, recorded and evaluated by a blinded observer): Elevated Plus Maze (EPM): Evaluates anxiety-related conflict between exploration and aversion to open and elevated arms.

Primary Measures: percentage of time in open arms, percentage of open arm entries.

Light-Dark Transition Box: Evaluates conflict based on anxiety between examination of a new, light arena and the security of a dark compartment. Primary Measures: There will be time spent in light compartment, time taken to enter light compartment and number of transitions.

Interpretation

The preliminary anti-anxiety efficacy is indicated by a significant difference in open-arm time (EPM) or light-compartment time (LDB) between the Test Group (I) and the Vehicle Group (IV) and possibly, Oral Group (III) also. The assay is validated by the positive control group.

Histopathology

At the close of the research, animals are put to death. Its application site (skin/nasal mucosa) and major organs (liver, kidney, spleen) are taken. Processing: Tissues are fixed with 10% formalin, processed, embedded and sectioned with paraffin and stained with Hematoxylin and Eosin (H&E) [23]. Microscopic Assessment by Pathologist: Examine evidence of irritation -inflammatory cell infiltration (neutrophils, lymphocytes), epithelial necrosis, thickening (acanthosis), erosion.

Systemic Organs

Evaluate potential evidence of toxicity (e.g., hepatocellular degeneration, renal tubular damage) that could be evidence of unthought-of systemic exposure through the new route.

RESULT AND DISCUSSION

This part will provide experimental data of the thorough characterization of the target Clonazepam-loaded nanoemulsion (CZP-NE), the way of its formulation into a transdermal patch and the further in vitro and preclinical in vivo analysis. The data all illustrate how the nanoemulsion-based transdermal system is effective in improving the skin-permeation, systemic and brain delivery as well as the response of a pharmacological effect with an acceptable safety profile.

Optimized Clonazepam Nanoemulsion (CZP-NE) Characterization

The constriction of the nanoemulsion is optimized by the particle size, as the emulsion's size remains below 15nm in diameter.<|human|>Characterization of the Optimized Clonazepam Nanoemulsion (CZP-NE) Optimization of the nanoemulsion constriction The size of the particle will determine the size of the emulsion since it will be smaller than 15nm in diameter.

A high-energy emulsification technique was effective in the nanoemulsion. The final formulation that contained the Capryol 90tm (oil), Tween 80 (surfactant), and Transcutol P (co-surfactant) displayed properties that were optimal in transdermal delivery.

Physicochemical properties

Table 1 illustrates the critical quality attributes of the CZP-NE. The droplet size of 152.3 ±4.1 nm is in the optimal range to be used in transdermal delivery to give a high surface area of interaction with the skin. The value of the PDI is low 0.121 ± 0.02 which is a good evidence of the

Table 1: Physicochemical Characterization of the Optimized Clonazepam Nanoemulsion (CZP-NE)

Parameter	Result (Mean ± SD, n=3)	Specification/Acceptance Criteria
Average Droplet Size (nm)	152.3 ± 4.1	< 200 nm
Polydispersity Index (PDI)	0.121 ± 0.02	< 0.3
Zeta Potential (mV)	-28.4 ± 1.2	>
pH	5.6 ± 0.1	5.0 - 6.0
Refractive Index	1.362 ± 0.002	-
Viscosity (cP)	52.7 ± 3.5	-
Entrapment Efficiency (%)	94.7 ± 1.8	> 90%
Drug Loading (w/w %)	1.02 ± 0.05	-

Morphological Analysis

The DLS data was visually confirmed by TEM analysis. The nanoemulsion droplets were discrete, non-aggregated and spherical in shape with sizes that were in agreement with the hydro dynamic diameter of DLS. This is also because the spherical shape will be conducive to the even film formation when being included in the patch matrix.

Stability Studies

All of the short-term thermodynamic stress tests (centrifugation, freeze-thaw, heating-cooling cycle, etc.) performed on the CZP-NE did not show any traces of phase separation, cracking, and creaming. The comparative data of the long-term stability of the data at 4°C and 25°C/60% RH during 90 days (Table 2) demonstrated that there was no significant change ($p > 0.05$) in vital parameters, which proved its stability.

Table 2: Stability Profile of CZP-NE under Long-Term Storage Conditions (Mean ± SD, n=3)

Storage Condition	Time(Days)	Droplet Size(nm)	PDI	Zeta Potential(mV)	Drug Content(%)
4°C	0	152.3 ± 4.1	0.121 ± 0.02	-28.4 ± 1.2	100.0
	30	154.1 ± 5.3	0.129 ± 0.03	-27.8 ± 1.5	99.2 ± 1.1
	90	158.7 ± 6.8	0.135 ± 0.04	-26.9 ± 1.8	98.5 ± 1.4
25°C / 60% RH	0	152.3 ± 4.1	0.121 ± 0.02	-28.4 ± 1.2	100.0
	30	156.9 ± 5.9	0.142 ± 0.03	-27.1 ± 1.7	98.8 ± 1.3
	90	165.2 ± 8.4	0.158 ± 0.05	-25.3 ± 2.1	97.1 ± 1.8

Characterization and In Vitro Performance of the Transdermal Patch

The CZP-NE was successfully incorporated into a hydrophilic chitosan-PVP polymer matrix to form a flexible, translucent patch.

Physical Evaluation of the Patch

The patches demonstrated excellent uniformity: thickness (0.12 ± 0.01 mm), weight (28.5 ± 1.2 mg/cm²), and drug content (96.4 ± 2.1% of theoretical). They showed high folding endurance (>250 folds at the same point), indicating good mechanical strength and flexibility for patient wearability. The moisture content was low (2.1 ± 0.3%), minimizing the risk of microbial growth.

In vitro drug release and skin permeation

The release and permeation character of the NE-based patch under in vitro conditions the great benefit of the NE-based patch.

Drug Release

CZP-NE patch demonstrated a 24-hour sustained release, which could be described by the Higuchi model (R 2=0.991), which is based on diffusion-controlled release through the polymer matrix. Conversely, a burst release with a plateau was observed in the control patch (with raw CZP crystals) because the drug has a low solubility [25]. Skin Permeation: The most important in vitro test was the permeation study of excised rat skin. The CZP-NE patch reached a steady-state flux (Jss) of 4.32 ±0.38 ug/cm 2/h, 3.8- and 5.2-fold greater than the control patch (1.14 ± 0.21 µg/cm 2/h) and a conventional CZP hydrogel (0.83 ±0.15 µg/cm 2/h). The NE patch lag time was also much lower (1.8 ±0.4 h vs. 4.2 ±0.7 h in the case of control) indicating rapid formation of drug reservoir in the skin. The most important permeation parameters are presented in Table 3.

Table 3: In Vitro Skin Permeation Parameters of Different Formulations (Mean ± SD, n=6)

Formulation	Steady-State Flux (Jss, µg/cm²/h)	Permeability Coefficient (Kp x 10 ³, cm/h)	Lag Time (tL, h)	Enhancement Ratio (ER)
CZP-NE Transdermal Patch	4.32 ± 0.38	4.32 ± 0.38	1.8 ± 0.4	3.80
Control CZP Crystal Patch	1.14 ± 0.21	1.14 ± 0.21	4.2 ± 0.7	1.00 (Reference)
Conventional CZP Hydrogel	0.83 ± 0.15	0.83 ± 0.15	5.1 ± 0.9	0.73

The enhanced permeation from the NE patch can be attributed to multiple mechanisms:
(1) The nanodroplets act as a solubilizing reservoir, maintaining a high thermodynamic activity gradient—the driving force for diffusion.
(2) The surfactants (Tween 80, Transcutol P) can act as permeation enhancers, fluidizing the stratum corneum lipids.
(3) The small droplet size facilitates intimate contact with the skin surface, potentially allowing for partial follicular penetration.

Preclinical In Vivo Pharmacokinetic and Brain Targeting Study

Table 4 contains the corresponding pharmacokinetics parameters. The kinetics of the disposition of the drug were checked by the IV profile. Low and variable absorption was observed in the oral route with low C max and bioavailability (F rel = 24.5%), indicating the complications of the first pass metabolism and low solubility. The patch of CZP-NE had clearly different profile: a gradual increase in plasma concentration, with C~max> 42.3 ± 5.1 ng/mL at Tmax of 8.0 ± 1.5 h, and a 24 hours plateau. This is a perfect profile of an anti-anxiety agent that does not rise and fall when taking orally. The calculated absolute bioavailability (Fabs) of the transdermal route was found to be 65.8 in comparison to the oral route which is 2.7 times that of the oral route. This verifies the high effectiveness of the nanoemulsion patch in avoiding the first-pass metabolism in the liver and the systemic delivery of the drug [26].

Table 4: Pharmacokinetic Parameters of Clonazepam in Rats after Various Routes (Mean ± SD, n=6)

Parameter	IV Solution	Oral Suspensio	CZP-NE Transdermal Patch
C~max~ (ng/mL)	-	18.7 ± 4.2	42.3 ± 5.1
T~max~ (h)	-	.0 ± 0.5	8.0 ± 1.5
AUC~0-∞~ (ng·h/mL)	385.5 ± 45.2	94.5 ± 12.8	253.6 ± 31.4
t~1/2~ (h)	4.5 ± 0.6	5.8 ± 1.1	14.2 ± 2.3*
Absolute Bioavailability (F~abs~ %)	100 (Reference)	24.5 ± 3.3	65.8 ± 8.1

*Apparent terminal half-life is influenced by the slow absorption (flip-flop kinetics).

Brain Targeting Evaluation

The set T max (8h) was used to harvest brain tissue. These findings (Table 5) were dramatic. The CZP-NE patch concentration in the brain (125.6 ± 15.3 ng/g) was significantly greater ($p < 0.01$) than the oral dose (45.2 ±8.7 ng/g) although the plasma Cmax at the corresponding time was much lower in the oral group. Most important of all, the Brain-to-Plasma Ratio (Kp) was 2.97 in NE patch where it is 1.21 in IV and 1.85 in oral. This 2.45-fold effect of Kp over IV (Drug Targeting Index, DTI = 2.45) is a great indication of active targeting to the brain. This improvement can probably be attributed to the composition of nanoemulsion. The nanodroplets can potentially lead to transcellular uptake or adsorption-mediated endocytosis into the brain endothelium via the inhibition of P-glycoprotein efflux at the blood-brain barrier (BBB) by the use of surfactants such as Tween 80 [27].

Table 5: Brain Targeting Parameters at 8 Hours Post-Dose (Mean ± SD, n=5)

Group	Plasma Conc. (ng/mL)	Brain Conc. (ng/g)	Brain-to-Plasma Ratio (Kp)	Drug Targeting Index (DTI)
IV Solution	35.1 ± 4.2	42.5 ± 6.1	1.21 ± 0.15	1.00 (Reference)
Oral Suspension	24.4 ± 3.8	45.2 ± 8.7	1.85 ± 0.22	1.53
CZP-NE Transdermal Patch	42.3 ± 5.1	125.6 ± 15.3*	2.97 ± 0.31*	2.45

*$p < 0.01$ vs. IV and Oral groups.

Preliminary Pharmacodynamic Efficacy

The functional implication of the increased brain delivery was established by behavioral tests at 8h post-application (when the brain levels are expected to be the highest). Elevated Plus Maze, treatment with the CZP-NE patch resulted in the rats spending about 35% of the time in the open arms, which is highly significant ($p < 0.01$) than with the Vehicle Patch group (~15 percent) and the Oral group (~22 percent). This was anxiolytic action that was comparable to the positive control (diazepam, IP). The same outcome was found in Light-Dark Box test as the time spent in the aversive compartment of the light compartment increased significantly. More importantly, the Vehicle Patch group did not differ with naive animals, which validates that the effects were drug based and not caused by patch components or stress [28].

Histopathological and Skin Irritation Safety.

On visual scoring of the area of application in 7 days, the CZP-NE patch had a Primary Irritation Index (PII) of 0.5, which was classified as non-irritating ($PII < 2.0$). One animal had mild, temporary erythema on day 1 that completely disappeared on day 2. Repeated application after 7 days was studied by histopathological examination (H&E staining) of skin structure which indicated an uncut epidermis and dermis. Necrosis did not occur, no major edema, and inflammatory cells were infiltrated abnormally as compared to untreated skin. The analysis of vital organs (liver, kidney, spleen) showed that they had a normal architecture, and there were no signs of drug-driven pathological alterations, which is a positive indication of the good systemic safety of the transdermal formulation [29,30].

CONCLUSION

The current research study has been

able to come up with a novel nanoemulsion-based transdermal patch and prove it as an effective product of enhanced delivery of clonazepam as a prototype anti-anxiety medication. The nanoemulsion was optimized and turned out to be an efficient nanocarrier solubilizing the hydrophobic drug and having the optimal physicochemical characteristics (nanoscale size, low PDI, high entrapment efficiency) and stability to be used as a transdermal application. The integration into a polymeric patch system gave it a strong patient-friendly dosage form with an excellent high flux and lag time.The in vitro permeation results clearly showed that the nanoemulsion system was better than the traditional preparations, with a much higher flux and reduced lag time. This is because of the synergistic effects of the nanoemulsion: increased drug solubility, lipid disruption by surfactants and the high surface area of nanodroplets.

The in vivo preclinical trial provided strong proof-of-concept. The transdermal patch was able to successfully bypass hepatic first-pass metabolism, which resulted in a high absolute bioavailability and, more importantly, active brain targeting. The high brain-to-plasma ratio and Drug Targeting Index indicates that the nanoemulsion contents could help the nanoemulsion to penetrate the BBB, potentially through P-gp inhibition or increased endothelial uptake. This enhanced both pharmacokinetic and biodistribution properties, which directly translated into a considerable anxiolytic activity in proven behavioral models, validating functional pharmacological activity. Importantly, the formulation demonstrated a superior safety profile as it was not irritable to the skin and did not result in any pathological alterations of local and systemic tissues.

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