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Review Article

Calcipotriol (Vitamin D Analogue) Loaded Coconut Oil Based Nanoemulgel for the Treatment of Psoriasis and its Future Research Perspectives

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Abstract

Psoriasis disease is manifested with the inflammation on skin and is characterized by the formation of plaques. The present article aims at the utilization of calcipotriol (synthetic vitamin D analogue) as therapeutic agent using coconut oil based nonoemulgel for the treatment of psoriasis. Calcipotriol acts by the inhibition of the cell growth like keratinocyte proliferation. Coconut oil consisting mostly of saturated fatty acids like lauric acids exhibit anti-inflammatory properties and suppress the inflammatory markers like TNF-α and Interleukin-6. Thus Calcipotriol and coconut oil exhibit synergistic action for the treatment of symptoms of psoriasis formulated in the form of nanoemulsion based gel. Thus this article directs the possibility of the use of calcipotriol loaded coconut oil based nanoemulsion gel for the treatment of psoriasis and its future research perspective.

Keywords: Calcipotriol; Coconut Oil; Nanoemulgel; Psoriasis; Inflammation

Introduction

Psoriasis is an inflammatory skin disorder characterized by the formation of plaques. It is an auto immune system related hyperactivity which later develops into a serious skin disease [1]. The present article discusses the formulation aspects of nanoemulsion gel of calcipotriol as a therapeutic drug using coconut oil as an oily phase of the emulsion system which has research prospect to be useful in the treatment of psoriasis. The formulated nanoemulsion is converted into a gel dosage form so that it is readily applicable on the affected skin areas in the psoriatic manifestation. Psoriasis exhibits thicker scales than that of eczema [2]. Coconut oil moistuirizes the affected skin that helps to cure the dryness, scaling and itching observed in psoriasis disease. It is a better remedy for scalp psoriasis as it contains the hardest scales with trapped hair thus coconut oil helps to loosen the scales on the scalp [2].

Synergistic action of therapeutic agent calcipotriol and coconut oil in psoriasis

As both exhibits anti-inflammatory properties thus they are a good option for design of some topical dosage form like nanoemulgel for anti-psoriatic action.

What is an inflammation?

Inflammation is a protective immunovascular response mediated by the production of different cell types. It is activated by various external and internal stimuli. The main purpose is to maintain homeostatis and the immune system produces both pro and anti-inflammatory markers in response [3].

Vitamin D3 and its metabolites and their role in inhibition of inflammation

Studies conducted on cell lines have shown the inhibitory role of vitamin D and its analogues in the inflammatory process.

Vitamin D receptor and various vitamin D metabolizing enzymes are expressed in most of the cells of the immune system such as T&B lymphocytes, dendritic cells, monocytes, macrophages and in the target tissue cells. These cells produce vitamin D3 metabolites that exert immonumodulating effects which regulates immune responses in a cell-type dependent manner. Thus vitamin D and its analogues help in regulating different inflammatory related molecular pathways. Thus vitamin D3,its analogues and metabolites have inhibition effect on psoriasis whereas vitamin D3 plays significant role in calcium metabolism of the cells and tissues, its metabolites and analogues like calcipotriol are devoid of any calcium metabolizing activity with retained anti-inflammatory activity. The VDR belonging to steroid/thyroid receptor superfamily exists on the cells of many different tissues like thyroid, bone, kidneys and T cells of the immune system. T cells play eminent role in psoriasis and it is thought that the binding of calcipotriol to the VDR modulates the T cells gene transcription (protein synthesis) of cell differentiation and proliferation related genes [4].

Chemistry of calcipotriol

Calcipotriol is a synthetic form of vitamin D3 which is used in topical dosage forms to treat the mild to moderate symptoms of psoriasis. It is structurally a seco-cholestane with a cyclopropyl group and three hydroxy groups (OH groups) at C1, C3 and C24 positions hence named triol at the end. It binds to the vitamin D receptor (VDR). Its key component, the cyclopropyl group reduces its calcium regulating activity (which is the major activity of calcitriol vitamin D), thus making it safer for skin treatment, though it still it still modulates skin cellular growth and differentiation, thus aids in reducing the psoriasis symptoms. It is a steroidal structural drug and has multiple double bonds in its chemical structure [5].

Mechanism of action of calcipotriol on skin cells

It binds to the VDR on the skin cells (keratinocytes). It inhibits the growth of keratinocytes thus preventing their proliferation and promotes their differentiation and helps in the normalization of the skin growth process in psoriasis. Thus it acts by retarding the growth of skin cells and also cause immonumodulation. It is readily combined with corticosteroids like betamethasone for severe plaque psoriasis [5].

Mechanism of action of coconut oil in psoriasis

Coconut oil, derived from the kernal (inner part of fruit) harvested from the coconut palm (*Cocos nucifera*). It consists of saturated fatty acids, 12- carbon chain (end chain methyl group and terminal carboxylic acid). The main chemical component of coconut oil (lauric acid) exhibits anti-inflammatory properties, may suppress inflammatory markers like TNF-alpha and interleukin-6, thus reduces the typical symptoms of inflammation like redness, swelling seen in the psoriasis. It can also reduce the symptoms of eczema (atopic dermatitis), a skin condition that looks similar to psoriasis [2].

Introduction to emulsions as topical dosage forms

Emulsions are thermodynamically stable liquids comprising of immiscible oily and aqueous phases which are made miscible with the addition of third agent called emulsifying agent. The emulsifying agents are the substances that lower the interfacial tension that exists between the layers of two phases and made them miscible to some extent. One phase exists in globule phase and the other phase exists as the continuous phase. The various emulsifying agents exhibit different mechanisms to undergo this action. Nano emulsions are the dosage form in which the droplet size exists in nanoscale (nanometer range). These can be created by the high speed homogenization of the two phases to create the globules in nano size range [6].

Advantages of nanoemulgels over conventional topical formulations in psoriatic skin disorder treatment

Conventional topical formulations have many limitations like adverse side effects, reduced skin penetration, reduced bioavaiability and variable kinetics. The novel topical dosage forms like nanoemulsions and noanoemulgels offer superior advantages for the treatment of skin disorders like psoriasis. The nanoemulsions are the homogenous systems with globule size in the nanorange size. The nanoemulgels combine the properties of both nanoemulsions and hydrogels offering better therapeutic efficiency and improved patient compliance. The nanoemulsions are converted into the nanoemulgels to improve the consistency by conversion from solution to gelled state for the readily application on the skin surface. The nanoemulsions improves the drug solubility as these exhibits reduced droplet size and enhanced surface area. Hydrogels form a good topical base and prolongs the release of drug into the skin barrier. The other benefits of nanoemulgels include the enhanced penetration due to large droplet surface and small droplets size leading to increased drug release and hence action. Further nanoemulgels offer controlled as well as sustained drug delivery and improved patient compliance [7].

Thus nanoemulgels are more functional than the conventional topical formulations and offer many benefits. The gel matrix of the hydrogel acts as a reservoir for maintenance of the required concentration of the drug at the delivery site and release the drug gradually over the time period. This also reduce the frequency of application of formulation. Also the water content within the gel matrix improve the skin hydration and form the occlusive layer on the skin. The hydrogel also prevents the transepidermal water loss leading to softening of the skin layer required in typical psoriasis symptoms. Moreover, hydrogels renders the formulation non-greasy, more biocompatible, easier application, more patient adherence to the treatment regimen [7].

Design of coconut oil based nanoemulsion of calcipotriol

Calcipotriol is a fat soluble compound with higher lipophilicity. The log P value is around 3.8-4.9. It has higher octanol/water partition coefficient indicating its lipophilic nature [8]. Accordingly as "like dissolves like", calcipotriol will be highly soluble in coconut oil thus stable emulsion can be formed consisting of water as aqueous phase, coconut oil as an organic phase and using surfactant tween 80 as an emulsifying agent.

Preparation of nanoemulsion

As the drug calcipotriol has significant solubility in coconut oil, hence the solubility studies can be omitted to be conducted.

Construction of pseudo-ternary phase diagrams

The pseudo ternary phase diagrams are the theoretical triangular diagrams consisting of water, oil and surfactant/co-surfactant mixture. These are constructed to find the concentration range of components to locate the nanoemulsion region. These are made by using water titration method at room temperature (25° Celcius). Oil and surfactant/cosurfactant (Smix) ratio are mixed thoroughly in different combinations of oil and Smix from 1:9 to 9:1. Titration with water is carried out for each ratio in a test tube with moderate shaking. After the equilibrium is reached, the mixtures are viewed visually and determined as emulsions or gels [9].

Thus the selected composition ratio from pseudo ternary phase diagram studies can be utilized for further nanoemulsion preparation.

High pressure homogenization method

The high energy methods can be used to formulate nanoemulsions. This is a high energy method where high pressure is applied to break the large droplets into nano-sized droplets and produce nanoemulsion with high kinetic energy. These use disruptive forces like turbulence etc applied together to give nanoemulsions with very small droplet sizes. In this technique, firstly the required amount of calcipotriol can be dissolved in small amount of coconut oil. Then surfactant can be added. The water can be introduced dropwise with high pressure homogenization (500psi) to form the nanoemulsion [10].

The selected ratio of coconut oil, water and Smix can be used for nanoemulsion preparation.

Formulation of nanoemulgel from nanoemulsion

Coconut oil based nanoemulsion can be suitably gelled using Carbopol 940 as a gelling agent. The procedure can involve first the preparation of Carbopol gel base. It can be prepared by the dispersion of weighed quantity of Carbopol 940 (1gm for 1%gel) into a specific amount of distilled water (99 ml). This mixture is allowed to stand and hydrate for at least 12-24 hours to ensure complete swelling of the gel with sufficient hydration. Then humectants like glycerine can be added to prevent the loss of moisture. The appropriate preservatives can be added to prevent the microbial growth during the life period of the gel because the microbial growth can occur due to the presence of moisture in the formulated gel. Then the neutralizing agent like triethanolamine can be added drop by drop to the dispersion while continuous stirring until the pH is adjusted to 5.5 -6.0 (suitable for skin) until thick transparent gel is formulated. Then the previously prepared coconut oil based nanoemulsion can be mixed with Carbopol gel base with continuous stirring using a high speed mixer or magnetic stirrer at 1000-1500 rpm speed for 10-15 minutes until a stable transluscent nanoemulgel is formed. After preparation check the gel for homogeneity, consistency, pH and viscosity as listed under the physicochemical characterization of the formed nanoemulgel. The formulated nanoemulgel should be free of any lumps, aggregates, should have sufficient consistency and viscosity to be readily applicable over the skin [11].

Physicochemical characterization and evaluation of the formulated nanoemulsion gel

Physical appearance

The ideal nanoemulgels should be clear or slightly opalescent in appearance. The clarity of the nanoemulgel indicate the homogeneity of the formulation and has well dispersed uniform sized globules. It can be checked visually. If any turbidity appears, it can be due to aggregation, instability or any possible degradation in the formulation [7].

Particle size distribution and zeta potential measurement

These parameters play an important in the optimal performance of the nanoemulgel. Particle size influences the appearance, stability and efficacy of the nanoemulgels. The smaller the size of droplets, better is the efficacy of the nanoemulgels. Techniques like scanning electron microscopy and dynamic light scattering can be used to measure particle size. This measurement is done to verify the particle size distribution of the nanoformulation that whether it lies in the optimal nanometer size range and thus prove the successful formulation and its performance in the biosystems. Zeta potential is the measure of electrostatic stability and thus prevents the aggregation of the dispersed globules and thus maintain the stability of formulation. It measures the density of charge over the globules using Zetasizer (Malvern instrument, UK) [7].

Free radical scavenging assay

It can be performed using DPPH method invented by Marsden Blois in 1958. DPPH(2,2-diphenyl-1-picrylhydrazyl is a dye that changes its color from deep violet to pale yellow upon reduction by antioxidants and the change in color can be quantitatively measured using spectrophotometrically at 517nm. DPPH accepts electrons or protons from radical scavengers and gets converted into the reduced or oxidized form causing rise or fall in visible spectral absorption. The reaction consists of taking 50 µl of 0.25 mg/ml DPPH in ethanol and 50 µl of various concentrations of sample can be added in a 96 well plate. Incubate the plate for 30 minutes in the dark at 27 ± 2 ° Celcius, an absorbance of 517 nm is measured on the plate.

The samples are calculated as follows:-

Scavenging activity = Absorbance control -Absorbance sample x 100

Absorbance control

The IC 50 VALUE is the concentration of the sample causing 50 % DPPH scavenging.

pH and conductivity measurements

pH, a chemical property of solutions, is defined as the negative logarithm of hydrogen ion concentration in the respective sample. It is indicative about the acidic or basic character of the formulation. The correct pH of the nanoemulsion based gel indicate the compatibility with the skin's chemical environment. The nanoemulgels should have a pH that is slightly acidic as that of skin. Otherwise unmatched pH of the formulation may cause skin irritation and uneffectiveness of the formulations. The pH is measured after the dilution of the gel with pure water. The pH of the of the formulated sample should be compatible with the skin so as not to cause any unwanted reaction or incompatibility with the skin pH. If unmatched, then the formulation pH should be adjusted accordingly with the addition of sufficient acid or base. It can be measured using previously calibrated pH meter (calibrated using standard buffer solutions).The conductivity is the measure of the presence of free ions in the given sample. Higher conductivity value shows the presence of higher concentration of electrolytes which can effect the formulation stability and release behaviour like electrolytes can cause aggregation and phase separation of the nanopemulgels. Conductivity can be measured with the calibrated conductivity meter.

Reological and Spread ability measurements

These are the two essential parameters to be studied and effect the performance of the nanoemulgel. Viscosity determines the thickness and flow properties of the emulgels and influence the drug release behaviour. The formulation intended to be applied on skin must have sufficient consistency. It can be measured using Brookfield viscosity using appropriate spindle at 100 rotations per minute. It shows reading in centipoises units. The viscosity should be in appropriate range to give sufficient consistency. Spreadability is defined as the spreading ability of the formulation over the applied skin surface. Good spreadability indicates the uniformity of formulation with uniform droplet size.it can be measured by

tests like "slip drag test" which measures the extent to which the nanoemulgel spreads under the given applied force using a glass slab apparatus [7].

Phase separation

It can be studied by centrifuging the sample at 3500 rpm for 30 minutes at 25° Celcius using a centrifuge. Observe for any phase separation of the nanoemulsion [7].

Stability studies

These are conducted to check for any creaming or other form of unstability in the formulated nanoemulsion under various storage conditions of temperature, humidity and light conditions that mimics the real storage conditions of the formulation. Also the changes in the formulation regarding visual appearance, viscosity, pH and drug content due to degradation can be studied. These can be performed by heating cooling test for 6 cycles. 1 cycle kept at 4 ± 2 ° Celcius for 24 hours and 45 ± 2 ° Celcius for 2 hours. These determine the samples shelf life and ensure that it remains effective, safe and stable throughout its life [7].

Morphological studies

These can be performed by the help of scanning electron microscopy to study the morphology of the globules of dispersed phase. They should not exist in the form of aggregates for an ideal nanoemulsion [7].

In-vitro drug release studies and release kinetics

Nanoemulgels should effectively release the drug at a desired rate for effective pharmacological action. Franz diffusion cell apparatus is employed to study the release behaviour of topical dosage forms. The nanoemulgel is applied superficially on the excised rat skin separating the upper and lower compartment. The lower compartment contains buffer and acts as a receptor whereas the upper compartment serves as a drug reservoir at specific time intervals, samples are withdrawn from the receptor compartment and filtered through 0.45 micrometer porosity membrane filters and analyzed using the UV-Visible spectroscopic method in order to measure the content of the drug released at different time intervals. The samples withdrawn at different time intervals can be diluted with 100% methanol and analyzed for the absorbance at 264 nm or 265 nm for calcipotriol measurement. The concentration of calcipotriol can be extrapolated from the absorbance readings using calibration curve preparation. After the data is obtained, the data is fitted in various mathematical models like zero order kinetics, first order kinetics, Higuchi, korsemeyer-peppas and Hixson -crowell kinetic models to study the release behaviour [7].

Flux calculation

This parameter is calculated to measure the skin permeation rate from the data obtained from the *in vitro* skin permeation evaluation. The plot of cumulative amount of Calcipotriol permeated versus time is plotted and its slope can be found to calculate the flux [9]. It can also be calculated using following formula:

$$Jss = \frac{\Delta Q}{\Delta t \times A}$$

Where Q is the cumulative amount of drug permeated, t is the time interval and A is the area. Thus the flux can be calculated as the slope of the linear portion of the cumulative amount of drug permeated per unit area versus time plot.

Permeability coefficient

It is measured to check how the given formulation can permeate through the skin. It is calculated by dividing the flux calculated in test 10 by the initial concentration of Calcipotriol in the donor cell.

Skin irritancy studies

These are performed either on human volunteers or animals like mice. The small amount of formulation can be applied on elbow or skin of animal and studied for any signs of itching, irritation, redness or swelling. The formulation should exhibit minimal skin irritancy for patient compliance, comfort and safety after application on the skin [7].

In vivo skin permeation studies

These are performed to study the permeability ability and study the formulation's ability to cure the inflamed skin symptoms in psoriatic skin disease. This test is performed on the healthy mice aged 8-11 weeks. The animals are divided into three groups, one with no formulation received, other with imiquimod cream applied to induce the psoriasis like inflammatory skin symptoms and the third group comprising of the one on which the formulation is applied. Then after some days the animals are sacrificed and the histopathological studies can be carried out to study the effect of

the applied formulation on the skin and compared with the control group i.e. on which no formulation is applied. This will indicate the effectiveness of the nanoemulgel's ability to help in the treatment and cure of psoriatic skin symptoms when applied in the humans.

Chemical interaction between calcipotriol and coconut oil

This formulation can exhibit the possible H-bonding interaction between the hydrogen atoms of the OH groups of Calcipotriol and free hydrogens of the methyl groups of the lauric acid (a fatty acid) component present in the coconut oil this possible chemical interaction can be detected using FTIR studies revealing the chemical compatibility between the calcipotriol and coconut oil combined together in the nanoemulgel formulation. If this interaction exists in this formulation, then it can contribute to the stability of the nanoemulsion gel and its successful formulation preparation.

Conclusion

Thus nanoemulgel of coconut oil loaded with Calcipotriol can be formulated and studied for physicochemical characterization. This formulation has prospect to be beneficial in the treatment of psoriatic skin symptoms and thus the research can be further carried out.

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