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Topical delivery of clobetasol propionate loaded microemulsion based gel for effective treatment of vitiligo: *Ex vivo* permeation and skin irritation studies

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ABSTRACT

The aim of the present investigation was to evaluate microemulsion as a vehicle for dermal drug delivery and to develop microemulsion based gel (MBC) of clobetasol propionate (CP) for the effective treatment of vitiligo. D-Optimal mixture experimental design was adopted to optimize the amount of oil (X_1), S_{mix} (mixture of surfactant and cosurfactant) (X_2) and water (X_3) in the microemulsion. The formulations were assessed for globule size (nm) (Y_1) and solubility of CP in microemulsion (mg/ml) (Y_2). The microemulsion containing 3% oil, 45% S_{mix} and 50% water was selected as the optimized batch (ME). The globule size and solubility of CP in ME were 18.26 nm and 36.42 mg/ml respectively. Transmission electron microscopy showed that ME globules were spherical in shape. Carbopol 934P was used to convert microemulsion containing drug into gel form without affecting its structure. *Ex-vivo* permeation studies showed that cumulative amount of CP permeated (Q_n) from ME, MBC and market formulation (MFCP) at 8 h after application were 53.6 ± 2.18, 28.43 ± 0.67 and 37.73 ± 0.77 $\mu g\,cm^{-2}$ respectively. MBC showed greater retention of CP in to skin layers than ME and MFCP. Skin irritation studies showed MBC to be significantly less irritating than MFCP. Photomicrographs and scanning electron micrographs of skin sections treated with MBC showed significant changes in the skin structure, which was attributed to the interaction of microemulsion components with skin resulting in permeation enhancement and retention of CP into skin layers. It was concluded that CP loaded gel could be a promising formulation for effective treatment of vitiligo.

1. Introduction

Vitiligo is a common idiopathic de-pigmenting skin disease characterized by white patches on the skin [1]. Vitiligo affects 1–3% of the total world population, with an incidence of 3–4% in India [2]. Asymptomatic white patches induce psychological stress in many individuals leading to attempted suicide in few cases. The exact etiology of vitiligo is still unknown but autoimmune factors and increased sensitivity to oxidative stress are believed to be responsible for its pathophysiology. The conventional therapy of vitiligo comprises of medical, surgical and adjunctive treatment. Medical treatment resists the de-pigmentation of the skin by targeting the immune system. Dermatosurgical techniques along with cosmetics are sometimes used to achieve the re-pigmentation. Since, dermatosurgical techniques are painful and traumatic, medical treatment remains as the most feasible option for the treatment of vitiligo. Drugs such as corticosteroids, calcineurin inhibitors, vitamin D derivatives, psoralens (phototherapy) *etc.* are most commonly used for the treatment of vitiligo. Moderately potent to potent topical corticosteroids are considered to be the first-line therapy [3].

Clobetasol propionate (CP), a highly potent drug of all the available corticosteroids is widely used in the treatment of various skin disorders including psoriasis, atopic dermatitis and vitiligo [4–6]. It is currently approved in different dosage forms such as ointment, gel, cream, solution and foam for topical use [7]. Effective treatment of vitiligo requires prolonged use of topical CP formulations which results in significant therapy related side effects such as skin atrophy, steroid acne, peri-lesional hypo-pigmentation and allergic contact dermatitis [8]. Thus, it was required to improve the benefit–risk ratio of CP by developing novel drug delivery strategies [9]. Several attempts have been made earlier in order to decrease the adverse effects of corticosteroids and increase their safety [9,10]. It has been studied that vehicles of topical formulations play an important role in minimizing the adverse effects and influencing the rate and extent of drug permeation across the skin [11]. It can be inferred that potency of topical corticosteroid formulations could be defined by characteristics of the vehicle system [12,13].

Various colloidal carriers such as poly(D,L-lactic-co-glycolic acid) (PLGA) microspheres [14], solid lipid nanoparticles [15], lipid nanospheres [16], nanostructured lipid carriers [17], polymeric

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nanocapsules [9] and lecithin/chitosan nanparticles [18] containing CP have been developed in order to minimize its side effects and improve the absorption and therapeutic concentration in the target skin tissues. However, among all the colloidal drug delivery carriers, microemulsions offers several advantages over other dosage forms in terms of ease of preparation, high solubilization capacity for hydrophilic and lipophilic drugs, long-term stability and improved dermal drug delivery [19]. Hence, in the present investigation microemulsion was selected as a colloidal drug delivery carrier for CP. Microemulsions are transparent and thermodynamically stable as their droplet size range from 10 to 100 nm and they do not coalesce [20]. Microemulsions are composed of oil, surfactant, co-surfactant and water in specific proportions. The ingredients of microemulsion could facilitate the permeation rate of the drug by reducing the diffusion barrier of the *stratum corneum* [21]. However, due to low viscosity of microemulsion, their less retention capacity in the skin restrains its application in the pharmaceutical industry [20,22]. To overcome this disadvantage, gelling agents such as Carbopol 940, xanthan gum and carrageenan have been added into the microemulsion for forming microemulsion based gel in order to increase its viscosity which could be suitable for topical application [23,24]. Moreover, microemulsion based gel prevents the absorption of drug in the blood stream and provide higher drug accumulation in the skin for efficient action. Barot et al. reported a higher retention of terbinafine in the human cadaver skin after topical application of microemulsion based gel when compared to microemulsion containing the same drug [25]. Rao and Murthy reported that HPMC gels containing CP loaded liposomes when applied topically showed lower absorption of the drug in the bloodstream when compared to the same formulation containing free drug [26]. Zhu et al. showed that penciclovir loaded microemulsion based gel has excellent sustained release capability and enhanced skin permeation and retention due to viscosity imparted by Carbomer 940 [22].

Clobetasol propionate possesses low water solubility (2 μg/ml) and has log *P* value 3 which makes it suitable for encapsulating it in microemulsion globules [27]. The topical delivery of CP encapsulated in microemulsion could enhance its percutaneous absorption and retention in the skin, which is necessary for the effective treatment of vitiligo. Enhanced retention in the skin and minimal absorption could minimize the side effects associated with the drug. Hence, the objective of the present investigation was to develop microemulsion based gel of CP. Pseudo-ternary phase diagrams were constructed to obtain the suitable ratio of surfactant and co-surfactant. Composition of microemulsion system was optimized using D-optimal design. The microemulsion with optimized component ratio was converted in to gel to enhance the permeation and retention of CP in *stratum corneum*. *Ex vivo* permeation studies were performed to assess the ability of using this system for dermal delivery. Skin irritation studies were performed to assess the irritation potential of the prepared formulation.

2. Materials and methods

2.1. Materials

Clobetasol propionate was obtained as a gift sample from Sumit Laboratories (Vapi, India). Isopropyl myristate (IPM) was received as a gift sample from Bombay Tablets Pvt. Ltd. (Gandhinagar, India). Oleic acid, isopropyl alcohol (IPA), isobutyl alcohol (IBA), polyethylene glycol (PEG) 400 and PEG 600 were purchased from National Chemicals (Vadodara, India). Tween 20, Tween 60, Tween 80, propylene glycol, Cremophor EL and Cremophor RH 40 were purchased from Sigma–Aldrich (Mumbai, India). Methanol was purchased from Baroda Chemicals Ltd. (Vadodara, India). Captex 200, Captex 300 and Captex 355 were received as gift samples from Abitec Corporation (OH, USA). Labrasol and Transcutol P were obtained as gift samples from Gattefosse (Lyon, France). Carbopol 934P was purchased from Corel Pharma (Ahmedabad, India). Double distilled water was used throughout the study. All other chemical reagents and solvents used were of analytical grade.

2.2. Screening of components for MEs

In order to find out the most suitable oil phase, surfactant and co-surfactant in microemulsion, the solubility of CP was determined in various oils such as oleic acid, IPM, Captex 200, Captex 300 and Captex 355; in surfactants including Tween 20, Tween 60, Tween 80, Labrasol, Cremophor EL and Cremophor RH 40; and in co-surfactants like PEG 400, PEG 600, Glycerol, Transcutol P, IPA and IBA. Excess amount of CP was added in 3 ml of oil/surfactant/co-surfactant in 5 ml capacity stoppered vials separately and the resultant mixture was mixed initially by vortex mixer. The vials were later shaken on magnetic stirrer (Remi Instruments, Mumbai, India) for 72 h followed by centrifugation (Remi Instruments, Mumbai, India) at 10,000 rpm for 15 min. The supernatant was filtered through a 0.45 μm membrane filter and the concentration of CP in filtrate was determined by High Performance Liquid Chromatography (HPLC) analysis after appropriate dilution with methanol. Appropriately diluted solutions of oil, surfactants and co-surfactants in methanol were taken as blank. The components that showed highest solubility of CP were used for further studies.

2.3. Construction of pseudo-ternary phase diagrams

Pseudo-ternary phase diagrams were constructed by employing aqueous titration method in order to get concentration range of components of microemulsion. The ratio of surfactant to co-surfactant (S_{mix}) was altered at 3:1, 2:1, 1:1 and 1:2. For the construction of pseudo-ternary phase diagram at each S_{mix} ratio, the oily mixtures containing oil, surfactant and co-surfactant were prepared with volume ratio of oil to S_{mix} at 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 respectively. Double distilled water was added drop by drop to the oil and S_{mix} mixture under magnetic stirring at ambient temperature. Transparent and clear microemulsion was taken as the end point of aqueous titration method. The concentrations of components were then calculated in order to plot the pseudo-ternary phase diagram (Fig. 1).

2.4. Preparation of CP loaded microemulsions

From the pseudoternary phase diagrams, S_{mix} ratio with maximum microemulsion region was selected. Different proportions of oil and S_{mix} were mixed depending upon the design points of the D-optimal design space (Table 1). Clobetasol propionate was dissolved in the mixture of oil and S_{mix} under magnetic stirring at ambient temperature. Appropriate amount of double distilled water was added drop wise to the oily mixture until clear and transparent microemulsion was obtained. The mixture was allowed to stabilize and attain the equilibrium with gentle magnetic stirring for 15–20 min. All microemulsions containing CP were then stored at ambient temperature.

2.5. Optimization of microemulsion formulation

The levels of experimental design could not be chosen arbitrarily, where the composition is a factor of interest, because the sum of all the fractions of components equals to unity. Classical experimental designs such as full factorial designs do not consider specific experimental constraints, and thus they lack prediction