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Transdermal delivery of betahistine hydrochloride using microemulsions: Physical characterization, biophysical assessment, confocal imaging and permeation studies

Rania M. Hathout*, Maha Nasr

Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

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ABSTRACT

Transdermal delivery of betahistine hydrochloride encapsulated in various ethyl oleate, Capryol 90®, Transcutol® and water microemulsion formulations was studied. Two different kinds of phase diagrams were constructed for the investigated microemulsion system. Pseudoplastic flow that is preferable for skin delivery was recorded for the investigated microemulsions. A balanced and bicontinuous microemulsion formulation was suggested and showed the highest permeation flux (0.50 ± 0.030 mg cm^{-2} h^{-1}). The effect of the investigated microemulsions on the skin electrical resistance was used to explain the high permeation fluxes obtained. Confocal laser scanning microscopy was used to confirm the permeation enhancement and to reveal the penetration pathways. The results obtained suggest that the proposed microemulsion system highlighted in the current work can serve as a promising alternative delivery means for betahistine hydrochloride.

1. Introduction

Betahistine hydrochloride [*N*-methyl-2-(pyridin-2-yl) ethanamine dihydrochloride], which acts as an active histamine analogue, is a very interesting drug that aroused the curiosity of scientists since it was first synthesized [1,2]. Early attempts for its use centred on the ability of this compound to cause vasodilatation in the cerebral vasculature region, and hence, it has been used to reduce and relieve the symptoms of vertigo, tinnitus, and hearing loss associated with Ménière's disease [3]. With the expanding knowledge on histamine, in general, and on its role as a neurotransmitter in particular, potential new interesting indications for this compound have emerged especially in the regulation of feeding behaviour and weight control [4].

Betahistine is usually given by the oral route as the hydrochloride salt. The short half life of this drug (3–4 h) necessitates its frequent dosing [3]. Usually, the initial dose ranges from 8 to 16 mg taken three times daily preferably with meals. Maintenance doses are generally in the range 24–48 mg daily. Unfortunately, betahistine has histamine like action on the secretory cells of the gastric mucosa and hence its use is un-preferred in patients with gastric irritation or peptic ulcer [5,6]. In a clinical study conducted on eleven patients diagnosed with a definite Ménière's disease where oral betahistine hydrochloride was administered, various related side effects including gastrointestinal complaints, fatigue and after taste in 46% of the patients were reported [7]. The aforementioned facts warrant the search for a new route of administration viz. the transdermal route. Moreover, the high aqueous solubility of the drug requires specific technologies in order to control its release and delivery maintaining the concentration of the drug within the therapeutic range and eliminating the intolerance drawback that is usually associated with the immediate release conventional oral dosage forms [5,8]. Development of transdermal formulations for betahistine hydrochloride is challenging again because of its high solubility which makes its penetration through the stratum corneum (SC) (epidermis outermost and highly lipophilic layer) a difficult task. To this end, topical microemulsions could be considered an ideal solution.

Microemulsions are monophasic, optically isotropic and thermodynamically stable systems with a droplet size typically in the range 10–100 nm. They are considered more stable than topical vesicular systems like liposomes, ethosomes, niosomes, etc. They are translucent mixtures of oil, surfactant, cosurfactant, and water, in which either the oil globules are dispersed in water (o/w) or water globules are dispersed in oils (w/o) or else domains of oil and water co-exist together (bicontinuous) [9]. The use of microemulsions in skin drug delivery has been frequently exploited and has proven highly successful for the delivery of hydrophilic and lipophilic compounds [10–12].

* Corresponding author. Tel.: +20 1 005254919/2 22912685; fax: +20 2 0224011507.
E-mail addresses: r_hathout@yahoo.com, rania.hathout@pharma.asu.edu.eg (R.M. Hathout).

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Many mechanisms were reported for the transdermal delivery from microemulsions; first, microemulsions allow for high drug loading capacities, second, the microemulsion components exhibit a powerful penetration enhancing effect [13,14], third, the microemulsion components can possibly enter into the skin as monomers [15,16], increasing the solubility of the drug in the skin, fourth, the microstructure of the system provides large surface area for drug transfer and diffusion and finally, the phase transition of microemulsions provides a possibility for producing a supersaturated system with a high thermodynamic activity [17].

The present study was attempted to investigate the potential development of novel nano-colloidal formulations for the successful delivery of betahistine hydrochloride through an alternative route; the transdermal route. In this perspective, a microemulsion system was developed and physically characterized. Microemulsion formulations were selected and were subjected to ex-vivo permeation testing and laser confocal microscopic imaging. Physical and biophysical assessments were also attempted to evaluate the performance of the investigated microemulsion formulations and to record their effects on the skin barrier.

2. Materials and methods

2.1. Skin

Full male albino mouse skin was used in all the experiments. The animal was obtained from a local farm. The skin was cleaned carefully under cold running water and was stored frozen at −20°C for a period no more than one month before use.

2.2. Materials

Betahistine hydrochloride was kindly provided by the Egyptian International Pharmaceutical Industries Co. EIPICO (EIPICO, ELSharqia, Egypt). Bisdemethoxycurcumin, ethyl oleate, orthophosphoric acid, sodium lauryl sulphate and triethylamine were purchased from Sigma–Aldrich (Sigma–Aldrich, Gillingham, UK). Diethylene glycol monoethyl ether (Transcutol®) and propylene glycol monocaprylate (Capryol 90®) were kindly provided by Gattefosse' (Gattefosse', Lyon, France). Acetonitrile, (HPLC grade) (Fisher Scientific, Loughbrough, UK), sodium chloride, potassium chloride, sodium phosphate (monobasic) and potassium phosphate (dibasic) were purchased from Acros Organics (Acros Organics, Geel, Belgium). Parafilm® was purchased from Pechiney Co. (Pechiney plastic packaging company, Chicago, IL, USA). All aqueous solutions were prepared using high-purity deionized water with conductance less than 1 $\mu S\,cm^{-1}$ (18.2 MΩ cm) (Barnstead Nanopure Diamond, Dubuque, IA, USA).

2.3. Methods

2.3.1. Phase diagrams

2.3.1.1. Kahlweit phase diagram. Kahlweit fish presentation, where the phase domains were determined with respect to two variables; concentration of surfactants and temperature, was used to construct a phase diagram [18]. This diagram helps in confirming the microemulsion forming ability of the chosen components at the skin temperature (32 °C) and at an equal proportion of oil and water.

Ethyl oleate® was chosen as the oily phase. The surfactant system included Capryol 90® as the surfactant and Transcutol® as the co-surfactant. Water comprised the aqueous phase.

The percentage of the surfactant mixture was varied for a fixed oil/water ratio = 1/1. The different microemulsions were prepared by mixing the different components (oil, water and surfactant mixture) under magnetic stirring. Preparations were placed in ovens at different temperatures (from 10 °C to 70 °C with 10 °C steps) for equilibration. Samples were designated as monophasic (clear) (1Φ), diphasic (2Φ) with an oil excess phase (o/w microemulsion + oil) or with a water excess phase (w/o microemulsion + water), or triphasic (3Φ) (bicontinuous microemulsion + oil + water). Samples were observed under polarized microscope (Olympus BX51 U-AN 360, Tokyo, Japan) between crossed polars to confirm the isotropic nature of the obtained microemulsions [19].

2.3.1.2. Pseudoternary phase diagram at constant temperature. The phase diagram was constructed using the water dilution titration method at ambient temperature (25 °C) as previously described elsewhere [20,21]. The Pseudo-ternary phase diagram was prepared with the weight ratio of 1:1 Capryol 90® to Transcutol®.

Selected microemulsion formulations were chosen from the microemulsion obtained domain.

2.3.2. Preparation of betahistine microemulsions

For all the selected formulae, first, betahisitine (3%, w/v) was dissolved gradually in the aqueous phase. Second, Ethyl oleate, Capryol 90® and Transcutol® were mixed under magnetic stirring. Third, the aqueous phase was added gradually to the oil/surfactants mixture. Magnetic stirring was used to aid rapid emulsification.

2.3.3. Particle size measurements

The particle size and the polydispersity index of the chosen microemulsions were determined using dynamic light scattering (Malvern Zetasizer, Malvern, Worcestershire, UK). The viscosity was adjusted at 0.05 Pa S. The scattering intensity data were obtained after pre-filtering (0.45 μm) the microemulsions. The samples were loaded into cuvettes 1 ml in volume and the temperature was set at 25 °C. Triplicate measurements were performed.

2.3.4. Rheological measurements

The viscosity of the prepared microemulsions was measured with a DV-E Viscometer (Brookfield Engineering Laboratories, Middleboro, MA, USA) using a 61 spindle [22,23] at speeds ranging from 0.5 to 100 rpm, at room temperature, and over 10% torque.

2.3.5. Ex-vivo permeation and data analysis

2.3.5.1. Ex-vivo permeation. Male albino mouse skin was defrosted, cut into square pieces that were mounted with the stratum corneum uppermost in Franz-type diffusion cells (Permegear, Hellertown, PA, USA). The receptor volume was 7.5 ml and the diffusional area was 1.77 cm^2. Before starting the permeation experiment, the receptor solution was filled with phosphate buffered saline (PBS) at pH 7.4 and magnetically stirred. The skin surface was covered with 1 ml of the same solution at 37 °C for 1 h. Subsequently, the PBS in the donor was replaced by 1 ml of the prepared microemulsions containing 3% (w/v) betahistine and the compartment was covered with Parafilm®. One millilitre sample was collected to be analyzed at 0.5, 1, 2, 3, 4, 6, 8 and 24 h and immediately replaced with fresh buffer. All the samples were filtered using 0.45 μm Nalgene® Millipore syringe filters (Thermo Fisher Scientific, Waltham, MA, USA) prior to analysis. All the permeation experiments were performed in triplicates. Betahistine in the samples was quantified using high performance liquid chromatography.

2.3.5.2. Detection of betahistine using high performance liquid chromatography. Quantification of betahistine was achieved using an HPLC system (Agilent, Santa Clara, CA) consisting of a pump, a UV detector, and a C18 column (Agilent Eclipse column XDB-C18 (5 μm, 4.6 × 150 mm)). The mobile phase consisted of a 60% aqueous solution: 40% acetonitrile. The aqueous solution consisted of 0.67% (v/v) triethylamine and 0.33% (w/v) sodium lauryl sulphate