Development of clove oil based nanoemulsion of olmesartan for transdermal delivery: Box–Behnken design optimization and pharmacokinetic evaluation

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ABSTRACT

The aim of this study was to develop and optimize a transdermal nanoemulsion formulation of olmesartan using Box–Behnken design and to evaluate it for pharmacokinetic study. Box–Behnken experimental design was applied for optimization of olmesartan nanoemulsions. The independent variables were clove oil (X_1), S_{mix} (X_2) and water (X_3) while particle size (Y_1), polydispersity index (Y_2) and olmesartan transdermal flux (Y_3) were the dependent variables. Further the optimized formulation obtained was then tested in rats for an *in vivo* pharmacokinetic study. Results indicate that the developed nanoemulsion carrier of olmesartan provides reasonable particle size, polydispersity index and transdermal flux. The optimized formulation has presented the particle size of 53.11 ± 3.13 nm, polydispersity index 0.335 ± 0.008 and transdermal flux $12.65 \pm 1.60\,\mu g/cm^2/h$. Confocal laser scanning microscopy revealed an enhanced penetration of Rhodamine B loaded nanoemulsion carriers to the deeper layers of the skin. Furthermore, *in vivo* pharmacokinetic study of optimized formulation showed a significant increase in the bioavailability (1.23 times) compared with oral formulation of olmesartan by virtue of better permeation through rat skin. The developed nanoemulsion formulation could be used as an antihypertensive dosage form for effective transdermal delivery of olmesartan.

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1. Introduction

Hypertension is one of the most common cardiovascular disorders throughout the world because of its high prevalence and it is a main factor for increasing the risk of developing kidney disease, hardening of the arteries, eye damage, and stroke [1]. The World Health Report 2002 identified hypertension, or high BP, as the third ranked factor for disability-adjusted life years. Hypertension is one of the leading risks for premature death and disability universally. Hypertension affects approximately 78 million individuals in the United States and approximately 1 billion worldwide [2–4]. Recent analyses have shown that the number of adults with hypertension in 2025 was predicted to increase by about 60% to a total of 1.56 billion [5–7]. As the population ages, the prevalence of hypertension will increase even further unless broad and effective preventive measures are implemented. The report from the Framingham heart study suggest that individuals who are normotensive at age of 55 years have a 90% lifetime risk for developing hypertension [8,9]. Overall, hypertension is an important public-health

challenge globally. Awareness, prevention, treatment and control of hypertension are significant public health measures. Patients with hypertension need long term treatment and sometimes lifelong therapy is advised, as transdermal therapeutic systems offer a better quality of life, they are more accepted than the oral dosage forms [10,11] therefore drug deliveries via transdermal route are ideally suited for ailments like hypertension that need chronic treatment [12–14].

The greatest obstacle for transdermal drug delivery is the barrier property of stratum corneum [15–17]. Many strategies [14,18–20] including used carrier vehicle, chemical permeation enhancers, and physical technologies such as electroporation, iontophoresis, ultrasound, and microneedle technologies either singly or in combination, have been used to facilitate the permeability of therapeutic compounds through the skin. It was reported that small droplet size provides a better chance for adherence to biological membranes transporting therapeutic compounds in a controlled manner [21]. Therefore, drug carriers of nano or micro size such as ethosomes, transfersomes, nanoemulsions, liposomes, and polymeric nanoparticles have been widely used to improve permeability of therapeutic agents through skin in recent years [22–24].

Nanoemulsion is a thermodynamically stable isotropically clear dispersion of two immiscible liquids, such as oil and water, stabilized by an interfacial film of surfactant molecules [25–27]. The dispersed

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phase is composed of small particles or droplets, with a size range of 5–200 nm, and has a very low oil/water interfacial tension [28]. Nanoemulsions are transparent because the particle size is less than 25% of the wavelength of visible light [29]. The size and polydispersity of nanoemulsions can affect properties such as particle stability, rheology, appearance, color; texture and shelf life [30]. Nanoemulsions have been reported to make the bioavailability of poorly soluble drugs more consistent. Nanoemulsions have also been reported as one of the most promising techniques for enhancement of transdermal permeation and bioavailability of poorly soluble drugs [31]. Hence, the nanoemulsions were used as the vehicle to enhance the olmesartan bioavailability via transdermal route of administration.

Olmesartan is a selective AT_1 subtype angiotensin II receptor antagonist. It exhibits more than 12,500-fold greater affinity for the angiotensin II receptor type 1 than for the angiotensin II receptor type 2, making it theoretically the second most potent agent [32]. Oral olmesartan 10–40 mg once daily is recommended for the treatment of adult patients with hypertension [33]. Orally administered olmesartan was rapidly absorbed from the gastrointestinal tract and converted during absorption to olmesartan, the pharmacologically active metabolite that was subsequently excreted without further metabolism. The absolute bioavailability of olmesartan from olmesartan medoxomil tablets was 28.6% [34]. It is a Biopharmaceutics classification system class II drug having molecular weight (446.50 Da) with a log partition coefficient (4.70), and half-life (10–15 h). Above properties of olmesartan make it a good candidate for the development of transdermal therapeutic system [35,36].

The aim of the present study was to optimize the nanoemulsion formulation for enhanced skin delivery of olmesartan, a lipophilic anti-hypertensive drug. Box–Behnken design was specifically selected for formulation optimization, because it requires fewer runs than a central composite design, in cases of three or four variables [22,23,37]. Independent variables were chosen as clove oil, S_{mix}, and water, while particle size, polydispersity index, and flux were selected as dependent responses. Objective function for the present study was selected as maximizing olmesartan transdermal flux, while minimizing particle size.

2. Materials and methods

2.1. Materials

Olmesartan medoxomil was received as a gift sample from Ranbaxy Research Laboratory (Haryana, India). Tween 20 and polyethylene glycol 400 (PEG) were purchased from Merck (Schuchardh, Hokenbrunn, Germany). Campul PG-8, Plurol olique CC 497, Labrasol, Labrafil M 1944 CS were received as gratis samples by Gattefosse (Saint Priest, Cedex, France). Ethanol was purchased from Changshu Yangquan Chemical (China). Other chemicals (oils, surfactants and co-surfactants) purchased from different vendors were of analytical grade.

2.2. Solubility of olmesartan

Solubility of olmesartan was determined in different oils (almond oil, olive oil, black seed oil, eucalyptus oil, sesame oil, babchi oil, clove oil, oleic acid, isopropyl myristate, Campul PG-8), surfactants (Plurol olique CC 497, Labrasol, Labrafil M 1944 CS, Tween 20, Tween 80, Span 20, Span 80) and co-surfactants (PEG and ethanol) by adding an excess amount of drug in 1 ml of selected vehicle in 2 ml capacity Eppendorf tubes, and mixed using a vortex mixer. These Eppendorf tubes were then kept at 25 ± 2 °C in a bath shaker for 72 h to equilibrate. The equilibrated samples were centrifuged at 4000 rpm for 15 min (REMI International, Mumbai, India) and supernatants were filtered through syringe membrane filter (0.45 mm, Axiva Sichem Biotech, New Delhi, India) and the concentration of olmesartan was determined

in each oil, surfactant and co-surfactant by UV spectrophotometer (Shimadzu, Japan) at 254 nm using methanol as diluting solvent [38].

2.3. Emulsification study

Selection of surfactant was done on the basis of %transparency and ease of emulsification, 0.5 ml of surfactant was added in 0.5 ml of oil phase. The mixture was homogenized thoroughly and 0.1 ml of the mixture was then diluted with 50 ml distilled water to yield fine emulsion. The resulting emulsions were observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was assessed by UV beam spectrophotometer using distilled water as blank.

2.4. Construction of pseudo-ternary phase diagram

On the basis of solubility studies, clove oil was selected as the oil phase. Tween 20 and PEG were selected as surfactant and co-surfactant, respectively. Distilled water was used as an aqueous phase. For the determination of existence zone of nanoemulsion, pseudo-ternary phase diagrams were constructed using water titration method (spontaneous emulsification method) Surfactant and co-surfactant (S_{mix}) were mixed in different weight ratios (1:1, 2:1, 3:1, 1:2, 1:3). These S_{mix} ratios were chosen in increasing concentrations of surfactant with respect to co-surfactant and increasing concentration of the co-surfactant with respect to surfactant for detailed study of the phase diagrams. For each phase diagram, oil and specific S_{mix} ratio were mixed well in different volume ratios ranging from 1:9 to 9:1. Sixteen different combinations of oil and S_{mix} (1:9, 1:8, 1:7, 1:6, 1:5 1:4, 1:3.5, 1:3, 3:7, 1:2, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were made so that maximum ratio could be covered for the study to depict the boundaries of the phases formed precisely in the phase diagrams. Slow titration with aqueous phase was done for each weight ratio of oil and S_{mix} under moderate stirring, and visual observation was used for transparent and easily flowable nanoemulsion. The physical state of nanoemulsion was marked on a pseudo three component phase diagram with one axis representing the aqueous phase, second representing oil, and the third representing a mixture of surfactant and co-surfactant at fixed weight ratio (S_{mix} ratio).

2.5. Preparation of olmesartan nanoemulsion systems

The olmesartan nanoemulsion formulations were prepared by spontaneous emulsification method [39]. The drug was accurately weighed and added to the clove oil then S_{mix} (surfactant Tween 20 and co-surfactant PEG) in an appropriate ratio was added and mixed well and stirred on magnetic stirrer, at room temperature for complete homogenization. The weighed amount of water then added drop wise with continuous mixing.

2.6. Thermodynamic stability testing of drug loaded nanoemulsions

To overcome the problem of metastable formulations, physical thermodynamic stability tests were performed [30].

2.6.1. Centrifugation study

The prepared formulations were centrifuged (REMI International) at 5000 rpm for 30 min and observed for phase separation, creaming or cracking. The formulations which did not show any instability (creaming, cracking, phase separation) were selected and subjected to heating–cooling cycle.

2.6.2. Heating–cooling cycle

It was used to see the effect of variations in temperature on the stability of nanoemulsions. Six cycles between refrigerator temperature (4 °C) and 40 °C with storage at each temperature for not less than 48 h