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An ionic liquid-in-water microemulsion as a potential carrier for topical delivery of poorly water soluble drug: Development, *ex-vivo* and *in-vivo* evaluation

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ABSTRACT

In this paper, we report an ionic liquid-in-water (IL/w) microemulsion (ME) formulation which is able to solubilize etodolac (ETO), a poorly water soluble drug for topical delivery using $BMIMPF_6$ (1-butyl-3-methylimidazolium hexafluorophosphate) as IL, Tween 80 as surfactant and ethanol as co-surfactant. The prepared ME was characterized for physicochemical parameters, subjected to *ex-vivo* permeation studies as well as *in-vivo* pharmacodynamic evaluation. The *ex-vivo* drug permeation studies through rat skin was performed using Franz-diffusion cell and the IL/w based ME showed maximum mean cumulative percent permeation of $99.030 \pm 0.921\%$ in comparison to oil-in-water (o/w) ME ($61.548 \pm 1.875\%$) and oily solution ($48.830 \pm 2.488\%$) of ETO. *In-vivo* anti-arthritic and anti-inflammatory activities of the prepared formulations were evaluated using different rodent models and the results revealed that ETO loaded IL/w based ME was found to be more effective in controlling inflammation than oily solution, o/w ME and marketed formulation of ETO. Histopathological studies also demonstrated that IL/w based ME caused no anatomical and pathological changes in the skin.

1. Introduction

Ionic liquids (ILs) are organic salts whose melting point is below 100 °C; some of them are liquid at room temperature, and whose melts are composed of cations and anions (Rogers and Seddon, 2005). Currently ILs are of high interest due to its fascinating properties including low melting temperature, marginal vapor pressure, non-inflammability, low toxicity, high conductivity and high thermal stability. These outstanding properties render them to use as a "green" replacement for traditional organic solvents. Their physiocochemical properties can be modified by using different combinations of cations and anions (Armand et al., 2009; Galinski et al., 2007; Ngo et al., 2000). ILs are receiving much attention due to their wide range of applications in pharmaceuticals such as solvents for poorly soluble drugs, drug reservoirs, drug carriers, antimicrobial agents, antibiofilm agents and ionogels (Smith et al., 2011). An IL based microemulsion (ME) have both the advantages of IL and ME, which can overcome the inability of conventional ME to dissolve number of chemicals including hydrophilic and hydrophobic substances. Recently, we have reported an IL based ME comprising 1-butyl-3-methylimidazolium bromide (BMIMBr) as IL and isopropyl myristate (IPM) stabilized by Tween 80 and Span 20 for dermal delivery of 5-fluorouracil, a poorly water soluble anticancer drug (Goindi et al., 2014).

Etodolac (ETO) is a poorly water soluble NSAID used to relieve inflammation, swelling, stiffness, and pain associated with rheumatoid arthritis, osteoarthritis (Simon et al., 2004) and juvenile rheumatoid arthritis (Boni et al., 1999). It shows poor oral bioavailability, gastrointestinal and cardiovascular side effects following oral administration (Barakat, 2006). Therefore, to overcome these side effects and obtain high drug concentration at the target site, topical application of drug seems to be an ideal route of administration which surpasses the gastric side effects, avoids first pass metabolism and dose dumping and cause increase in patient compliance.

Thus, the present study was aimed at development of an ionic liquid-in-water (IL/w) ME for the topical delivery of ETO using 1-butyl-3-methylimidazolium hexafluorophosphate ($BMIMPF_6$) as an IL, and combination of surfactant and co-surfactants. The prepared formulation was then characterized for particle size, zeta potential, Transmission electron microscopy (TEM), pH and conductometric studies. *Ex-vivo* permeation and *in-vivo* pharmacodynamic studies were carried out and then compared with the oily solution, oil-in-water (o/w) ME and available commercial

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formulation (Proxym gel®) of ETO. Histopathological study and cytotoxic study were also performed to assess the dermal safety of prepared formulations.

2. Materials and methods

2.1. Chemicals

Tween 20, Tween 40, Tween 60 and Tween 80 were purchased from S.D. Fine Chemicals Ltd., Mumbai, India. Capmul, Captex 300, Captex 355 (Abitec Corporation, Columbus, US) and ETO (Akum Drugs and Pharmaceuticals, Haridwar, India) were received as a generous gift samples. IPM (Loba Chemie Pvt., Ltd., Mumbai, India) and oleic acid (Qualigens Fine chemicals, Mumbai, India) were purchased. Proxym gel® was procured from a local pharmacy store. All other reagents used in the experiment were of analytical grade.

2.2. Animals

Wistar rats (250–300 g) and Laca mice (20–25 g) were obtained from Central Animal House, Panjab University, Chandigarh, India. These were housed in polypropylene cages, kept at ambient temperature with a 12 h night/day cycle and supplied with a standard pellet diet and water *ad libitum*. The protocol of the experiment was approved by Institutional Animal Ethics Committee (IAEC), Panjab University, Chandigarh, India.

2.3. Synthesis and characterization of $BMIMPF_6$

BMIMBr was synthesized following a previously reported method (Goindi et al., 2014) and subjected to anion exchange using hexafluorophosphoric acid to obtain $BMIMPF_6$, which was further extracted using dichloromethane and then the organic phase was evaporated under vacuum to obtain the pure IL ($BMIMPF_6$). Further, its characterization using Infrared spectroscopy (IR) and proton nuclear magnetic rasonance (^{1}H NMR) techniques was carried out and data were compared with previously reported spectra to confirm the formation of $BMIMPF_6$.

2.4. Solubility studies

Solubility of ETO was determined in $BMIMPF_6$ in order to develop IL/w type ME using the shake flask method (Avdeef, 2007). Similar studies were also carried out in various oils (IPM, oleic acid, Captex 355, Captex 300, Capmul, castor oil) and surfactants (Tween 20, Tween 40, Tween 60, Tween 80) to formulate o/w ME (control) to be used for comparative studies. An excess of drug was added to a flask containing 10 mL of each solvent, and kept at 37 ± 1 °C in a thermostat water bath shaker for 48 h. Then, the solutions were filtered through 0.45 μm filter and analyzed spectrophotometrically at λ_{max} 279 nm after appropriate dilution. The studies were performed in triplicate.

2.5. Screening of formulation ingredients

The different components for developing IL/w and o/w based MEs of ETO were selected primarily based on the various studies like drug solubility, surfactant efficiency (S_{min}% w/w), co-surfactant efficiency ($SCoS_{min}$), emulsification capability of surfactants, and calculated molecular volumes (v). The emulsification capability of various surfactants namely Tween 20, Tween 40, Tween 60, Tween 80 was screened by homogenizing 1:1 mixture of surfactant and IL phase. This isotropic mixture (50 mg) was diluted with double distilled water to 50 mL to yield fine emulsion. The resulting mixture was observed visually for the relative turbidity. The emulsions were allowed to stand for 2 h and their transmittance was assessed at 638.2 nm by UV spectrophotometer using double distilled water as blank (Date and Nagarsenker, 2007). Different co-surfactants were also screened. The values of co-surfactant efficiency ($SCoS_{min}$) were determined using titration method. Previously, mixtures of the surfactant and co-surfactants in predetermined weight ratios (K_m) were made. Each surfactant/co-surfactant mixture was added to mixtures of IL and water (1:1), in a drop-wise manner under mixing until clear, single phase ME was formed. The transparent ME was maintained at 25 ± 1 °C for a minimum of 72 h to reach the equilibrium. Similar procedure was carried out to screen the surfactant and co-surfactant for formation of o/w type ME, the only difference being the addition of oily phase instead of IL phase.

2.6. Construction of pseudo ternary phase diagrams

The pseudo ternary phase diagrams were obtained by titrating mixture of IL and surfactant mixture (S_{mix}) with water, then titrating mixture of S_{mix} and water with IL, and assessed visually for being a ME, an emulsion or a gel (Talegaonkar et al., 2008; Chen et al., 2004). The S_{mix} consisting of Tween 80 and ethanol used was in weight fractions of 1:1, 2:1 and 3:1. Based on these results, appropriate percentages of IL, water and S_{mix} were selected to prepare ME. Likewise, the entire process was repeated using 1:1, 2:1 and 3:1 weight ratios of S_{mix} with oil and water in order to find out the ME region for o/w type ME.

2.7. Preparation of ME formulations

ETO loaded IL/w based ME (A1) was prepared by incorporating ETO (0.3% w/w) into the $BMIMPF_6$ (2% w/w) in a beaker magnetically stirred at room temperature; Tween 80: ethanol (3:1) S_{mix} was then added to the mixture and stirred. Subsequently, accurately weighed amount of water (80% w/w) was added drop wise under constant stirring into the above solution and clear IL/w type ME was obtained spontaneously. Similarly, blank IL/w based ME (A2) without drug was prepared. Also, an o/w based ME (A3) containing IPM (2% w/w) and oily solution (A4) containing same amount of drug were prepared and used for comparison.

2.8. Characterization of ETO loaded IL/w based ME

2.8.1. Morphology and micromeritics

Morphology and structure of formulation A1 were determined with the aid of TEM (H-7500 Hitachi, Japan). Particle size, polydispersity index (PDI) and zeta potential of A1 were also analyzed using Malvern's Zetasizer™ by employing dynamic light scattering method.

2.8.2. Electrical conductivity

The conductivity measurements were carried out following previously reported method (Li et al., 2012). The electrical conductivity (κ) of formulation A1 was measured as a function of weight fraction of IL using a conductivity meter (Max. electronic model ME 976). The dependence of conductivity on the amount of IL was carried out by dropwise addition of IL phase into the selected mixture at 25 °C.

2.8.3. Drug content and pH

The percent drug content of formulation A1 was determined spectrophotometrically at λ_{max} 279 nm by diluting it 100 times with ethanol. Also, the pH of A1 was determined at room temperature using pH meter (Eutech Instruments pH 510, India).