RAPID PAPER

Formulation and *in vitro* Evaluation of Ketoprofen in Palm Oil Esters Nanoemulsion for Topical Delivery

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Abstract: The aim of the present study is to formulate and investigate the potential of nanoemulsion formulation for topical delivery of ketoprofen. In this study, Palm Oil Esters (POEs) a newly introduced oil by Universiti Putra Malaysia researchers was chosen for the oil phase of the nanoemulsion, because the oil was reported to be a good vehicle for pharmaceutical use. Oil-in-water nanoemulsion was prepared by spontaneous emulsification method. The droplets size was studied by laser scattering spectroscopy (Nanophox) and Transmission Electron Microscopy (TEM). Franz diffusion cells were used, to determine the drug release and drug transferred through methyl acetate cellulose membrane (artificial membrane). The results of droplets size analysis shows the droplets are in the range of nanoemulsion which is below than 500 nm. The *in vitro* release profile shows a sufficient percentage of drugs released through the methyl acetate cellulose membrane. This initial study showed that the nanoemulsion formulated using POEs has great potential for topical delivery of ketoprofen.

Key words: palm oil esters, ketoprofen, topical delivery

1. INTRODUCTION

The unique composition of palm oil, the leading agricultural product of Malaysia, makes it versatile in its application in food manufacturing, chemicals, cosmetics and in pharmaceutical industries. Palm oil consists of triglycerides, a combination of glycerol and different fatty acids. Palm oil is derived from the fruit of the palm tree *Elaeis guineensis* and is rich in C₁₆ and C₁₈ fatty acids^{1,2}. Research bodies in Malaysia such as Malaysia Palm Oil Board (MPOB), Palm Oil Research Institute of Malaysia (PORIM) and Universiti Putra Malaysia (UPM) are actively modifying palm oil in to better constituents having improved properties and new applications. Among of the works are syntheses of esters from palm oil. Alcoholysis of triglycerides from palm oil to produce palm oil esters using lipase as a catalyst is a relatively simple process and moreover

the starting materials are cheap³⁻⁶. Synthesis or modification of lipids for example; modification of Palm Oil into Palm Oil Esters (POEs) has grown due to the possibility of obtaining a wide variety of high quality natural products under mild reaction conditions utilizing selective enzyme substrate as environment friendly biocatalysts^{2,7}.

In this study we undertook the project in collaboration with Basri M. *et al.* of Universiti Putra Malaysia to produce topical nanoemulsions by using Palm Oil Esters (POEs) as the oil phase and ketoprofen which is an excellent candidate among the NSAIDs as the model drug for transdermal delivery⁸. Nanoemulsion is chosen as the transdermal dosage form because it is a promising novel dosage form in terms of controlling droplet size, relatively long-term stability and powerful solubilization ability⁹. Nanoemulsion is defined as emulsion with the droplet size of 20-500 nm¹⁰.

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Due to their characteristic size, nanoemulsion appears transparent or translucent to the naked eyes and possesses stability against sedimentation or creaming.

Ketoprofen is an aryl propionic acid derivative and a potent non-steroidal anti-inflammatory drug (NSAID), which also has analgesic and antipyretic activities. This drug has been widely used for the treatment of rheumatoid arthritis and osteoarthritis¹¹. However it accompanies adverse side effects including gastrointestinal irritation usually when administered orally in chronic doses and also in acute administration in some case. Efforts to reduce the adverse effects have been attempted. One promising method is to administer the drug topically near to the effected site(s). Several topical delivery dosage forms containing ketoprofen have been reported. For example patches¹².

The aim of this study is to formulate the nanoemulsion containing ketoprofen as a model drug and POEs as the oil phase and evaluate the release and transfer of ketoprofen by *in vitro* in Franz diffusion cells through methyl acetate cellulose membrane. The results obtained could be used to predict the suitability of the dosage form formulated for topical drug delivery.

2. EXPERIMENTAL

2.1 Chemicals and reagents

Tween 80[®] was chosen as the surfactant (Sigma pharmaceuticals, USA). Ketoprofen was purchased from Eurochem Asia limited, Shanghai, China. Palm Oil Esters (POEs) was provided by co-researcher in Universiti Putra Malaysia. Fastumgel[®] (A. Menarini industrie Farmaceutiche Riunite, Italy) was bought from a retail pharmacy in Penang, Malaysia. Water used in this study was distilled water and all other solvents and chemicals were either of analytical reagent or high performance liquid chromatography (HPLC) grade.

2.2 Preparation of nanoemulsion

In this study nanoemulsions were prepared by spontaneous emulsification process¹³ (titration method). Palm Oil Esters and Tween 80 are left for 30 min under magnetic stirring 600 rpm at 25°C to mix thoroughly. Then weighed amount of ketoprofen was added into the solution and mixed thoroughly, until a clear dispersion was formed, which indicated that all the drug solubilization is completed. To the resulting mixture water was added drop by drop while mixing with the aid of magnetic stirrer at 600 rpm and temperature of 25°C. The final compositions of the nanoemulsion are Oil 24.37% (w/w), Surfactant 37.05% (w/w), ketoprofen 2.5% (w/w) and water 36.1% (w/w). The dosage form formulated was subjected to the following studies.

2.3 Nanoemulsion droplet size analysis

The droplet size distribution of the prepared samples were measured by laser scattering spectroscopy technique (Nanophox, SympaTec, GmbH, Germany). The samples were placed in the temperature-controlled sample holder at least 5 min before starting the measurement. Light scattering was set at an angle of 90° and the temperature was maintained at 25°C. From each sample, three correlation functions were measured over periods of at least 450s. Second cumulant method of analysis was used to calculate the mean droplet size according to the intensity of scattered light (Z-average diameter). The results are the mean and standard deviation (S.D.) of at least 3 measurements of the samples.

2.4 Transmission electronic microscopy (TEM)

Morphology and structure of the nanoemulsion were studied using Transmission Electron Microscopy (TEM) LEO 912AB EFTEM. To perform the TEM observations, samples were placed on a formvar carbon-coated copper grid (200 mesh in⁻¹) and then stained with 1% phosphotungstic acid. The excess phosphotungstic acid on the sample was gently wiped off using filter paper and examined after drying for about half an hour at room temperature.

2.5 *In vitro* release or transfer of ketoprofen through methyl cellulose membrane.

The diffusion of ketoprofen across artificial membranes was investigated by using Franz diffusion cells (LG-1083-PC; Erweka). The pore size of the membrane was 0.2 µm. Cellulose acetate membrane was soaked in receptor medium (pH=7.4) for 24 h prior to the experiment¹⁴. After fixing the membrane between the donor and receptor compartments 0.2 g of the ketoprofen formulations were applied evenly onto the membrane. The effective area available for diffusion was 0.636 cm² (d=9 mm). The receptor compartments were filled with pH 7.4 phosphate buffer which was stirred by a magnetic bar and its temperature was maintained at 37±0.1°C. After the application of ketoprofen formulations, 400 µL of the receptor medium was withdrawn every hour up to 8 h, and replaced immediately with an equal volume of fresh phosphate buffer. These samples were analyzed by HPLC for ketoprofen content. All experiments carried out in triplicates.

2.6 Analysis of ketoprofen

The quantity of ketoprofen in samples collected from Franz Diffusion Cell was determined by HPLC method which was developed and validated¹⁵. The HPLC system consisted of an isocratic pump (Shimadzu Co, Japan, Model LC-10-ATVP), a degasser, an auto-sampler (Shimadzu, Japan, Model SIL-10 ADVP) and a UV visible detector (Shimadzu, Japan, Model SPD 10 A). Samples were chro-

Fig. 1 Laser Scattering Spectroscopy Results for Ketoprofen Unloaded POEs Nanoemulsion.

Fig. 2 Laser Scattering Spectroscopy Results for Ketoprofen Loaded POEs Nanoemulsion.

Fig. 3 Transmission Electron Microscopic Image of Ketoprofen Nanoemulsion Showing Size of Some Oil Droplets.

(A)

(B)

Fig. 4 Examples of Chromatograms of Samples Collected from the Receiver Cells after (A) 1 h and (B) 8 h.

Fig. 5 Percentage of Cumulative Release of Ketoprofen Transferred through Methyl Cellulose Acetate Membrane.

matographed into a column, 250 mm long and internal diameter 4.65 mm containing 5 μm C₁₈ ODS hypersil as stationary phase. The samples were eluted by the mobile phase consisting a mixture of 52% acetonitrile: 10% methanol (HPLC grade): 38% phosphate buffer (0.005 M Na₂HPO₄ adjusted to pH 3.0 with orthophosphoric acid) which was filtered through 0.45 μm membrane filter. Detection wavelength was at 260 nm and the run time was 10 min. The mobile phase flow rate was 0.9 mL/min. The injection volume was 10 μL .

3. RESULTS AND DISCUSSION

POEs are a new ingredient for pharmaceutical industry^{16,17}. The oil is non irritant on human skin², increases the skin hydration due to its moisturizing properties², shows high thermal stability², and it could be used in cosmetic or medicinal formulations to deliver poorly water-soluble lipophilic actives or drugs¹⁷. It has been reported that only very specific pharmaceutical excipients combinations lead to efficient nanoemulsion formulations¹⁸. Abdurrahman *et al.*¹⁶, in their study have shown that POEs can be used to produce nanoemulsions and suggested that palm-based nanoemulsion can be used in transdermal delivery.

Droplet size is the most important characteristic for nanoemulsion. The defined size of nanoemulsion varies in literature. However a well accepted typical droplets size falls in the range of 20 - 500 nm¹⁰. The results obtained by laser scattering spectroscopy are in the range of nanoemulsion. Droplet size of ketoprofen unloaded 84.75 nm (Fig. 1) and droplet size of ketoprofen loaded 229.64 nm (Fig. 2). Droplets size results of blank formulation (drug unloaded) are in agreement with the results obtained by Basri *et al.*, (2008)¹⁷, they have reported POEs and nonionic surfactants mixtures namely Span 20[®] and Tween60[®] have produced droplets below 200 nm. The increase in droplet size (Fig. 2) could be due to the drug loading.

TEM determination is one of the studies conducted in order to confirm the particle size obtained by the laser scattering spectroscopy. Because recently people started to have doubts about measurements made by using laser scattering spectroscopy method which usually need significant dilution of samples. In the TEM image (Fig. 3), the nanoemulsion appeared dark and the surroundings were bright. The micrograph exhibits, the droplets size of the sample were in the range of nanoemulsion.

It was predicted in this study reduction of nano-droplets size will lead to the increase in total surface area for release, transfer and absorption of the drug. Nano delivery systems have been reported to increase the bioavailability and efficacy of a number of compounds such as anti-inflammatory agents¹⁹. It would be of interest if nano delivery

system consisting POEs as the oil phase would also have these abilities.

In this preliminary study, a 2.5% ketoprofen was incorporated in nanoemulsion containing POEs as the oil phase and having viscoelastic or gel properties was developed. It is targeted to be used as transdermal dosage form with 2.5% ketoprofen in the formulation in order to compare with marketed product (Fastum gel[®], containing 2.5% of ketoprofen).

Figures 4 (A) and (B) are examples of HPLC chromatograms showing the peaks of ketoprofen resulting from analysis of sample collected 1 h and 8 h after application of the product onto the methyl acetate cellulose membrane respectively. The chromatogram showed that the retention of ketoprofen peaks at 5.8 min. Increase in height of the peaks from 1 h to 8 h (Fig. 4) ensures increase in ketoprofen release with time.

Figure 5 showed the cumulative percentage of ketoprofen release or transferred from POEs nanoemulsion and marketed formulation through the methyl acetate cellulose membrane into receptor of the Franz diffusion cells. The percentage of ketoprofen release increased from $3.65 \pm 0.01\%$ at 1 h to $33.49 \pm 0.08\%$ at 8 h in POEs nanoemulsion and the percentage of ketoprofen release in marketed formulation was $4.73 \pm 0.01\%$ at 1 h and $33.06 \pm 0.24\%$ at 8 h. Cumulative percentage release of drug from formulated nanoemulsion mimic the marketed product at different interval of time (Fig. 5). Thus POEs nanoemulsion has potential to deliver ketoprofen with similar release pattern of marketed formulation through the artificial membrane.

Studies are in progress on the transfer of ketoprofen through excised rat skin of the rats to get a clearer idea on what might happen when the POEs nanoemulsion is applied topically.

4. CONCLUSION

Ketoprofen in POEs / water nanoemulsion was successfully prepared by the spontaneous emulsification method (titration method). The droplets size of nanoemulsion was found to be 229.64 nm. *In vitro* studies reveal that, sufficient percentage of ketoprofen was released and transferred through the artificial membrane, which in turn might result in good permeation when applied in skin. It can be concluded, that palm oil esters is a good candidate for use as a pharmaceutical ingredient to produce topical dosage form to deliver ketoprofen and possibly for other drugs also.

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