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Research Article

ASSESSING THE CHARACTERIZATIONS OF KETOPROFEN LOADED AND UNLOADED VIRGIN COCONUT OIL BASED CREAMY NANOEMULSION

SURAWEERA RK, PASANSI HGP, SAKEENA MHF*

Department of Pharmacy, Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka. Email: Sakeena.hameem@pdn.ac.lk

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ABSTRACT

Objective: The aim was to analyze the characterization of the formulations prepared with and without ketoprofen in virgin coconut oil (VCO) based emulsions.

Methods: The primary emulsions were prepared by magnetic stirring, and the process was continued with high sheer homogenization, aiming for nanoemulsion. The present study evaluated the characteristic properties of emulsions such as; droplets size, pH, viscosity, creaming index and microscopic features.

Results: Droplets size falls within the range; 144.9-664.7 nm and pH value was 4.91-5.66. It was noted homogenization process improved the characteristics of the emulsion, mainly droplets size and viscosity. Further, found the incorporation of ketoprofen affects the droplets size of emulsions significantly, this could be due to the amphiphilic nature of drug that can affect the core of the surfactants. Characteristic properties of VCO based emulsions were affected by high shear homogenization and incorporation of drug.

Conclusion: Characteristic properties of 2.5% (w/w) ketoprofen loaded 23.60% VCO: 30.53%, Tween 80®: 45.87% water creamy emulsion is suitable for topical application.

Keywords: Ketoprofen, Virgin coconut oil, Nanoemulsion.

INTRODUCTION

Emulsions have gained high attention in many industries as food, pharmaceutical, agrochemical, paint and energy industries [1,2] and in recent years, in pharmaceutical industries enormous interest on emulsions improved greatly with the development of new types of emulsions [3]. The advantages of emulsion over other dosage forms it is highly recommended to be produced in the pharmaceutical industry. Emulsions are liquid/semisolid preparations having homogeneous dispersions of two immiscible liquids one liquid phase as droplets throughout in the other another immiscible liquid phase, a stable emulsion system is produced with the addition of suitable emulsifying agent [2,4].

Emulsion systems are classified based on droplets size as macroemulsions, microemulsions and nanoemulsions. Nanoemulsions have tremendous applications in many areas and recently characterized as a promising drug delivery system in the pharmaceutical industry [5,6] due to their favorable characteristics over other dosage forms. Nanoemulsions are colloidal dispersions, usually in the 20-500 nm size range, which are transparent or translucent [6-8]. It is exploded as an effective dosage form due to its advantages as improving the bioavailability and bioefficacy [8], preventing physical destabilization by minimizing creaming, sedimentation and flocculation which occurs during storage [5], containing biophysical and sensorial benefits highly valued by consumers [8], and uniform spreading [9]. These properties make them efficient transdermal drug delivery systems for the active ingredients incorporated in many personal care and health products [10].

Concerning factors of oil:water ratio, electrolyte concentration, temperature and type of emulsifier/surface active agent [1]; emulsions can be further classified into two types named as oil in water (o/w) where internal oil phase (less polar) is dispersed throughout the continuous aqueous phase (high polar) and water in oil (w/o) where oil acts as the continuous phase and water droplets as the internal phase [4,11]. In emulsions, depending on the consistency of either internal or external phase; the formulations vary from liquid to semisolid characteristics. Therefore, different pharmaceutical emulsions are available from a range of low viscosity lotions to high viscosity creams [12].

Efficiency of delivering the drug molecules depend on the development of high-quality emulsions with effective characteristics [13]. In pharmaceutical industry, characterization of emulsions and manipulation of its properties is imperative [2]. In this study main characteristics of emulsions were studied; such as droplets size, viscosity, pH, microscopic analysis and creaming index using pre-developed analytical techniques and methodologies. The droplets size of emulsions mainly affects stability, appearance and texture [5] and in emulsions with small droplets size due to its long-term kinetic stability; shelf life of product particle size can be enhanced [10]. pH value monitoring is also crucial as it affects the stability and quality of the final product [5] and viscosity affects the droplets size of the formulation [14]. Microscopic analysis is important in this study as oil-in-water emulsion was to be formulated because ketoprofen has mainly lipophilic properties and creaming index provides information on stability [14].

Ketoprofen is a potent non-steroidal anti-inflammatory agent (NSAID) having analgesic and antipyretic activities and is used in the treatment of rheumatoid arthritis, osteoarthritis and mild to moderate pain [15-17]. Compared to other NSAIDs; ketoprofen is considered to be an effective drug in transdermal drug delivery due to its appropriate partition coefficient [17] and due to its limitations in clinical oral use, its topical delivery was studied in past years [16,18].

Tween 80® which is a surfactant is used as an emulsifier to prevent separation of the emulsion into two distinguished phases on standing [19]. In ketoprofen loaded emulsions, Tween 80® improves the stability of the formulation [20] as surfactants decline the destabilizing effects through steric effects, enhancing viscoelastic

properties, lowering the interfacial tension and promoting interfacial gradients [2,11].

The objective of this study was to assess the characterization studies with and without Ketoprofen incorporated virgin coconut oil (VCO) based creamy emulsions containing Tween 80® as the surfactant prepared using high sheer homogenization and magnetic stirring.

METHODS

Emulsion preparation

Tween 80® and ketoprofen were obtained from Analytical Instruments Pvt. Ltd., Sri Lanka and VCO was obtained from Serendipol (Pvt.) Ltd., Sri Lanka. Initially in pre-formulatory studies, ratios that resemble oil-in-water, creamy emulsions were selected by constructed ternary phase diagrams using CHEMIX software and formulas were optimized (Table 1) [21]. 10 g of each emulsion was prepared by two methods as Method 1 (magnetic stirrer/spontaneous emulsification) and Method 2 (high shear homogenization) with and without incorporation of ketoprofen [22].

Without incorporating ketoprofen

In Method 1, according to ratios of stable samples A, B and C; VCO and Tween 80® were mixed thoroughly under magnetic stirrer (1 MLH magnetic stirrer, Rajendra Electrical Industries Limited, Mumbai, India) at 600 rpm and 25°C for 15 minutes until a clear mixture was formed. To the resulting mixture distilled water was added drop-wise with the constant stirring at 600 rpm and 25°C. In Method 2, the primary emulsion prepared by Method 1was mixed further under high shear homogenizer (Homogenizer OV5, VELP Scientifica, Italy) at 10,000 rpm and 25°C for 5 minutes.

Method of ketoprofen incorporation

About 2.5% ketoprofen emulsions were prepared in both methods concerning drug loaded stable formulation sample B. In Method 1, magnetic stirring was applied at 600 rpm and 25°C for 15 minutes to VCO and Tween 80® mixture to form a clear mixture and to that ketoprofen was added following thorough mixing for 5 minutes. Then, distilled water was added to it drop-wise with the constant stirring at 600 rpm and 25°C. In Method 2, ketoprofen was incorporated with magnetic stirring to the magnetic stirrer mixture of VCO and Tween 80® as in Method 1 and distilled water was added drop by drop with constant stirring at 600 rpm and 25°C.

Characterization of emulsion

Drug unloaded preparations

Identified stable formulations from ternary phase diagrams were subjected to characterization by analyzing droplets size, creaming index, viscosity, pH value and microscopic studies. Creaming index was measured in the Pharmaceutical Laboratory of Department of Pharmacy, Faculty of Allied Health Sciences, University of Peradeniya, pH value was measured in the laboratory of department of Radiography, Faculty of Allied Health Sciences, University of Peradeniya and microscopic studies were performed in the Laboratory of Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Peradeniya. Viscosity measurements were taken at the Laboratory of Department of Mechanical Engineering, Faculty of Engineering, University of Peradeniya.

Droplets size analysis

The droplets size analyses of the stable emulsions were performed for freshly prepared samples at Sri Lanka Institute of Nano Technology (SLINTEC) using a laser light scattering apparatus (Malvern Zetasizer Ver. 6.00, Malvern Instruments, Worcestershire, UK) at the temperature of 25°C. Electrophoretic light scattering technology/laser Doppler micro-electrophoresis was used to measure velocity of dispersed particles after applying an electrical field and electrophoretic mobility, zeta potential and size distribution were calculated.

Table 1: Emulsions of different compositions after optimization (samples of 10 g)

Sample	VCO	Tween 80®	Water
A	2.620	3.392	4.095
B	2.360	3.053	4.587
C	1.772	3.543	4.686

Creaming index

To measure creaming index, 5 g of each stable emulsion was transferred into universal bottles that were tightly sealed with a metal cap. Then they were left to stand at 25°C for 24 hrs and checked for the creaming index. Creaming occurs due to the migration of the dispersed phase of an emulsion, under the influence of buoyancy. In storage emulsions, unstable separate into two layers as an opaque layer at the top and a transparent layer at the bottom. As VCO has low density than distilled water phase, oil particles move upward. Creaming extend measured using creaming index (100 [HD/HE]) where HE is the total height of the emulsions in the tubes, and HD is the height of droplet-depleted lower layer. If creaming index is high, droplets will move upward faster and more droplet aggregation occurs.

Viscosity analysis

Viscosity analysis was performed for freshly prepared stable emulsions. For this purpose Redwood Viscometer (Seta Redwood Viscometer, Stanhope-Seta, London Street, UK) was used at 25°C. Time taken to flow 1 ml of sample through the viscosity meter into a 10 ml measuring cylinder was measured from a stopwatch and time for 50 ml sample to flow was calculated.

pH value analysis

pH values of freshly prepared selected stable emulsions were determined by a pH meter (Mi 150 pH/temperature bench meter, Milwaukee, Martini Instruments, Hungary) at 25°C. First, pH meter was calibrated using pH 4.01, 7.01 and 10.01 buffer solutions respectively and then for each Sample C measurements were taken for the accuracy of values and to minimize effects of external factors for the measurements.

Microscopic analysis

Microscopic analysis of stable emulsion samples was performed using a microscope (Olympus Vanox S Microscope, Japan). It was to determine the emulsion type (O/W or W/O) of each sample. A drop of lipid soluble dye Sudan IV was added to the drop of the emulsion placed on a slide with a dropper and mixed using a spatula. Then it was covered with a cover slip and observed under a microscope for the staining pattern. As the dye is lid lipid soluble, orange-brownish external phase with clear droplets will indicate water-in-oil type emulsions, whereas clear external phase with stained droplets indicates oil-in-water emulsion.

Drug loaded preparations

The most stable 2.5% ketoprofen incorporated emulsion (Sample B) [20] was studied for pH value, microscopic analysis and creaming index concerning samples prepared via both methods (Sample B3 and B4) and droplets size analysis was performed only for Sample B4 as Sample B1 was a microemulsion and Sample B2 was a nanoemulsion.

RESULTS

Characterization of drug unloaded preparations

Droplets size analysis

According to Fig. 1 in both methods Sample B (B1 and B2) showed the highest droplets size whereas Sample A (A1 and A2) showed the lowest droplets size. And comparative samples prepared by Method 2 had lower droplets size than samples prepared by Method 1.

Creaming index

All the emulsion samples formulated via Method 1 and Method 2 did not show creaming properties at 25°C after 24 hrs. So creaming index was "zero" for all emulsions.

Viscosity analysis

According to Fig. 2, viscosity of emulsions was increased in Method 2 with high shear homogenization than in Method 1. However, the relationship between the varying composition of VCO, Tween 80® and water with the viscosity of the emulsion was not clearly represented. However, it was shown that in both methods Sample A had the highest viscosity and Sample B had the lowest.

pH value analysis

In all A, B and C samples; samples prepared by Method 2 had higher pH values than Method 1. In both methods Sample B (B1 and B2) had the highest pH whereas Sample A (A1and A2) had the lowest (Fig. 3). And it could be seen that in both methods pH of the sample increase with the increase amount of water.

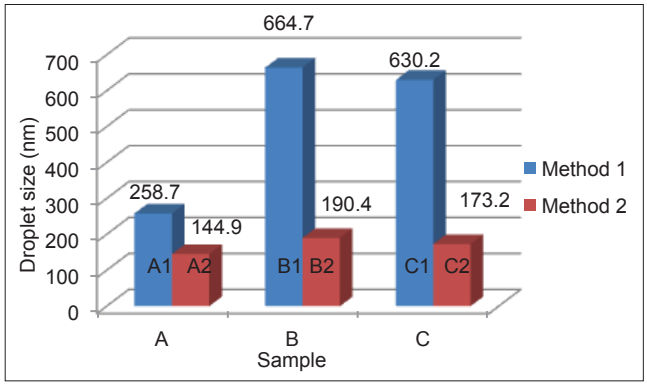

Fig. 1: Droplets size of stable emulsions

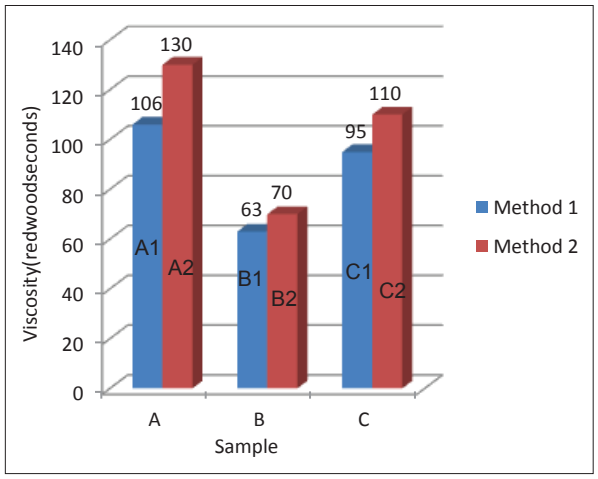

Fig. 2: Viscosity of stable emulsions

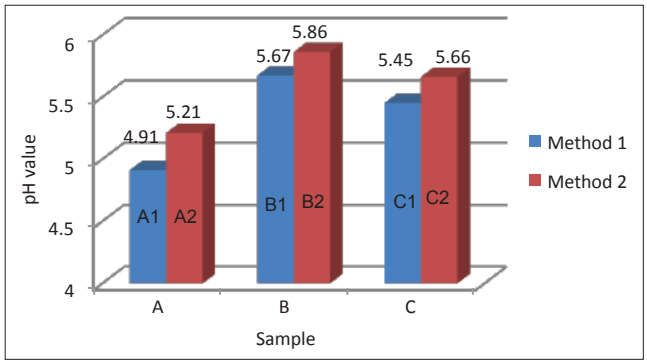

Fig. 3: Comparison of pH values of stable emulsions of Method 1 and Method 2

Microscopic analysis

In all emulsions, samples formulated via Method 1 and Method 2; the dispersed droplets were stained in brownish orange color and the surrounding remains transparent under microscopic observation. As Sudan IV oil soluble dye was used in staining all the studied emulsion samples proved to be O/W emulsions.

Characterization of the drug loaded preparations

Droplets size analysis

Method 2 - Sample B

Droplet size of the emulsion without ketoprofen (B2) was decreased when ketoprofen was incorporated into the sample (Fig. 4).

Creaming index

B3 and B4 ketoprofen loaded emulsion samples formulated via Method 1 and Method 2 did not show creaming properties at 25°C after 24 hrs. Creaming index was "zero" for both emulsions.

pH value analysis

According to Fig. 5; ketoprofen 2.5% loaded emulsion samples prepared by Method 2 had higher pH values than Method 1. In both Method 1 and Method 2 stable emulsions without ketoprofen (B1 and B2) had higher pH values than stable emulsions with 2.5% ketoprofen (B3 and B4). And also in both with and without drug loaded samples; emulsions formulated by Method 2 (B2 and B4) had higher pH values than emulsion formulated by Method 1 (B1 and B3).

Microscopic analysis

In both B3 and B4 emulsion samples formulated via Method 1 and Method 2; the dispersed droplets were stained in brownish orange color and the surrounding remains transparent under microscopic

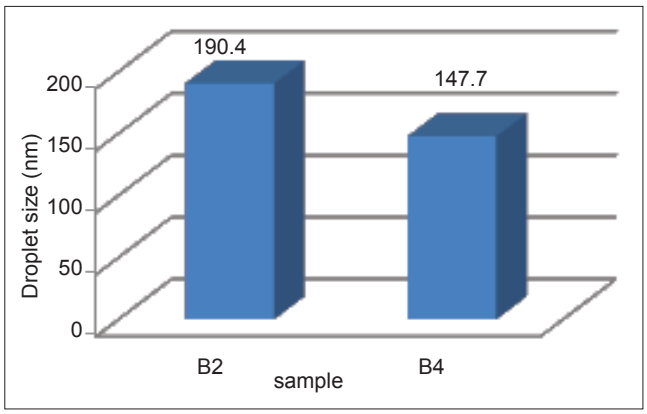

Fig. 4: Droplets size of emulsion without (B2) and with (B4) ketoprofen via Method 2

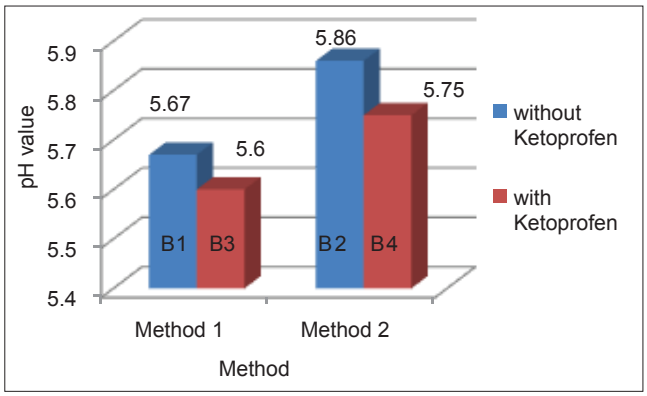

Fig. 5: Comparison of pH values of stable sample C emulsions with and without ketoprofen in Method 1 and Method 2

observation. As Sudan IV oil soluble dye was used in staining both emulsion samples proved to be O/W emulsions.

DISCUSSION

Characterization of emulsions was crucial in this study because specifically oil-in-water (O/W), creamy emulsion with nano-size droplets was to be formulated as O/W emulsion was more preferable in transdermal application because of non-greasiness. And the low interfacial tension of the O/W droplets enhances the penetration of active agent [9]. As ketoprofen is a poor water soluble compound, its transdermal absorption can be enhanced through improved solubility by dissolving them in an oil phase [18]. Creamy emulsions were preferred in this study as transdermal drug delivery system was to be formulated and according to literature, concerning the characteristics as the phase and texture behavior of oil/water/surfactant system, new emulsion types have been developed recently [3]. Comparably emulsions with nano-size droplets have been used recently in many personal care and health products [10] due to their advantages as high kinetic and physical stability, high solubility, rapid penetrability and ultimately improving bioavailability in transdermal drug delivery.

Emulsion characteristics are mainly affected by stirring speed and time [23]. It can be justified as the increased viscosity of emulsion was achieved with higher stirring speed (Fig. 2) resulting decreasing particle size (Fig. 1) led to an increase in the interfacial surface which is also proved in literature [24,25]. Well-mixed fine emulsions are more stable than coarse emulsions. Therefore, utilization of high stirring intensity could improve the characteristics of the emulsion system and it was identified that in ketoprofen loaded emulsions homogenization was more effective in stable emulsion formation than spontaneous emulsification [20].

When concerning the effect of viscosity on droplets size, it was illustrated in literature that the decreased viscosity can increase the droplets size [14,24]. When comparing Figs. 1 and 2, it can be observed that in all the cases Method 2 samples have higher viscosity and lower droplets size than Method 1 samples. When considering sample B1 and B2, at same VCO: Tween 80®: Water composition; nanoemulsions had higher viscosity than in microemulsions, which was reported in literature [14]. And an order was observed from those graphs where viscosity of samples decreased in the order of A, C, B and droplets size reduction was in inverse order of B, C, A. Decreasing droplets size with increasing viscosities is due to hydrodynamic interactions, effective dispersed-phase concentration, polydispersity and degree of flocculation. Due to hydrodynamic interactions, viscosity is more when the distance of separation between the droplets is less and due to effective dispersed-phase concentration it increases as a result of reduction in the droplets diameter causing the ratio of the emulsifier thickness to the droplets size significant. Polydispersity is decreasing with the decreasing droplets size and degree of flocculation increases with decreasing particle size [25].

Droplets size of the emulsion is crucial in this study as nanoemulsion was to be formulated to increase the effectiveness of the formulation. The minimum droplets size that can be achieved depends on stirring speed and pressure, viscosity of components, oil composition and surfactant properties [8]. When concerning without drug loaded stable samples [20], Sample B has the highest droplets size of 664.7 nm and 190.4 nm respectively in Method 1 (B1) and Method 2 (B2) whereas Sample A has the lowest droplets size of 258.7 nm and 144.9 nm respectively in Method 1 (A1) and Method 2 (A2) (Fig. 1). According to literature survey nanoemulsions comprise of droplets size between 20 and 500 nm [16] and microemulsions generally fall between 500 and 2000 nm [26]. Except Samples B1 and C1, which were microemulsions, all the other samples were nanoemulsions (Fig. 1). When ketoprofen was incorporated to emulsion Sample B2, droplets size was reduced according to Fig. 4. This is because when ketoprofen was added, in some cases viscosity of the sample increased [27] thus droplets size reduced [25].

pH measurements are also important in this study as creamy emulsion was formulated for topical application. To be compatible with the skin and to minimize the destruction of skin proteins and enzymes emulsion pH should be similar to the pH of the skin [28]. Earlier findings on the effect of formulation's pH on the skin showed that more alkaline formulations having pH 9-10, caused skin irritation, swelling and skin dehydration [29]. Skin pH ranges between 5 and 6, where pH 5.5 considered being the ideal average pH [28]. According to Fig. 3 all the stable emulsions without ketoprofen had the pH within the range 4.91-5.86 making them suitable for further studies. Especially 2.5% ketoprofen incorporated emulsion had pH of 5.6 and 5.75 in Method 1 and Method 2 respectively proving the formulation is suitable for topical application (Fig. 3). The decrease in pH of emulsions after incorporation of ketoprofen (Fig. 5) in both methods is due to the acidic nature of the drug as it is a propionic acid derivative [16]. And Fig. 3 shows that pH of samples prepared by Method 2 had higher pH values compared with which prepared by Method 1. Similar results reported in literature; the reduction of droplets size by Method 2 than in Method 1 (Fig. 1) led to increased surface area, increased solubility and ultimately decreased the acidity of the medium [14].

According to microscopic studies all the selected and stable samples were O/W and specially the final 2.5% ketoprofen incorporated emulsion was also an O/W emulsion. It is because the samples B, C and D had suitable VCO: Tween 80®: Water compositions and Tween 80® is a hydrophilic surfactant (hydrophilic-lipophilic balance value of 15) [11]. Emulsion type depends on volume fractions of oil and water phases [2] and in this study; it did not change with the addition of ketoprofen because the drug is incorporated in oil globules. Only when the drug is excess phase inversion can occur. And also microscopic studies did not provide clear images as particle sizes are in micro and nano range. In literature from earlier findings it was proven that spherical droplets assuring the emulsion droplets flow freely [28].

CONCLUSION

It can be concluded that, characteristics of emulsion dosage such as droplets size, pH, creaming index and viscosity of 2.5% (w/w) ketoprofen loaded 23.60% VCO: 30.53% Tween 80®: 45.87% water creamy emulsion is suitable for topical application. High shear homogenization provided more favorable emulsion characteristics than spontaneous emulsification.

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