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Formulation, stability evaluation and characterization of Tween 80® consisting of Neem oil-based emulsion

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Abstract

The pharmaceutical properties of an emulsion ranges from lotion to cream instead of the consistency of the content and the particle size. Neem oil is widely used in Ayurveda medicine due to the presence variety of bioactive compounds which is used in various cosmetic preparations. Due to having considerable Sun Protection Factor value in Neem oil and leaves extract, Neem is used in development of a sunscreen formulation. The objective of this study was to formulation, optimization, stability evaluation and characterization of Tween 80® consisting of Neem oil-based emulsion. Primary emulsions were prepared using Neem oil, distilled water, and Tween 80® according to randomly selected ratios and secondary emulsions were obtained by high shear homogenization of respective primary emulsions. The short term, long term and accelerated stability were visually observed. Creaming, sedimentation, flocculation, coalescence, phase separation and phase inversion were considered as unstable conditions to evaluate stability. The microscopic analysis, pH, viscosity, creaming index and colour change were used to characterize the emulsions. All optimized primary and secondary emulsions were stable throughout the short-term stability evaluation period. All optimized secondary emulsions were stable throughout the accelerated stability evaluation period. Creaming was observed as the major unstable condition in long term stability evaluation. The formula consisted of 30% Neem oil, 40% water and 30% Tween 80® in Oil-in-Water type secondary emulsion was the best formula obtained with the viscosity of 2750 Redwood seconds which is within the non-skin irritating range of pH 4-7.

Keywords: Neem oil; Emulsions; Stability evaluation; Characterization

1. Introduction

Creams, ointments, gels, suspensions, and emulsions are commercially available pharmaceutical products intended to apply on skin to get localized effect. In pharmaceutical industry, emulsions provide a liquid drug delivery in the form of minute globules and permits easy drug administration to the human body. An emulsion is an opaque heterogeneous system of two immiscible liquid phases (oil and water) where one phase is dispersed in the other as drops of microscopic or colloidal size [1]. The Oil-in-Water (O/W) and Water-in-Oil (W/O) type emulsions are commercially available depending on which phase compromises the drops [2]. Both emulsion types are used to apply on the skin depending on the factors like emollient on the skin, nature of the therapeutic agents and the nature of the skin [1]. In addition, Water-in-Oil-in-Water (W/O/W) and Oil-in-Water-in-Oil (O/W/O) emulsions are formulated to obtain controlled drug delivery and targeting [3]. Primary emulsions are prepared in small scale and large scale by primary homogenization of oil, water, and emulsifying agents whereas secondary emulsions are prepared by secondary homogenization of prepared primary emulsions [1].

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Neem which is belongs to the family Meliacea is a tropical ever green tree native to India. This plant is used in Ayurveda medicine due to its medicinal properties. The seeds contain 45% of fixed oil which is brownish yellow in colour. It has characteristic acrid taste and a persistent unpleasant odor [4]. Since the Neem leaves extract and Neem oil have been proven with considerable Sun Protection Factor (SPF) value [5], affordability, less toxicity and high availability, this study was aimed to formulate stable emulsions consisting with Neem oil as the oil base to evaluate the stability and characteristic properties with the aim of expanding this study to formulate a Neem oil-based sunscreen formulation.

Emulsifying agents are being considered when formulating emulsions to protect newly formed drops from re-coalescence thus stabilizes the emulsion [2]. The prime importance of the emulsifying agent is to promote the emulsification as well as to maintain the stability throughout the shelf life of an emulsion. The wetting agents are the mostly used emulsifying agents in pharmaceutical formulations. Anionic, cationic, and non-ionic agents are the main types of wetting agents [1]. Among them, Tween 80® is a non-ionic polysorbate which is widely used as an emulsifying agent to formulate O/W emulsions [6]. In addition, it is an odourless, light brown viscous liquid [7]. Hydrophilic Lipophilic Balance (HLB) is used to categorize the emulsifying agents that indicates the polarity of the substances either as hydrophilic or lipophilic. The usual range of HLB value is 1-20. The highly lipophilic emulsifying agents are within the HLB value of 3-6 whereas highly hydrophilic emulsifying agents are within the HLB value of 8-18 [8, 1]. The study conducted by Mahdi *et al*., [9], based on "The effect of surfactant and surfactant blends on pseudo ternary phase diagram behavior of newly synthesized Palm kernel oil esters" mentioned that non-ionic surfactants are safe, biocompatible and are not affected by pH changes in the media due to their uncharged nature and it is important to consider the proper HLB value when selecting a surfactant for specific oil.

Since the initial properties of an emulsion are changing over the time, stability evaluation and characterization should be considered to determine the quality of an emulsion. Stability of an emulsion is defined as the maintenance of the initial state that was attained after homogenization of two or more immiscible liquids. In general stability is measured as short term and long term periods. [10, 11]. In addition, accelerated stability and effect of temperature are considered to determine the stability [12]. Creaming, sedimentation, flocculation, coalescence, phase separation and phase inversion are the main unstable conditions of an emulsion to determine the stability [13]. Creaming or sedimentation is the migration of particles either to top or bottom of the formulation and are considered as the reversible mechanisms which can be redistributed upon shaking [1]. Flocculation is the process where two or more droplets come closer and form aggregates without losing their individual integrity. Coalescence is the mechanism where two droplets come closer to each other for an extended time and become distort due to thinning of the liquid film between the two droplets [14, 15]. Phase inversion occurs due to high volume fraction in an emulsion and spontaneously revert either from W/O to O/W or O/W to W/O [14, 16, 17]. Short term and long term stability evaluations are mainly determined by the absence of creaming and coalescence [18]. Since the kinetic stability of an emulsion is an important parameter, centrifugation is carried out to determine its accelerated stability [19]. As far as the extreme of temperature can cause emulsions cracking, it is essential to determine the stability upon different temperatures [1].

Characterization of an emulsion is important to determine the granulometric distribution of the emulsions. The microscopic analysis, pH value, viscosity, droplet size analysis and creaming index are considered to characterize the emulsions [20, 21]. Microscopic analysis is used to determine whether the emulsion is O/W or W/O or O/W/O or W/O/W type [22] and to analyses the size distribution of the dispersed droplets in an emulsion [23]. Measuring of the pH value is important since it has a strong influence on emulsion stability. The pH value of the skin is range from pH 4-7 and average pH is 4.7 [24]. The viscosity of the dispersed phase in an emulsion is mainly affected by large droplets in the dispersed phase at high phase volume fraction [25]. The viscosity of an emulsion is substantially greater than the viscosity of either oil or water and changes can occur due to the temperature, and it has a significant influence on stability of an emulsion. Therefore, it is essential to measure the viscosity [23]. Since creaming index can provide indirect information about extent of droplet flocculation, measuring of the creaming index in an emulsion is important to measure the creaming percentage after 24 hours of its formulation [26]. The colour observation is considered as the easy process of characterization of the emulsions since it is depending on the content and characteristics of oil and water. Emulsions with large surface area of dispersed droplets are in light in colour whereas emulsions with low surface area of dispersed droplets appear in dark in colour [23].

The main objective of this study was to formulate, optimize the emulsions containing Neem oil as the oil phase, Tween 80® as the emulsifying agent, and study the effect of high shear homogenization on the optimized formulas and to analyze the stability and characteristics of optimized formulas.

2. Materials and Method

Materials used in this study were, Neem oil, Tween 80®, distilled water, methylene blue staining solution, carbon tetra chloride (CCl_4) and 1% potassium chloride buffer solution (KCL).

Commercially available Neem oil was obtained from Ayurveda Osusala in Pettah, Colombo, Sri Lanka. Tween 80® (Sigma Aldrich), Methylene blue staining solution, Carbon tetra chloride (CCl_4) and 1% Potassium chloride (KCL) buffer solution were obtained from Chemistry Laboratory, Department of Pharmacy, FAHS, KDU, Rathmalana, Sri Lanka.

2.1. Preparation of primary emulsions

Primary emulsions were prepared at Chemistry Laboratory, Department of Pharmacy, FAHS, KDU, Rathmalana, Sri Lanka. Neem oil, distilled water and Tween 80® were used to prepare the primary emulsions. Initially, 10 ratios were selected randomly, and series of primary emulsions were prepared using the magnetic stirrer under 300 revolutions per minute (rpm) (Stuart- Hotplate & Stirrers CB161) at room temperature (28 ±5 °C). Water was added drop by drop and stirred for 10 minutes. Prepared emulsions were kept at room temperature (28 ±5 ºC) for short term stability evaluation to obtain the best compositions for further studies.

Based on the short term stability evaluation results, 04 formulations were identified as stable emulsions. Among them 01 formulation was provided a very thick stable emulsion compared to the other selected formulations. Two new formulations were prepared to reduce the viscosity by slightly changing the values of oil: water: emulsifying agent in aforesaid formulation to evaluate which the most stabilized ratio that provide stable emulsion is. Short term stability of these two formulations were observed for 14 days.

Based on the short term stability results, 06 different optimized ratios were selected to observe long term stability, accelerated stability and desired characteristics. Primary emulsions of selected ratios were prepared according to the aforesaid method. However, the stirring time was increased up to 15 minutes. Prepared emulsions were transferred to labelled containers and kept at room temperature (28 ±5 °C) to observe the long term stability throughout the study period of 90 days.

2.2. Preparation of secondary emulsions

Secondary emulsions were prepared at Pharmaceutical Chemistry Laboratory, Department of Pharmacy, FAHS, University of Peradeniya, Sri Lanka. Optimized 06 primary emulsions were prepared and subjected to high shear homogenization using the high shear homogenizer (Homogenizer OV 5, VELP, Scientifica, Italy) under 10000 rpm for 05 minutes to obtain the secondary emulsions. Homogenized emulsions were transferred to containers and labelled accordingly. Those emulsions were kept at room temperature (28 ±5 °C) to observe the long term stability.

2.3. Short term and long term stability evaluations of primary and secondary emulsions

Prepared primary and secondary emulsions were observed visually for the unstable conditions such as creaming, sedimentation, flocculation, coalescence and phase separation on 1^{st}, 3^{rd}, 6^{th}, 10^{th}, and 14^{th} days for short term stability evaluation and on 1^{st}, 3^{rd}, 6^{th}, 10^{th}, 14^{th}, 20^{th}, 30^{th}, 45^{th}, 60^{th}, 75^{th} and 90^{th} days for long term stability evaluation.

2.4. Accelerated stability evaluation of primary and secondary emulsions

This was carried out at Pharmaceutical Chemistry Laboratory, Department of Pharmacy, FAHS, University of Peradeniya, Sri Lanka. Freshly prepared optimized primary and secondary emulsions were subjected to centrifugation (Universal Centrifuge- Model: PLC-036H) at 1200 rpm for 03 minutes. Centrifuged emulsions were kept at room temperature (28 ±5 °C). Emulsions were observed on 1^{st}, 3^{rd}, 6^{th}, 10^{th}, and 14^{th} days respectively.

2.5. Microscopic analysis

This was carried out at Chemistry Laboratory, Department of Pharmacy and Pharmaceutical Sciences, FAHS, University of Sri Jayewardenepura, Sri Lanka. The freshly prepared stable primary and secondary emulsions were examined under an optical microscope (LABOMED, Labo America, Inc, USA) with a camera (Magnus Analytics, MIPS-USB, and 7M 0439, New Delhi, India). A drop of an emulsion was placed on a glass slide and stained with a drop of Methylene blue and covered with a cover slip. Observations were made at 100X magnification under the microscope.

2.6. Measuring the pH

This was carried out at Microbiology Laboratory, Faculty of Medicine, KDU, Rathmalana, Sri Lanka. Freshly prepared stable primary and secondary emulsions were subjected to 50% (w/v) dilution with distilled water. The pH of diluted emulsions was measured using pH meter (CE-pH 510/425, EUTECH pH 510).

2.7. Measuring the viscosity

This was carried out at Department of Mechanical Engineering Laboratory, Faculty of Engineering, University of Peradeniya, Sri Lanka. Freshly prepared stable primary and secondary emulsions were subjected to viscosity measurement using Redwood viscometer (SETA Redwood viscometer, Stanhope-Seta, London, UK). The viscosity was measured by Redwood seconds which is an obsolete unit of kinematic viscosity.

2.8. Measuring the creaming index

Freshly prepared stable primary and secondary emulsions were transferred into transparent airtight containers. Samples were stored at room temperature (28 ±5 °C) for 24 hours. The Height of the total Emulsion (HE) and the Height of the Droplet- depleted lower layer (HD) were measured and the creaming indices were calculated.

2.9. Observation of colour

All the freshly prepared stable primary and secondary emulsions were subjected to visual colour observations and checked for colour changes.

3. Results

3.1. Stability evaluation of primary and secondary emulsions

Out of 12 initial ratios of primary emulsion formulations, 06 ratios were optimized. All the 06 optimized primary and secondary emulsions were stable during the short term stability evaluation period. All the optimized primary emulsions were stable up to 45 days and 02 formulations were stable up to 75 days during long term stability evaluation. All the optimized secondary emulsions were stable up to 60 days and only 01 formulation was stable throughout the long term stability evaluation. Creaming was the main unstable condition that was observed during long term stability evaluation.

Only 01 optimized primary emulsion was subjected to phase separation upon the centrifugation and other optimized primary and secondary emulsions were stable throughout the accelerated stability evaluation.

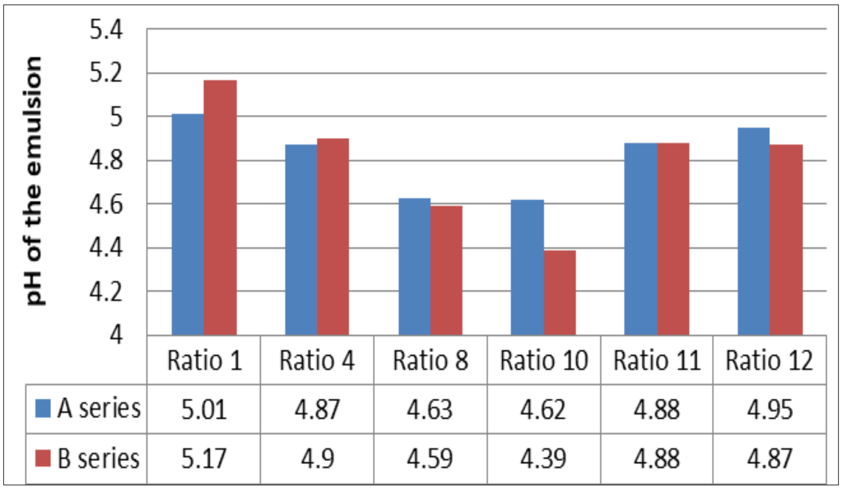
3.2. Characterization of primary and secondary emulsions

3.2.1. Microscopic Analysis

Both O/W and W/O type primary emulsions were observed. But phase inversion was observed in the most stable formulation as W/O type in primary emulsion and O/W type in its respective secondary emulsion.

3.2.2. The pH values

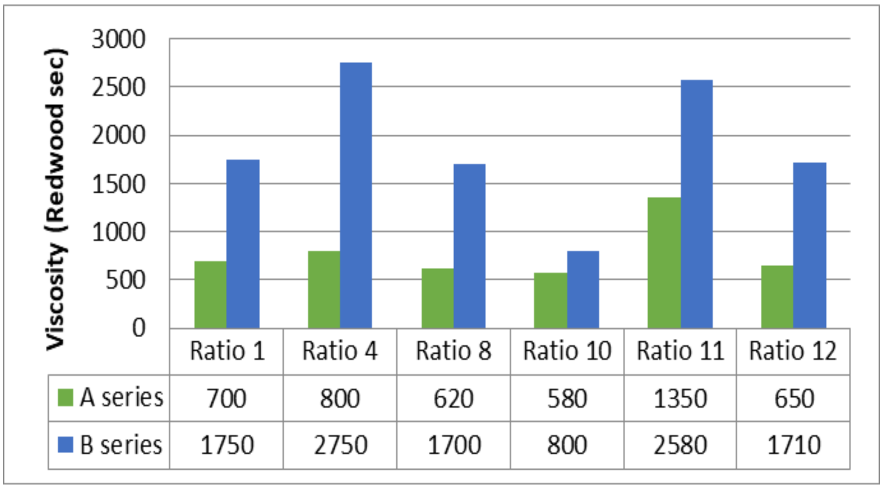
In primary emulsions, all the observed pH values were in the range between 4.60- 5.20. In secondary emulsions, all the observed pH values were in the range between 4.35- 5.20.



A: Primary emulsions B: Secondary emulsions

Figure 1 The pH values of optimized primary and secondary emulsions

3.2.3. Viscosity



A: Primary emulsions B: Secondary emulsions

Figure 2 The viscosity values of optimized primary and secondary emulsions

According to the mentioned values, it clearly shows that the secondary emulsions provide viscous emulsions than the primary emulsions. In addition, the increments of the values were not equal in primary and secondary emulsions among the above different ratios.

3.2.4. Creaming index

None of the primary and secondary emulsions showed creaming property, after 24 hours. Therefore, creaming indices of these samples were zero.

3.2.5. Colour observations

Light brown colour was observed for prepared primary emulsions and light cream colour was observed for prepared secondary emulsions. Very light colour was observed in secondary emulsions due to decreased droplet size.

4. Discussion

Selection of the components is the most crucial step in the preparation of an emulsion, as the formulation, stability and characterization mainly depend on the components of the emulsion. Neem oil, Tween 80® and distilled water were chosen as the components to formulate series of emulsions. Neem has high number of bioactive compounds which have the potentiality to use in public health and animal care [27], many cosmetics, pharmaceuticals, and bio pesticide industries due to variable activities [28]. Different emulsifying agents mainly ionic, nonionic, and amphoteric are used in the formulation of topically applied emulsions [1]. Since Tween 80® is a non-ionic, odorless, well stabilizing, solubilizing [7, 29], hydrophilic in nature with the HLB value of 15 [30], non-skin irritant and easy availability [26], this was used in this study as the emulsifying agent to formulate emulsions. Pre-formulation studies were carried out to choose the best ratios and selected optimized ratios were used to prepare series of primary and secondary emulsions.

Since the emulsions are thermodynamically unstable, stability evaluation is an important parameter to determine the stability of an emulsion [31]. Stability of pharmaceutical emulsion is mainly determined by the absence of creaming and coalescence in dispersed droplets [18]. As the short term and long term stabilities of an emulsion provide an idea about the emulsion shelf life, in this study those were considered to determine the best emulsion base that provide high stability. As unstable conditions, creaming and phase separation were observed within the study period. All the primary emulsions were resulted in creaming at the end of the 90 days of study period. Considering secondary emulsions, one emulsion was resulted in creaming by its 60th day and other emulsions except one were resulted in creaming by 75th day and remained in creaming throughout the 90 days of study period. According to the literature, stability of an emulsion is affected by processing conditions such as mixing efficiency and energy input during emulsification [10, 11]. The results were proven that the unstable conditions occurred earlier in primary emulsions than the secondary emulsions. There is evidence that reduction in droplet size of the dispersed phase of secondary emulsions provide the most stable emulsions due to increased viscosity and enhance the stability compared to the primary emulsions [17, 18]. This has been proven in the literature that changes in size of the dispersed droplet is taken as a key indicator for losing the stability of an emulsion [32], and the stability of an emulsion can be enhanced by reducing the droplet size [17]. Furthermore, increased concentration of the emulsifying agent also leads to increasing the viscosity of an emulsion, thus reducing the coalescence and creaming rate by reducing the mobility of the dispersed droplets [21]. Moreover, reduced droplet size provides good stability against creaming and sedimentation due to increase in viscosity [26]. When the emulsions are under the high-speed centrifugation force, they tend to undergo phase separation faster than the normal conditions [33]. It has been documented that the visual observation of the stability testing including instability mechanisms such as creaming, phase separation can be accelerated directly by applying a centrifugal field [26, 33]. In this study high speed centrifugation was applied for the samples to assess the kinetic stability throughout the study period of 14 days. According to the results obtained by centrifugation, it is clearly proven that reduced in particle size by high shear homogenization, not only increasing the dynamic stability, but also increasing the kinetic stability of an emulsion.

According to the literature, the hydrophilic emulsifying agents (Tween 80®) are soluble in aqueous phase thus, use for the formulation of O/W emulsions [6]. However, according to this study, both O/W and W/O type emulsions were observed. This may be due to the difference in the amounts and the type of hydrophilic emulsifying agent that was taken to formulate the emulsions. The reason is stated as the hydrophilic surfactant that is taken in lesser amount for the emulsion formulation, weakens the interfacial film of the O/W interface and in most cases will form W/O emulsions except the O/W emulsions as the effect of using reduced amount of hydrophilic surfactant towards the final emulsion [2, 30]. Since O/W type emulsions are increasing the compliance than W/O type emulsions, the phase inversion occurred during the formation of secondary emulsions, can be taken as an advantage for formulation of novel pharmaceutical emulsion. The pH values of all freshly prepared primary emulsions were within the range of 4.60-5.20 and pH values of the secondary emulsions were within the range of 4.35-5.20, which compatible with the range stated in the literature which is pH 4-7, the non-skin irritating pH range. Considering the viscosity, the most stable secondary emulsion during the study period was provided more viscous creamy emulsion compared to other emulsions, that is 2750 in Redwood seconds. These results agree with literature, where using high shear homogenization reduces the droplets size in the dispersed phase, thus increasing the viscosity and immobilize the dispersed droplets and enhance the stability of the emulsion [17, 26]. Creaming index is important to get an idea about the percentage creaming of an emulsion. It also provides indirect information about the extent of droplet flocculation in an emulsion which is a reversible unstable condition [26]. In this study none of the emulsions were shown creaming property after 24 hours of formulation and this indicates that all the emulsions were stable within the 24 hours after the preparations. Even though, the creaming property is taken as a characteristic of freshly prepared emulsions, during the period of stability evaluation, some of the primary and secondary emulsions were resulted in creaming. Since it is a reversible unstable condition, there is a possibility to enhance the stability of those already creamed emulsions by re-modifying the time duration of mixing of emulsions and speed of the magnetic stirrer. Visual observation of colour is a key indicator of characterization of an emulsion [23]. In this study, the reason for this slight colour difference may be due to the decreased in droplet size in dispersed phase of secondary emulsions compared to primary emulsions. It is proven in the literature that, emulsion which has large, dispersed droplets appears dark in colour whereas emulsion which has small, dispersed droplets appears light in colour [23]. The main purpose of this research study was to determine the composition of most stable Neem oil-based emulsion.

5. Conclusion

High shear homogenization leads to substantial reduction of the particle size and increase the stability of the secondary emulsions compared to the method of formulation of primary emulsions. Composition 30% Neem oil: 40% water: 30% Tween 80® in O/W type secondary emulsion was the best formula with the viscosity of 2750 (Redwood seconds). This formula was within the non-skin irritating range of pH 4-7. A preparation of a Neem oil-based sunscreen and carry out *in-vitro* and *in-vivo* studies for topical delivery is recommended.

Compliance with ethical standards

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Disclosure of conflict of interest

There is not any conflict of interest.

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