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Formulation Screening of Palm-based Nanoemulsion for an Oral Drug Vehicle of Phyllanthin†

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Phyllanthus amarus is commonly used in traditional medicine, and the major lignins of the plant, namely, phyllanthin and hypophyllanthin, have been reported to possess hepatoprotective activity. However, phyllanthin exhibits low oral bioavailability and requires a large quantity of herbal extract for a longer duration of treatment, limiting the clinical use of phyllanthin. To overcome these drawbacks, nano-sized emulsion systems have gained increasing importance due to their ability to improve the gastrointestinal absorption of hydrophobic drugs. Proper selection of oils and surfactants, along with their suitable hydrophilic-lipophilic balance (HLB) value, is crucial to obtain stable, mild and clinically acceptable nanoemulsions. The focus of the present study is to provide an efficient screening approach for the excipient selection for the optimum nanoemulsion formulation development containing phyllanthin. The selection of oil was based on the solubilizing capacity of phyllanthin by HPLC analysis. The drug solubility in the oil was taken as the criterion for oil selection, and palm kernel oil ester was chosen as the oil phase in the nanoemulsion formulation. Emulsification ability of the surfactant was assessed for surfactant screening. Among them, Tween® 80 and Span® 80 were found to be an effective emulsifier for the oil phase. The desired HLB value for oil-in-water nanoemulsion is between 8 and 18. The nanoemulsion formulation was thermally stable and could be effectively used for drug delivery applications.

Key words: Nanoemulsion; *phyllanthus amarus*; phyllanthin; screening; palm-based oil; non-ionic surfactant

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Phyllanthus amarus (*P. amarus*) is highly valued in traditional medicine for its healing properties. The plant has been shown to work as an antifungal, antibacterial, antiviral, antidiabetic, anticancer and anti-inflammatory agent, as well as in the treatment of liver diseases, asthma, cardiovascular problems, dropsy, and jaundice. *P. amarus* is alleged to be a restoration herb by traditional medicine practitioners, and thus, is recommended for regular consumption during meals as a blood tonic for the detoxification of human systems and ultimately, the prevention or cure of infective and degenerative diseases.

Biochemical constituents of plants are relevant sources of natural antioxidants, and the efficacy of plant extracts is more significant when they are consumed as a crude extract [1]. Phyllanthin (Figure 1), a major bioactive lignan component of *P. amarus*, has been found to possess several therapeutic properties. A study demonstrated that phyllanthin possessed hepatoprotective activities against carbon tetrachloride, galactosamine and ethanol treatment [2]. Phyllanthin significantly lowered the plasma uric acid in hyperuricemic animals to a normal level; this activity was comparable to the effects of allopurinol, benzbromarone and probenecid. However, a pharmacokinetic study in rats revealed that oral bioavailability of phyllanthin was incomplete, with only 0.62% in rats; it has been suggested that the poor oral bioavailability of phyllanthin was due to poor aqueous solubility [3]. Despite the promising pharmacological effects of phyllanthin, poor oral absorption, resulting in low oral systemic bioavailability, limits its clinical use. Other major limitations of phyllanthin include the high quantity of herbal extract required for treatment and the long

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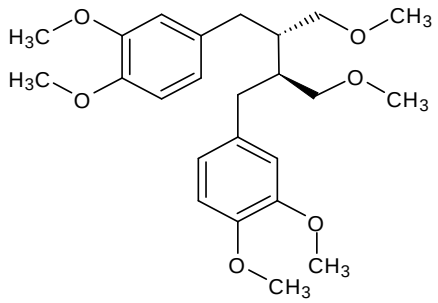

Figure 1. Chemical structure of phyllanthin.

duration of treatment needed due to the degradation of various plant constituents in the gastrointestinal tract, in addition to poor absorption of these constituents in the intestine [4]. Therefore, an attempt has been made to enhance the dissolution rates and bioavailability of the *P. amarus* extract by preparing its nanoparticles.

Nanotechnology is an attractive area of research regarding their possible benefits in clinical medicines [5-7]. Traditional biomedical applications incorporate the use of nanotechnology in a wide range of sectors. Among them, nanoemulsion encapsulation is used in implant design and controlled release systems. Several nano-oriented approaches are being used to optimize the technological aspects of drugs. The use of these processes has dramatically enhanced dissolution rates *in vitro* and bioavailabilities *in vivo* of many drugs [8].

Nanoemulsions are isotropic, thermodynamically stable, transparent (or translucent) systems of oil, water and surfactants with a droplet size primarily in the range of 10 – 200 nm [9, 10]. Their long-term stability, ease of preparation (spontaneous emulsification), and high solubilization of drug molecules make them promising as a drug delivery tool. They have found wide applications in oral drug delivery to enhance the solubility and bioavailability of lipophilic drugs [11-13]. Recently, there has been a surge in the exploration of nanoemulsions for transdermal delivery [14-16]. They are also being investigated for potential applications in ocular [17, 18], pulmonary [19], nasal [20, 21], vaginal [22, 23], and parenteral drug delivery [24-26].

The use of nanoemulsions in drug delivery has been reviewed. The main objective of this study was to provide an efficient screening approach for the proper selection of oils and surfactants for the nanoemulsion formulation development. These systems often require high surfactant concentration, which may lead to toxicity and irritancy problems. Therefore, proper selection of surfactants, along with their optimum concentration, is required, which has been discussed in this report. Determination of the hydrophilic-lipophilic balance (HLB) values of mixed surfactants on the nanoemulsion formation region also played an important role in the study. The most stable emulsions are achieved with emulsifiers or a combination of emulsifier agents having HLB values close to that required for the oil phase. Excellent selection would aid in better formulation with desirable attributes.

EXPERIMENTAL

Chemicals

Phyllanthin was purchased from Sigma Aldrich. All oils medium-chain triacylglycerol, long-chain triacylglycerol, isopropyl myristate, isopropyl palmitate, palm oil ester, palm kernel oil ester, and non-ionic surfactants of the Tween® and Span® series used were all obtained from the Malaysian Palm Oil Board (MPOB). The distilled water used in this study was mixed with sodium benzoate (0.1%). All other chemicals and solvents were of analytical grade.

Oil Screening

The saturation solubilities of phyllanthin in various oils were determined by a shake flask method. Phyllanthin was added in excess to each screw-capped glass vial, containing 1 ml of each vehicle. After capping, mixing was performed using a vortex mixer (Vortex-2 Genie, Scientific Industries, Bohemia, NY). The mixtures were then shaken by a reciprocating shaker (LabQuake, Barnstead, Thermolyne) at 25±1.0°C for 72 h to attain equilibrium. After reaching equilibrium, the samples were centrifuged at 4000 rpm for 10 min using a high-speed centrifugal machine (Centrifuge 5403, Eppendorf, Hamburg, Germany) and the supernatants were filtered through a 0.22 mm membrane to remove the excess insoluble phyllanthin. Filtrates were then diluted 200 times with absolute ethanol, and the concentrations of phyllanthin were quantified using liquid chromatography [27]. The selection of oil was based on the solubilizing capacity of phyllanthin.

Surfactant Screening

Selection of a surfactant is critical to obtain the desired formulation, with each surfactant and oil having a specific HLB. The corrected HLB of the selected surfactant or blend of surfactants that match the HLB of the chosen oil provides the lowest interface tension between the oil and water phases. The HLB of the surfactant chosen reflects the stability of the system at lower levels and can be obtained when the HLBs of the surfactant and oil are similar.

Nonionic surfactants are generally recognized as being safe and biocompatible and not affected by pH changes in media because they are uncharged. The nonionic surfactants shown in Tables 1 were chosen for screening to select a suitable single surfactant or pair of surfactants that would best match the selected oil. The water solubilization capacity, i.e. the minimum content of surfactant required to form a nanoemulsion system with the selected oil, was performed as a criterion for optimization using the water titration method. We used our solubilization results to select the best emulsifier.

Nonionic surfactants of the Tween® series and selected oil were weighed and put into a series of screwcap test tubes in the range of 0.1:0.9, 0.2:0.8, 0.3:0.7, 0.4:0.6 and 0.5:0.5 w/w g of 1 g per batch, mixed, and vortexed thoroughly. After that, distilled water (100 µl, 0.1 g) containing sodium benzoate (0.1%) was added to each oil-surfactant mixture in 20–25 µl drops using a micropipette. After each drop of water was added, the system was vortexed for 10–20 seconds. The final mixtures, after complete addition of water, were vortexed for 2–3 minutes at room temperature. Visual observations were made, and the clarity or turbidity of each sample was recorded. The surfactant that formed a nanoemulsion or a cloudy system was selected as the surfactant that best matched the selected oil chosen.

Selection of Surfactant Blend

A blend of hydrophilic and lipophilic surfactants is needed to obtain longer stability of the dispersion phase at the lowest concentration levels. Blends of surfactants were investigated, as well as single surfactants. A blend of surfactants with an HLB that matches that of the oil phase will provide better solubilization and stability of the dispersion system produced. Therefore, the selection of surfactant that blends at lower and higher HLB, matching the HLB of oil, is important in the formulation of a colloidal system. The individual nonionic hydrophilic surfactant, Tween® 80, was blended with the lipophilic surfactants of the Span® series in ratios of 3:2, 7:3, 4:1, and 9:1 w/w to produce blends of surfactants with various HLBs in the range of 10.7–14.0. The solubilization capacities of the surfactant blends were studied using the same method used to study other surfactants individually. The blend of surfactants forming a cloudy system at the minimum concentration (water-in-oil nanoemulsion) was selected as the blend that best matched the HLB of palm kernel oil esters (oil chosen).

RESULT AND DISCUSSION

The most important criteria for the selection of oils and surfactants in nanoemulsion components is that all excipients should be pharmaceutically acceptable for oral administration or topical application, depending on the requirement and falling under the generally regarded, safe category. The high solubility of drugs in the oil phase is the important parameter when designing stable nanoemulsion formulations. The drug should possess good solubility in a solvent, so precipitation during the shelf-life of the formulation and after dilution in the water phase can be avoided. Surfactants can cause gastrointestinal irritation. Therefore, their selection is a significant factor in nanoemulsion design. The non-ionic surfactants are less toxic than ionic surfactants and are characterized by lower critical micelle concentration values.

Another important criterion is the selection of blend surfactant with proper HLB value. Hydrophilic surfactants and cosurfactants are considered to prefer the interface and lower the necessary energy to form nanoemulsions, consequently, improving the stability. The right blend of low and high HLB surfactants will lead to the formation of a stable nanoemulsion upon dilution with water. This also helps to lower interfacial tension to a minimal value, which will aid in the dispersion process during the preparation of the nanoemulsion.

Lipophilic drugs preferably solubilize in oil-in-water (o/w) nanoemulsions, whereas water-in-oil (w/o) systems can be a better choice for hydrophilic drugs. Drug loading per formulation is a critical design factor in the development of nanoemulsion systems for poorly soluble drugs, which is dependent on the drug solubility in various formulation components. The formulation volume should be minimized as much as possible to deliver a therapeutic dose of the drug in an encapsulated form. The solubility of the drug in the oil phase is an essential criterion for the selection of oils. This is particularly important in the case of oral formulation development, in which the ability of a nanoemulsion to maintain the drug in a solubilized form is greatly influenced by the solubility of the drug in the oil phase. If the surfactant or cosurfactant is contributing to drug solubilization, there could be a risk of precipitation, as a dilution of the nanoemulsion

in the gastrointestinal tract will lead to lowering of the solvent capacity of the surfactant or cosurfactant [28, 29].

Solubilities of phyllanthin in various vehicles are presented in Figure 2. The water solubility of phyllanthin, at 9.5 ±0.21 mg/ml, is very low; however, the solubilities of the tested vehicles were found to be high. Among the six tested oils, maximum solubility of phyllanthin, i.e., 43.86 ±0.24 mg/ml, could be achieved with palm kernel oil ester (PKOE). Since the drug loading capacity is the main factor when screening the oil phase, PKOE was chosen as the oil component for phyllanthin-loading in this nanoemulsion system.

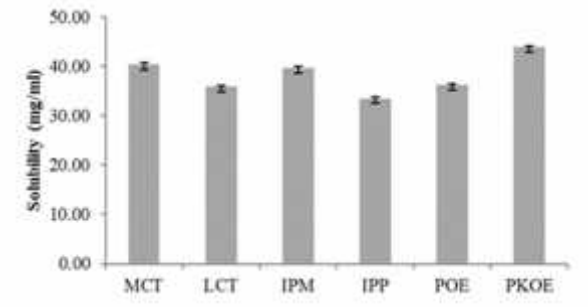

Figure 2. Excess velocity oftrichloroethylene+1-pentanol.

The most important problem related to nanoemulsion-based systems is the toxicity of the components. Large amounts of surfactants may cause gastrointestinal and skin irritation when administered orally and topically, respectively. Therefore, it is vital to determine the surfactant concentration and use the minimum concentration in the formulation. Nonionic surfactants are relatively less toxic than their ionic counterparts and typically have lower critical micelle concentration. Also, o/w nanoemulsion dosage forms for oral or parenteral use, based on nonionic surfactants, are likely to offer *in vivo* stability [30]. Reverse micelle systems have been an interesting area of research within various fields of science and technology, resulting from their ability to solubilize water in organic solvents in the presence of surfactants [31]. It is known that ethoxylated nonionic hydrophilic surfactants tend to form reverse micelles in organic media [32]. The results for the reverse micelle systems in this study, formed by screening Tween® series surfactants, are shown in Table 1. Tween® 80 shows a high solubilization capacity compared with Tween® 20, Tween® 60, and Tween® 85. The Tween® series (Table 1) are structurally similar in the head group for polyoxyethylene but differed in the tail group.

Tween® 80 is derived from poly-oxyethylenesorbitan (head group) and oleic acid (tail group) (Table 1). The Tween® 80 tail group is composed of unsaturated oleic acid, which is structurally different from the tail group in palm kernel oil esters and is made up of fatty acid esters, primarily medium-chain lauric acid and myristic acid, but is similar to the oleyl moiety of palm kernel oil esters. The Tween® 80 tail group is composed of a long chain (C18) unsaturated oleic acid, while Tween® 20 is structurally composed of a medium-chain carbon tail (C12, lauric acid), the main fatty acid of palm kernel oil esters. Despite the structural similarity between the lipophilic tails of Tween® 20 and palm kernel oil esters, Tween® 20 shows the lowest solubilization capacity compared with Tween® 80 (Table 1). This indicates a weak interaction between the oil and surfactant from the same fatty acid derivative. Similarly, Tween® 60 has a lower solubilization capacity in water with palm kernel oil esters despite sharing a carbon chain tail of similar length to Tween® 80 but lacking the carbon double bond at the C9 position in the tail group (monostearate) chain. Therefore, the presence of the carbon double bond at position 9 in the tail group has a great effect on the solubilization capacity of the Tween® series surfactants for water in palm kernel oil esters.

This result showed the importance of esterification of palm kernel oil esters with oleyl alcohol because it was the source of the structural similarities between palm kernel oil esters and the Tween® 80 tail group. Also, when the number of oleate groups increased, as in Tween® 85, the solubilization capacity decreased. This indicates the importance of a minimum number of hydroxyl groups of polyoxyethylene in the head group configuration with the oleate group. The results of this study are consistent with those of a previous study showing that the maximum solubilization capacity of water depends upon the oxyethylene chain, the configuration of the polar head group and hydrocarbon moiety of nonionic surfactants and the type of oil [33].

However, nonionic hydrophilic surfactants tend to form reverse micelle systems [32], and the HLB of a single surfactant (Tween® series) has no significant effect on the solubilization capacity. The results of this study showed that the high HLB of Tween® 20 (16.7) retained the least solubilization capacity (36.4%), compared with Tween® 80 at HLB 15.0, Tween® 60 at HLB 14.9, and Tween® 85 at HLB 11.0 which reached solubilization capacities of 18.2%, 27.3%, and 27.3%, respectively (Table 1). This is clearly shown by the solubilization capacity of Tween® 85 at HLB 11.0, in which oleate groups replaced two other hydrogens in the hydroxyl groups attached to polyoxyethylene, and

retained a stronger solubilization capacity compared with Tween® 20, which had a similar head group to that of Tween® 80 and Tween® 60. This showed that the head group of the Tween® series for single-use did not affect the solubilization capacity of these surfactants.

A single surfactant is not sufficient to form a single-phase nanoemulsion. Thus, an adequate mixture of surfactants may be required. The use of nonionic surfactant mixtures is an interesting approach, from the pharmaceutical point of view, because they are generally regarded as having low toxicity and irritancy and most importantly, considered to be acceptable for oral administration. Additionally, the use of mixtures allows the individual concentration of each surfactant to be decreased, which may increase the biocompatibility of the final formulations. Hydrophilic surfactants and cosurfactants are considered to prefer the interface and lower the necessary energy required to form the nanoemulsions, consequently improving the stability. For example, the value of HLB needed to form an o/w nanoemulsion is greater than 10 [34]. The results obtained for the solubilization capacity of the blends of surfactants are shown in Table 2. From this study, Tween® 80/Span® 80 at HLB 13.9 had the highest solubilization. These results indicated the importance of unsaturated trioleates because of the more lipophilic tail group, which was structurally similar to the oleyl group on the palm kernel oil esters, which enabled the surfactants to be well packed at the interface. Thus, these results reflected the effect of the type of surfactant blend on the solubilization capacity.

CONCLUSION

The present study could be summarized as the successful development of a nanoemulsion system for phyllanthin. PKOE was chosen as the oil component, along with Tween® 80 blended with Span® 80 as a surfactant. Identifying the suitable oil and surfactant/cosurfactant is crucial to an efficient nanoemulsion formulation development and optimum drug loading. Attention should be paid to the tolerability of the constituting excipients. Solubilization capacity appeared to be useful as a criterion for the selection of a single surfactant and blends of surfactants. The micelles discussed in this study have potential applications, advantages, and usefulness in the pharmaceutical industry as delivery systems through various routes of administration, as well as in cosmetics and personal care products.

Table 1. The solubilization capacity of selected surfactants.

Surfactant	HLB Value	The minimum % of surfactant formed reverse micelle (solubilization capacity of surfactant to water)
T20	16.7	36.4
T60	14.9	27.3
T80	15.0	18.2
T85	11.0	27.3

Table 2. The solubilization capacity of selected surfactants.

Surfactant	HLB Value	The minimum % of surfactant formed reverse micelle (solubilization capacity of surfactant to water)
T80/S20	13.7	27.3
T80/S60	14.0	36.4
T80/S80	13.9	18.2
T80/S85	13.7	27.3

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