Formulation and Characterization of Virgin Coconut Oil (VCO) Based Emulsion

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Abstract- An emulsion is defined as a heterogeneous system, consisting at least two immiscible liquids or phases, one of which is dispersed as droplets in the other liquid. VCO is gaining wide popularity in the scientific field and among the public due to its various health benefits and special characteristics. The main objectives of this study were to formulate an emulsion containing VCO as the oil phase and Tween 20® as the surfactant and to optimize the emulsion with appropriate ratio of oil: water: surfactant. The effects of high shear on the optimized formulae and their characterizations were also studied in this project. Based on stability studies the proper ratios of oil, water and surfactant were selected. The optimized formulae were further studied for its characterization such as Droplets size measurement, pH value, Creaming index, Viscosity and Microscopic studies. In conclusion best formulae with and without high sheer homogenization is o/w emulsion and it is consisting with oil 32 % (w/w), surfactant 32 % (w/w) and water 36 % (w/w).

Index Terms- Characterization, High shear homogenization, Nano-emulsion, VCO

I. INTRODUCTION

Emulsions are occur either as end products or during the processing of products in a huge range of areas including the food, agrochemical, pharmaceutical, paint and oil industries. An emulsion is defined as a heterogeneous system, consisting at least two immiscible liquids or phases, one of which is dispersed as droplets (dispersed phase = internal phase) in the other liquid (continuous phase = external phase). They are referred to as either w/o emulsions, water droplets dispersed in an oil medium or o/w emulsions, oil droplets dispersed in an aqueous medium. Nowadays emulsions are widely available in pharmaceutical market and usage is popular among the people. The design and development of effective formulations for drugs has long been a major challenge, because efficacy of a drug can be severely limited by instability or poor solubility in the vehicle [1]. Nano-emulsions are referred as the droplets size range in 20-500nm [2]. To design a new formulation, it is very important to identify both formulation and process parameters in the preparation, since both these variables will affect the properties and performance of the developed dosage form. Characterization and stability evaluation of newly formulated product is very important because initial properties of the product will change over time.

The process of converting two immiscible liquids into an emulsion is known as homogenization, and a mechanical device designed to carry out this process is called a homogenizer. Homogenization can be conveniently divided into two categories as primary and secondary. Primary homogenization is the conversion of two baulk liquids into an emulsion, whereas secondary homogenization is the reduction in size of the droplets in an existing emulsion. Emulsion which is undergone secondary homogenization usually contains small droplets than those which have undergone primary homogenization.

Various methods have been developed to extract coconut oil, either through dry or wet processing. Rather than going to the normal dry process, this oil is obtained by wet processing which entails the extraction of the cream from the fresh coconut milk and consequently breaking the cream emulsion. This process is more desirable as no chemical or high heat treatment is imposed on the oil. The coconut oil produced through the wet method is known as virgin coconut oil (VCO) and it is gaining wide popularity in the scientific field and among the public due to its various health benefits and special characteristics [3 - 5].

Emulsions are heterogeneous mixtures that consist of droplets of a liquid dispersed in a second continuous immiscible liquid phase. The liquid/liquid immiscibility creates an interfacial tension between the two liquids that assign thermodynamic instability to such systems. The droplets in emulsions are stabilized by emulsifiers, which are surface active molecules that rapidly adsorb to the surface of the oil droplets created during homogenization. The emulsifiers play two key roles in emulsions. They facilitate the emulsion formation and improve emulsion stability [6]. Non-ionic surfactants are generally recognized as being safe and biocompatible, and are not affected by pH changes in media because they are uncharged. Tween 20® is non-ionic and it called as Glycol Sorbitan Monolaurate, Polyoxyethylene Sorbitan Monolaurate.

II. OBJECTIVES

The main objective of this study was to formulate and to optimize an emulsion containing VCO as the oil phase and Tween 20® as the surfactant with appropriate ratio of oil: water: surfactant. This study further aimed to analyze the effect of high shear on the optimized formulae, to study the characterization of optimized formulae with and without high shear homogenization.

III. MATERIALS AND METHODS

Materials

VCO was purchased from Serendipol (pvt) Ltd, Pannala Road,

Dandagamuwa, Kuliyapitiya, Sri Lanka. Tween 20® and distilled water were taken from the Pharmaceutical Laboratory of Faculty of Allied Health Sciences, University of Peradeniya.

Emulsion preparation

The emulsion was formulated in the laboratory of Department of Pharmacy, Faculty of Allied Health Sciences, University of Peradeniya.

Method 1

In this study emulsions were prepared in different ratios of oil, water and surfactant. VCO and Tween 20® were left for 20 min under magnetic stirring 600 revolutions per minute (rpm) (1MLH magnetic stirrer, Rajendra Electrical Industries Limited, Mumbai, India) at 25^0C to mix thoroughly. To the resulting mixture water was added drop by drop while mixing with the aid of magnetic stirrer 600 rpm at 25^0C.

Method 2

Secondary homogenization was done in 10000 rpm by applying high shear homogenization (homogenizer OV5, VELP scientifica, Italy) for 5 min to formulations.

Initially 5 grams of nine formulations were prepared with different ratios of oil, water and surfactant for the preliminary studies.

Table 1 - Composition ratios of different formulations

Sample name	Oil (g)	Water (g)	Surfactant (g)	Oil : Water : Surfactant % (w/w)
A	2.0	2.0	1.0	40 : 40 : 20
B	1.0	2.0	2.0	20 : 40 : 40
C	2.0	1.0	2.0	40 : 20 : 40
D	1.5	2.0	1.5	30 : 40 : 30
E	0.5	0.5	4.0	10 : 10 : 80
F	0.5	1.5	3.0	10 : 30 : 60
G	1.5	0.5	3.0	30 : 10 : 60
H	3.0	1.5	0.5	60 : 30 : 10
I	3.0	0.5	1.5	60 : 10 : 30

For the preliminary study, after completion of visual observations of the nine formulations for one week, the best formulation was selected. Sample D was the best formulation among the nine formulations and other formulations shows the unstable conditions such as phase separation, sedimentation, creaming and flocculation. Due to the high aqueous nature of the selected stable formulation (Sample D), three new formulations were prepared for further studies with different ratios of oil, water and surfactant. Water content of the three new formulations was less than the sample D.

Table 2.2 - Composition ratios of different formulations

Sample name	Oil (g)	Water (g)	Surfactant (g)	Oil : Water : Surfactant (% w/w)
J	1.6	1.8	1.6	32 : 36 : 32
K	1.7	1.6	1.7	34 : 32 : 34
L	1.8	1.4	1.8	36 : 28 : 36

Characterization

After the optimization of best formulae based on proper oil, water and surfactant ratio, it was studied further for its characterization such as pH value, creaming index, droplets size measurement, viscosity and microscopic studies. Creaming index and pH values were determined in the laboratory of Department of Pharmacy, Faculty of Allied Health Sciences, University of Peradeniya. Microscopic studies were done in the laboratory of Department of Medical Laboratory Sciences, Faculty of Allied Health Sciences, University of Peradeniya and viscosity measurements were taken at the laboratory of Department of Mechanical Engineering, Faculty of Engineering, University of Peradeniya.

Creaming index

Ten grams of emulsion samples were transferred into universal bottles and tightly sealed with a cap, and then stored at 25 ^{0}C for 24 h. The oil droplets, in general, have a lower density than the surrounding aqueous phase and therefore move upwards during storage leading to creaming. The height of total emulsion (HE) and the height of the droplet-depleted lower layer (HD) were measured. Creaming index was reported as a "creaming index" = 100 (HD/HE). The creaming index provides indirect information about the extent of droplet flocculation in an emulsion: the more flocculation, the larger the particles and the faster the creaming rate.

pH value

pH values were measured at 25^0C by using a pH meter (Mi 150 pH / temperature bench meter, Milwaukee, Martini Instruments, Hungary). Three measurements were taken for one sample. Before the readings were observed pH meter was calibrated by using pH 7.01, 4.01 and 10.01 buffer solutions respectively.

Droplet size measurement

The droplet size distribution of the emulsions was measured in Sri Lanka Institute of Nano Technology (SLINTEC) using a laser light scattering instrument (Malvern Zetasizer Ver. 6.00, Malvern Instruments, Worcestershire, UK) at 25^0C. It uses micro-electrophoresis / electrophoretic light scattering technology to measure zeta potential and electrophoretic mobility and it measured size distributions by intensity. Laser Doppler Micro-electrophoresis is used to measure zeta potential. An electric

field is applied to a solution of molecules or a dispersion of particles, which then move with a velocity related to their zeta potential. This velocity is measured using a patented laser interferometric technique called M3-PALS (Phase analysis Light Scattering). This enables the calculation of electrophoretic mobility, and from this, the zeta potential and zeta potential distribution. A range of disposable and reusable cells are available to optimize the measurement in terms of sample volume, concentration and flow measurement. Other options include filters to improve the measurement of fluorescent samples; a temperature range extension to 120ºC and a viscometer to determine the sample viscosity to the accuracy required for the techniques used.

Viscosity measurement

Viscosity of the selected stable emulsion samples were measured by using the Redwood Viscometer (Seta Redwood Viscometer, Stanhope-Seta, London Street, UK) at 25^0C. This instrument is use for determining the viscosity of oils, expressed in Redwood Seconds at the testing temperature. Redwood Second is obsolete unit of kinematic viscosity. The time in seconds for 50 mL of the sample liquid to flow through a Redwood Viscometer is a measure of its viscosity and is given the units of Redwood Seconds. Samples were poured in to the brass oil cup and water was poured into the surrounding space. Heat was supplied to water up to 50^0C; then turn off the heater and allowed to rise down the temperature to 25^0C to get the equalling temperature of both the sample and water. After the equalling temperature was reached valve was opened and time was measured to fill the 50 mL of receiver by using the stop watch.

Microscopic study

The formulated selected stable emulsion samples (J, K L) were analyzed under a microscope (Olympus Vanox S microscope, Japan) to determine their types (o/w or w/o). A drop of the emulsion was placed on a slide and mixed with a drop of methylene blue (water soluble dye) by using a spatula, covered with a cover slide and observed under the microscope. Continuous blue phase surrounding the droplets indicate the emulsion is oil-in-water type and clear outer phase with dark bluish droplets indicate the water-in-oil type.

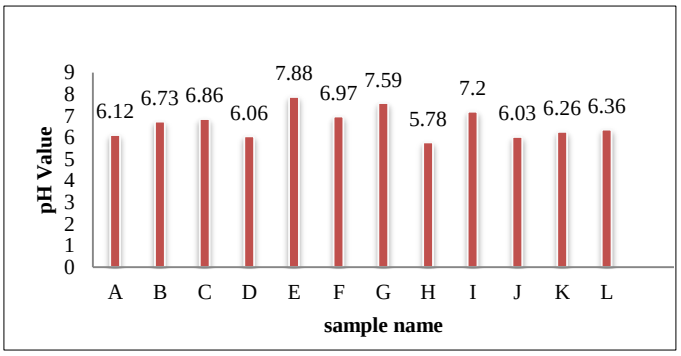
IV. RESULTS

Characterization

Creaming index

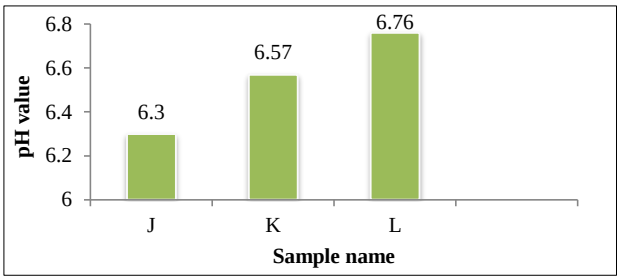
None of the samples (A - J) showed creaming property, after 24 hours. Therefore creaming indices of the samples were zero.

pH value

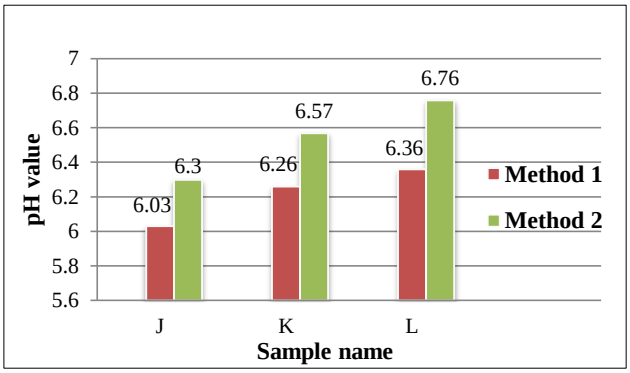


Graph 1 - pH values from Method 1

According to the graph all the resulted pH values are in range between 5.78– 7.88. These values are suitable for topical application.



Graph 2 - pH values from Method 2



Graph 3- pH values comparison from two different methods

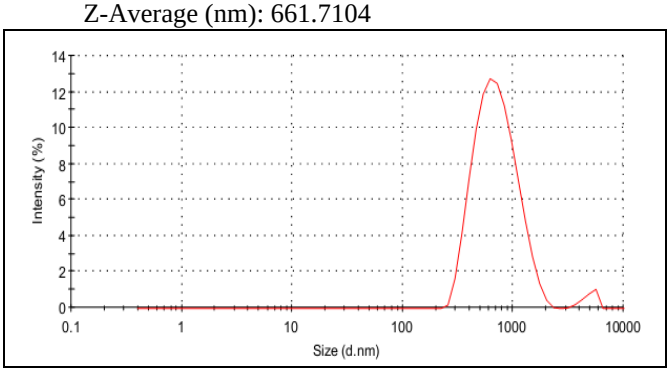
Method 1 and Method 2 give different pH values for the same formulations. Resulting values from Method 2 is always higher than the values from Method 1. The differences between the values of the two different methods were not similar in different formulations. Sample J shows a less difference than the other two formulations and sample L shows the highest difference of pH value.

Droplet size measurement

Method 1

1st reading

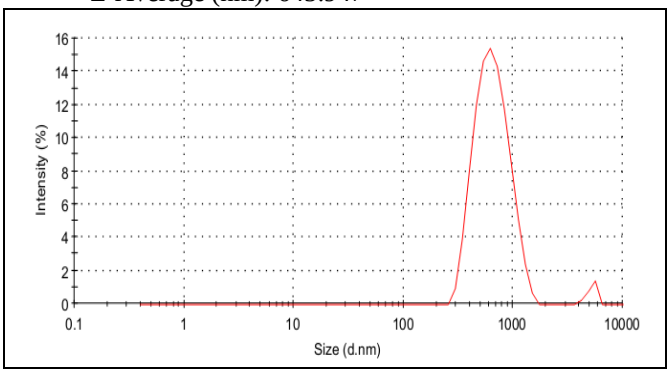
Z-Average (nm): 661.7104



Graph 4 - sample J size distribution by intensity

2nd reading

Z-Average (nm): 645.947



Graph 5 - sample J size distribution by intensity

Average particle size of sample J from Method 1;

= (661.71+ 645.94) nm / 2

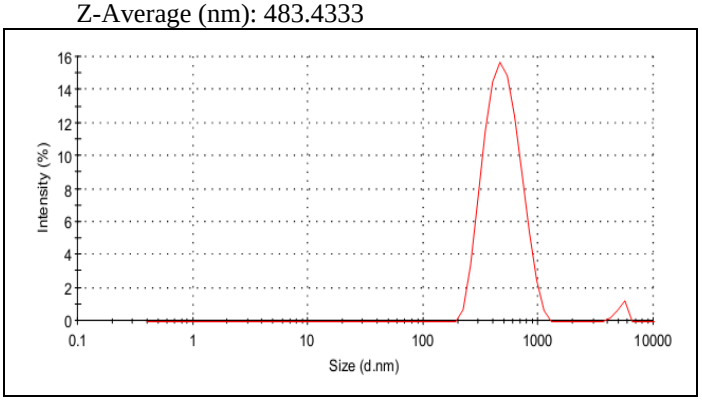
= 653.83 nm

In graph 4 size distribution range is 250-2200 nm and in graph 5 it is 250-1800 nm. Both show the peak intensity around 600-700 nm. Resulting particle size is in range of micro-emulsion.

Method 2

1st reading

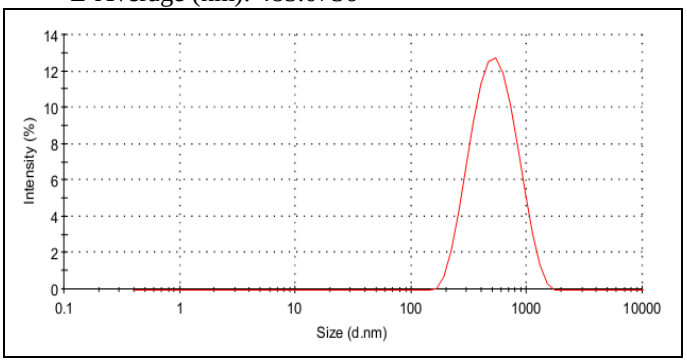
Z-Average (nm): 483.4333



Graph 6 - sample J size distribution by intensity

2nd reading

Z-Average (nm): 485.0736



Graph 7 - sample J size distribution by intensity

Average particle size in sample J from Method 2;

= (483.43+ 485.07) nm / 2

= 484.25 nm

In graph 6 size distribution range is 200-1500 nm and in graph 7 it is 180-1800 nm. Both show the peak intensity around 400-520 nm. According to the results, it gives a nano-emulsion which is below in 500 nm.

Viscosity measurement

Table 3- Viscosity measurement

Sample name	Time (Redwood Seconds)	
	Method 1	Method 2
J	186	960
K	851	2907
L	1800	3495

According to the resulting values it is clearly shown that from Method 2 it gives a high viscous emulsion than the Method 1 and viscosity increases with respect to the increase of the oil, surfactant and also due to the application of high shear homogenization. Increments of the values are not equal in Method 1 and 2 among the three different formulations.

Microscopic study

Continuous blue phase surrounding the droplets indicate the emulsion is oil-in-water type but clear outer phase with dark bluish droplets indicate the water-in-oil type. Sample J from both methods shows continuous blue phase surrounding the droplets and sample K and L does not show the clear images.

V. Discussion

The important criterion for selection of components for emulsion formulation is their pharmaceutical acceptability. It has been demonstrated that only specific pharmaceutical excipients combinations lead to efficient emulsion formulations [2]. The solubility of the drug in oils is most important, as the ability of the emulsion to maintain the drug in solubilized form is greatly

influenced by the solubility of the drug in the oil phase. Thus, VCO was selected as the oil phase for the development of the formulation. In recent years, VCO has gained popularity as a nutraceutical. Promoted as a dietary supplement designed to optimize health through improved nutrition, it is said to be of benefit for patients with various ailments. Anecdotal reports and testimonials on the health benefits of VCO have sprouted in various print and web publications. This has influenced a lot of people to join the bandwagon despite scarce scientific evidence of its efficacy in humans. In 2003, Indian biochemists set out to investigate the effect of VCO on various lipid parameters in oil-fed rats. VCO, at 8 g/100 g weight, had a beneficial effect in lowering lipid component compared to copra oil and ground nut oil. It reduced total cholesterol, triglycerides, phospholipids, LDL and VLDL cholesterol levels and increased HDL cholesterol in serum and tissues. The polyphenol component of the VCO prevented in vitro LDL oxidation, implicated as a risk factor for atherosclerosis and coronary heart disease [7]. VCO obtained by wet process may have more beneficial effects than coconut oil due to its higher unsaponifiable components like polyphenols and α-tocopherols. Several studies have revealed the anti-oxidant activity of polyphenolic substances, especially from red wine and olive oils in oxidation of LDL. However all of the skin care products that Tropical Traditions offers are made with organic VCO and Virgin Palm Oil.

The surfactant chosen must be able to lower the interfacial tension to a very small value to aid the dispersion process during the preparation of the emulsion provide a flexible film that can readily deform around droplets and be of the appropriate lipophilic character to provide the correct curvature at the interfacial region for the desired emulsion type (o/w, w/o or bicontinuous). It is well reported that large amounts of surfactants particularly ionic surfactants cause irritation so for drug delivery nonionic surfactants are preferred with low concentration [1]. Due to the various properties such as non-ionic, non-skin irritant, availability, allow to formulating nano-emulsion and solubility of the drug in this surfactant; Tween 20® was selected to my study as the surfactant. Early stage of this study was planned to use both Tween 20® and Tween 80® as the surfactants for emulsion formulation but the unavailability of Tween 80® only Tween 20® was used for the study.

Preparation of emulsion was adopted by the acceptable method which was previously followed to study the effect of oil and drug concentrations on droplet size of palm oil esters nano-emulsion [2]. Stability evaluation and characterization methods are also acceptable methods and it also previously used in development and evaluation of emulsions from Carapa guianensis (Andiroba) Oil [8]. In Method 1, only the magnetic stirrer was used for the preparation of the formulations and in Method 2, high shear homogenization was applied to formulations after preparing the formulations by using magnetic stirrer. It was shown in previous studies that the water/Cremophor myristate system formed o/w nano-emulsions by stepwise addition of water to the surfactant/oil mixture and in this study o/w emulsion was obtained as the result by following the same procedure. As this surfactant mixture is hydrophilic it was thought that by changing the surfactant ratio towards a lipophilic mixture w/o nano-emulsions could be formed by stepwise in addition to oil to surfactant/water mixture [9].

After the stability evaluation period, final compositions of the best formulae from Method 1 and Method 2 is sample J and which is consisting with oil 32 % (w/w), surfactant 32 % (w/w) and water 36 % (w/w). Resulting best formulae from both methods is o/w emulsion. It is suitable for topical applications because of the good moisturising property. Most of the previously studied vegetable oil based emulsions were also o/w emulsions.

Droplets size is the most important characteristic of nano-emulsion. The defined size of nano-emulsion is varied in literature. However a well-accepted typical droplets size falls in the range of 20 - 500 nm. The results obtained by laser scattering spectroscopy are in the range of nano-emulsion. Droplet size of the best formulae was 653.8 nm from Method 1 and 484.25 nm from Method 2. The results indicate that the Method 1 gives the micro-emulsion and Method 2 gives the nano-emulsion. It is well proven that emulsion which is undergone secondary homogenization usually contains small droplets than those which have undergone primary homogenization [6]. Under the given conditions primary homogenization gives a micro-emulsion and applying of high shear homogenization leads to give a nano sized emulsion which is below 500 nm. The nano-range droplets are enhancing the solubility and bioavailability of lipophilic drugs and leading to a significant increase in interfacial area associated with nano-emulsion would influence the transport properties of the drug. Nano-emulsions are only kinetically stable. However, the long- term physical stability of nano-emulsions (with no apparent flocculation or coalescence) makes them unique and they are sometimes referred to as 'Approaching Thermodynamic Stability' [10]. Due to the small droplets size nano-emulsions are stable against creaming and sedimentation. However, irreversible destabilization can occur by Ostwald ripening and/or coalescence mechanisms. It has been reported that the main nano-emulsion destabilization mechanism is mainly by Ostwald ripening [11].

Monitoring the pH value is important for determining the stability of the emulsion because pH changes indicate the occurrence of chemical reactions that can compromise the quality of the final product. Emulsions produced with vegetable oils may experience a decrease in pH due to the hydrolysis of fatty acid esters into free fatty acid degradation products [12]. Resulting pH of sample J from Method 1 and 2 are 6.03 and 6.3 respectively. All the selected formulations from Method 2 showed pH increment comparing to the Method 1 respected formulations. This may be due to the increasing of solubility of the ingredients in the emulsion due to the reduction of particles size when applying high shear homogenization and it leads to decrease the acidity of the medium. Resulting pH values are suitable for topical application. High temperature might have destabilised the nano-emulsion by hydrolysis, but it did not affect the overall quality of the nano-emulsions because the pH values remained around pH 6.0, which is an acceptable, non-skin irritating pH value [12].

Creaming indices of both formulations were zero. It is a good indicator of a stable emulsion. Best formulae from Method 1 showed creaming property after 95 days and from Method 2 didn't show any unstable condition even after 250 days. This is due to the proper mixing of the materials by the homogenization and high stability of nano-emulsion particles size.

According to the microscopic studies it was proven that the resulting best formulation (sample J) is o/w emulsion. Due to the very small droplets size of the formulations unable to obtained the clear images of the morphological features of the formulations.

The viscosity of the selected formulations was determined. The values are shown in Table 3. Sample J had the lowest viscosity, perhaps of its lower oil and surfactant content. The differences in viscosities between the formulations from Method 1 and 2 were significant. Though the same formulation from two different methods had the same oil, water and surfactant content viscosities were showed significant differences. According to the results, viscosities of the formulations which were obtained from Method 1 were always less than the formulations which were obtained from the Method 2. It means nano-emulsions have higher viscosity than the macro-emulsions though the samples having same compositions. The viscosity of the nano-emulsion increased with increasing concentration of Tween 20® [13]. It was shown from the results that the values were also increased with increasing of the surfactant content.

Nano-emulsion, a multipurpose drug delivery system can be used to deliver drugs by oral, topical, parenteral and many other routes [14]. Ultimate goal of this study develop a nano-emulsion based on the VCO for topical drug delivery and as this is the pre formulation study the release study of the system based on the route of administration of the formulation will be performed later.

Application and usage of the nano-emulsions are widely popular among the people and development of the resulting best formulae is necessary for further evaluation. We can conclude this research as an important research finding to develop a formulation for VCO based nano-emulsion for topical drug delivery.

VI. CONCLUSION

VCO is suitable for preparation of nano-emulsion and it gives an o/w emulsion. Best formulae consisting with oil 32% (w/w), surfactant 32% (w/w) and water 36% (w/w). High shear homogenization leads to substantial reduction of the particle size and increase the stability of the formulation comparing to the conventional emulsion preparation method. As this is the pre formulation study stage and further studies will include the need for incorporation of the drug to the best formulations and *in vitro* evaluation for topical delivery.

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REFERENCE

[1] P. B. Bernard, *Modern aspects of emulsion science*, Royal Society of Chemistry, pp 1

[2] M.H.F. Sakeena, F.A. Muthanna, Z.A. Ghassan, S.M. Elrashid, "Formulation and in vitro evaluation of ketoprofen in palm oil esters nano-emulsion for topical delivery", *J. Oleo Sci*.,59(4), (2010), pp 223-228

[3] A.M. Marina, Y.B. Che, I. Amin, "Virgin coconut oil: emerging functional food oil", *Trends in Food Science & Technology*, Volume 20 (2009), pp 481-487

[4] A.M. Marina, Y.B. Che, S. A. H. Nazimah, I. Amin, "Chemical Properties of Virgin Coconut Oil", *J Am Oil Chem Soc*, Volume 86 (2009), pp 301–307

[5] Z. A. Zakaria, M. S. Rofiee, M. N Somchit, A. Zuraini, M. R. Sulaiman, L. K. The, M. Z. Salleh, K. Long, "Hepatoprotective Activity of Dried- and Fermented-Processed Virgin Coconut Oil", *Evidence-Based Complementary and Alternative Medicine*, (2011), DOI: 10.1155/2011/142739

[6] C. Qian, E. A. Decker, H. Xiao, D. J. McClements, "Comparison of Biopolymer Emulsifier Performance in Formation and Stabilization of Orange Oil-in-Water Emulsions", *Journal of the American Oil Chemists' Society*, 88(1) , (2010), pp 47-55

[7] K.G. Nevin, T. Rajamohan, "Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation", *Clinical Biochemistry*, Volume 37, Issue 9 (2004), pp 830-835

[8] M. R. A. Ferreira, R. R. Santiago, T. P, Souza, E. S. T. Egito, E. E.Oliveira, A. L. Soares, "Development and Evaluation of Emulsions from Carapa guianensis (Andiroba) Oil", *AAPS PharmSciTech*. Volume 11, Issue 3 (2010), pp 1383–1390.

[9] M.J. Garcia, C. Solans "Formation of water-in-oil (W/O) nano-emulsions in a water/mixed non-ionic surfactant/oil systems prepared by a low-energy emulsification method, Colloids and Surfaces", *Physicochem. Eng. Aspects*, Volume 250 (2004), pp 415–42

[10] T. Tharwat, P. Izquierdo, J. Esquena, C. Solans, "Formation and stability of nano-emulsions", *Advances in Colloid and Interface Science*, Volume 9 (2004), pp 108–109

[11] N. U. Sadurn, S. Conxita, N. U. Azemar, C . Garc, "Studies on the formation of O/W nano-emulsions, by low-energy emulsification methods, suitable for pharmaceutical applications", *European Journal of Pharmaceutical Sciences*, Volume 26 (2005), pp 438–445

[12] D. S. Bernardi, T. A. Pereira, N. R. Macie,l J. Bortoloto, G. S. Viera, G. C. Oliveira, P. A. Rocha-Filho, "Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments", *Journal of Nanobiotechnology*, Volume 9 (2011), pp 44, Doi 10.1186/1477-3155-9-44

[13] C.H. Anjali, Y. Sharma, A. Mukherjee, N. Chandrasekaran, "Neem oil (Azadirachta indica) nanoemulsion--a potent larvicidal agent against Culex Quinquefasciatus", *Pest Manag Sci*, Volume 68, Issue 2 (2012), pp 158-163

[14] S. N. Sheikh, S. Faiyaz, S. Talegaonkar, J. Ali, S. Baboota, A, Ahuja, K. K. Roop, M. Ali, "Formulation Development and Optimization Using Nanoemulsion Technique": A Technical Note, *AAPS PharmSciTech*, 8(2), (2007), pp 12-17

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