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Research Article

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Preparation and characterization of virgin coconut oil nanoemulgel

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ABSTRACT

VCO Emulsions were prepared using high-speed stirrer with various surfactant concentrations and mixing ratio. The resultant mixture was added with distilled water. Among the formulation, best optimum formulation was selected for secondary homogenization using High Pressure Homogenizer (HPH) to produce of VCO Nanoemulsion in the range 20-200nm. The secondary homogenization process involves using variable parameter of pressure and homogenization cycle number. According to the results the VCO Nanoemulsion consists of 20% of VCO, 5% surfactant and 75% distilled water. 5% of the optimum VCO Nanoemulsion formulations with the smallest droplet size 153nm and Poly Dispersed Index (PDI) value of 0.139 were selected to incorporate with a 0.5% hydrogel concentration and distilled water mixed to produce VCO Nanoemulgel. Based on the characterization of VCO Nanoemulgel with droplet size of 163nm and 0.211 PDI value, low viscosity and pH value of 5.6-6.0 were suitable for topical usage and accelerated stability study indicates that VCO Nanoemulgel is a stable formulation.

Keywords: VCO, Nanoemulsion, Nanoemulgel, Formulation, Nanotechnology, Oils

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INTRODUCTION

Nanoemulsion is one of the growing technologies especially in food, cosmetic [1] and pharmaceutical industries as a novel delivery system for drugs and lipophilic materials such as essential oils, flavours, colours, fatty acids etc. The technological application of nanoemulsions have been used in various applications due to their characteristic properties of small droplet size (in the range 20-200nm) with high interfacial area, transparent or translucent appearance, high solubilization capacity, low viscosity, and high kinetic stability of sedimentation, flocculation, and in some cases, the coalescence [2].

Nanoemulsions have been used as a drug delivery system through various systemic routes mainly: oral, topical and parenteral nutrition [3]. Thus, the nanoemulsification technique is achieved with several modification and enhancement of the limitation routes encounter through the drug delivery processes [4]. Therefore, the modification related the incorporation of nanoemulsion and hydrogel it is term as nanoemulgel. With the gelling system, it promotes a better stability of nanoemulsion by reducing the surface and interfacial tension and also enhancing viscosity of the aqueous for better administration topically [5].

The advance knowledge in production and stability of dispersed systems enables the development of different vehicles such as nanoemulsions and nanoemulgels, which have been effectively used to increase the bioavailability and improve the stability of the active ingredients. Nowadays there is an intense usage of natural bioactive materials as medicinal agent in pharmaceutical industries [6].

Natural bioactive materials widely used such as virgin coconut oil (VCO) are gaining wide popularity in the scientific field and among the public, due to its various health benefits and special characteristics [7]. VCO is obtained by wet process may have more beneficial effects than coconut oil due to its higher unsaponifiable components like polyphenols and α-tocopherols. In this study adopted a preparation using high-pressure homogenizer to produce VCO nanoemulsion and incorporated with hydrogel to formulate VCO Nanoemulgel. The objectives of the present work were to prepare and characterize the formulation of VCO Nanoemulgel.

EXPERIMENTAL SECTION

MATERIAL

Virgin coconut oil (VCO) was obtained from Institute of Bioproduct Development, Universiti Teknologi Malaysia (UTM). Tween 20 and Span 80 were purchased from Sigma–Aldrich, UK. Carbopol Ultrafez 20 was purchased from Grace Cosmetic, Industries GMP SDN .BHD, Selangor, Malaysia. The Distilled water was obtained on the site from a Sartorius Arium 611 water system.

METHOD

3.1 VCO EMULSION PREPARATION

Emulsions were prepared in five different concentration (4%, 5%, 6%, 8% and 9%) with different ratio (4:1, 3:2 and 2:3) of surfactant (Tween 20: Span 80) and 20% VCO was added with distilled water. VCO emulsion was prepared by using high-speed mixer (IKA T25 digital ultra-Turrax Mixer) for 15 minutes at 6000-rpm. The droplet size of the Emulsion was measured using photon correlation spectroscopy (Zetasizer Nano Z version 7.00, Malvern Instruments Ltd, Malvern, UK). Each formulation was diluted with water (1:100) at 25°C. All size measurements were done in triplicate. The average droplet size distribution and size distribution was presented by the poly dispersed index values (PDI) were measured [8]. The samples were kept in a glass bottle under the room temperature for visual observation (phase separation, sedimentation creaming or flocculation) for one week [9].

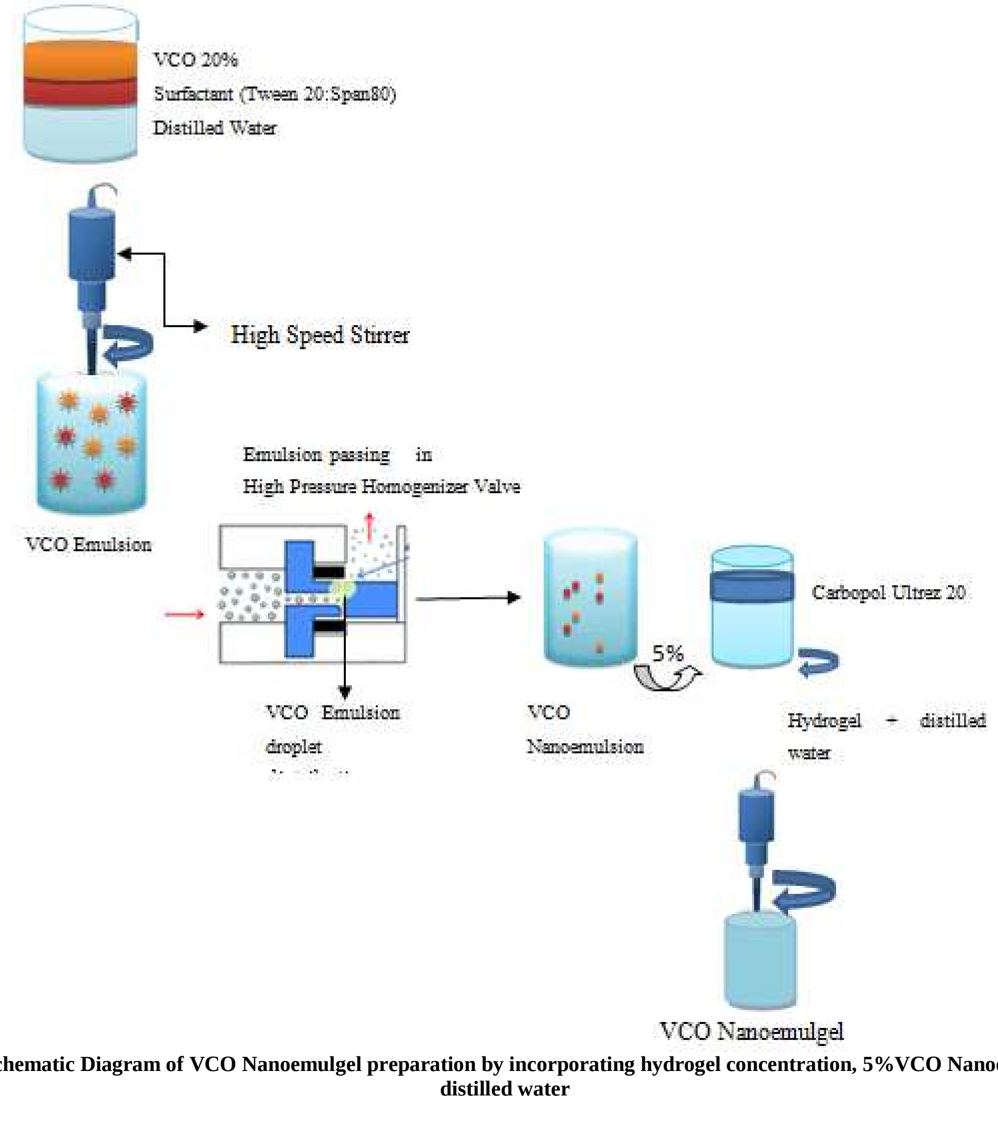

Figure 1: Schematic Diagram of VCO Nanoemulgel preparation by incorporating hydrogel concentration, 5%VCO Nanoemulsion and distilled water

3.2 VCO NANOEMULSION PREPARATION

Nanoemulsion is prepared using high-pressure homogenizer (HPH). The best optimum VCO Emulsion formulation with the smallest droplet size and with stable condition formulation was selected to process in (HPH) at different pressures (500, 1000 and 1500 bar) and cycles (1, 2, 3, 4, 5, 10 and 15). The droplet size of the VCO Nanoemulsion was measured using photon correlation spectroscopy (Zetasizer Nano Z version 7.00, Malvern Instruments Ltd, Malvern, UK). Each formulation was diluted with water (1:100) at 25°C. All size measurements were done in triplicate. The average droplet size distribution and size distribution was presented by the poly dispersed index values (PDI) were measured [8].

3.3 HYDROGEL PREPARATION

Hydrogel was prepared through the addition of Carbopol Ultrez 20 in water and constantly stirred by using high speed mixer. The mixture will undergo continuous stirring and be left overnight for 24 hours. The hydrogel pH will be adjusted to pH 5.6 using 2M NaOH, which will be added under constant stirring [10].

3.4 VCO NANOEMULGEL PREPARATION

Nanoemulsion of VCO will be incorporated with hydrogel as shown in figure 1 by mixing the nanoemulsion with different amount of hydrogel to produce formulation of nanoemulgels containing different concentration of hydrogel, 5% VCO Nanoemulsion and DI water.

3.4 CHARACTERIZATION OF VCO NANOEMULGEL

3.4.1 DROPLET SIZE AND POLY DISPERSED INDEX (PDI) MEASUREMENT

The droplet size and PDI of the nanoemulgel was measured using photon correlation spectroscopy (Zetasizer Nano Z version 7.00, Malvern Instruments Ltd, Malvern, UK).

3.4.2 VISCOSITY MEASUREMENT

The viscosity of nanoemulgel measured by using a Brookfield Viscometer (Model RVT, USA) at 100, 150, 200 and 250 rpm by using spindle number 61, 62, 63 and 64 [10].

3.4.3 pH DETERMINATION

pH values were measured at 25˚C.The measurement were taken three times for one sample. Before the readings were observed pH meter were calibrated by using pH 7.00, 4.00 and 10.00 buffer solutions respectively [9].

3.4.4 CENTRIFUGATION TEST

Centrifugation test was performed to observe phase separation in extreme condition. 30g of VCO nanoemulgel was load in the centrifuge tube. The sample was centrifuge directly at room temperature [11].

3.4.5 FREEZE-THAW TEST

150g of VCO nanoemulgel was filled into a container. The sample was first put into the freezer at 4˚C for 24 hours. The frozen sample was then, left at room temperature for 24 hours to thaw. One cycle of freeze thaw was completed in 48 hours. The properties of the samples were evaluated before the freeze-thawing process and after every cycle until the forth cycle [11].

RESULTS AND DISCUSSION

4.1 VCO EMULSION

Table 1 shows the results of mean droplet size and PDI value of VCO Emulsion preparation with different surfactant concentration and mixing ratios. All the formulation shows droplets size range between 251.83 ± 2.93 nm to 433.1 ± 2.10 nm and PDI value with 0.258 ± 0.084 to 0.459 ± 0.112. Physical conditions of the VCO emulsion produced using the high-speed stirrer was observed for seven days before proceeding to the secondary homogenization of the VCO Emulsion. In table 2 shows the physical observations of VCO Emulsions.

Table 1: Droplet size and Poly Dispersed Index (PDI) of VCO Emulsion formulation with different surfactant concentration and mixing ratio using high-speed stirrer

All the data are presented in ±SD (n=3)

Sample	Ratio	Surfactant Concentration (%)	Droplet Size (nm) ±SD	Poly Dispersing Index (PDI) ± SD
A	4:1	4	423.5 ± 7.56	0.524 ± 0.110
B	3:2	4	295.7 ± 1.53	0.381 ± 0.03
C	2:3	4	334.8 ± 5.14	0.406 ± 0.028
D	4:1	5	278.8 ± 0.30	0.268 ± 0.013
E	3:2	5	251.83 ± 2.93	0.258 ± 0.084
F	2:3	5	327.7 ± 6.68	0.258 ± 0.01
G	4:1	6	286.7 ± 0.79	0.30 ± 0.0204
H	3:2	6	363.8 ± 19.33	0.455 ± 0.047
I	2:3	6	279.2 ± 2.2	0.357 ± 0.08
J	4:1	8	307.8 ± 3.8	0.36 ± 0.03
K	3:2	8	360.6 ± 2.7	0.41 ± 0.01
L	2:3	8	433.1 ± 2.1	0.459 ± 0
M	4:1	9	327.63 ± 0.45	0.398 ± 0.001
N	3:2	9	375.1 ± 13.8	0.50 ± 0.001
O	2:3	9	377.6 ± 0.38	0.38 ± 0.007

Table 2: Physical observation analysis (colour, odor and phase separation) VCO Emulsion formulations using high-speed stirrer for 7 days

Sample	Surfactant concentration	Surfactant Ratio (Tween20 :Span 80)	Colour	Odor	Phase Separation On time
A	4:1	4	Milk White	—	—
B	3:2	4	Milk White	—	—
C	2:3	4	Milk White	—	╋
D	4:1	5	Milk White	—	—
E	3:2	5	Milk White	—	—
F	2:3	5	Milk White	—	╋
G	4:1	6	Milk White	—	—
H	3:2	6	Milk White	—	—
I	2:3	6	Milk White	—	╋
J	4:1	8	Milk White	—	—
K	3:2	8	Milk White	—	—
L	2:3	8	Milk White	—	╋
M	4:1	9	Milk White	—	—
N	3:2	9	Milk White	—	—
O	2:3	9	Milk White	—	╋

Symbols Abbreviations:

╋ : Phase Separation	—: No Odor Changes	—: No Phase Separation

4.2 VCO NANOEMULSION

High energy homogenizer was used to produce nanoemulsions. The production of nanoemulsion, basically requires the right surfactant at appropriate concentration, which enhances the stability of nanoemulsion. Therefore, VCO Emulsion processing using high pressure homogenizer (HPH) requires several times of homogenization cycles at high pressure in order to achieve nanoemulsion in nanometer size range of 20-200nm. At three different pressures (500, 1000 and 1500 bar) and repeated homogenization up 15 cycles for each pressure point was measured to obtain the smallest droplet size and PDI value. Table 3 shows the impact of high-pressure homogenizer on the droplet size and PDI value of VCO Emulsion at variable parameter of pressure (500, 1000 and 1500 bar) and passing time (1, 2, 3, 4, 5, 10 &15 cycles). The results obtained showed nano-droplets size in the range from 153.6 ± 0.91 nm to 261.5 ± 2.61 nm. The optimum nanoemulsion was selected based on the droplet size and PDI value. The smallest droplet size of 153.6 ± 0.91nm and lowest PDI value 0.139 ± 0.012 obtained at 1500 bar pressure with five cycles after processing in HPH.

4.3 VCO NANOEMULGEL

In this study, different concentration (0.5, 1 and 1.5 %) of hydrogel was prepared to be incorporated with the optimum formulation of 5% VCO nanoemulsion and distilled water to prepare VCO Nanoemulgel. From the result presented 0.5 % w/w hydrogel concentration consists of droplet size (186.3 ± 1.32 nm) and (0.189 ± 0.057) PDI value. At 1% hydrogel the droplet size was more than 200nm (364.5 ± 18.36 nm) and high PDI value of (0.772 ± 0.07) and at 1.5 % hydrogel w/ w concentration droplet size was (510.6 ± 10.39 nm) with high PDI value (0.658 ± 0.0176). Based on the results described above 0.5% hydrogel concentration was suggested to be the optimum concentration to incorporate with nanoemulsion and distilled water to form VCO Nanoemulgel as shown in table 4.

Table 3: Results of droplet size and poly dispersing index PDI in variable parameter of pressure and passing time (cycle number) using high-pressure homogenizer (HPH)

All data is presented in ±SD (n=3)

Sample	Pressure	No. cycle	Mean droplet size(nm) ± SD	PDI ± SD
F	500	1	261.5 ± 2.61	0.288 ± 0.288
		2	198.4 ± 1.11	0.174 ± 0.15
		3	198.3 ± 0.2	0.174 ± 0.16
		4	194.3 ± 2.52	0.174 ± 0.028
		5	192.5 ± 0.5	0.159 ± 0.13
		10	175.2 ± 0.473	0.15 ± 0.05
		15	171.9 ± 1.55	0.109 ± 0.011
G	1000	1	248.2 ± 1.21	0.351 ± 0.019
		2	198.3 ± 1.69	0.198 ± 0.019
		3	199.9 ± 1.78	0.22 ± 0.005
		4	172.7 ± 1.16	0.134 ± 0.012
		5	170.5 ± 0.87	0.138 ± 0.020
		10	169.9 ± 0.85	0.146 ± 0.009
		15	175.1 ± 1.13	0.128 ± 0.013
H	1500	1	177.1 ± 0.038	0.185 ± 0.007
		2	172.8 ± 1.86	0.201 ± 0.006
		3	163.9 ± 1.82	0.171 ± 0.005
		4	157.1 ± 1.40	0.162 ± 0.148
		5	153.6 ± 0.91	0.139 ± 0.011
		10	159.7 ± 0.76	0.135 ± 0.003
		15	158.7 ± 1.35	0.129 ± 0.0015
Control		0	294	0.341

Table 4: Droplet size and Poly Dispersing Index (PDI) value of VCO Nanoemulgel prepared at different hydrogel concentration, 5% VCO Nanoemulsion concentration and distilled water

Sample	Hydrogel (%)	Oil (%)	Distilled Water (w/w)	Dropt Size ± SD	PDI ± SD
I	0.50	5	67.5	186.3 ± 1.32	0.189 ± 0.057
J	1	5	30.5	364.5 ± 18.36	0.772 ± 0.0689
K	1.50	5	37.5	510.6 ± 10.39	0.658 ± 0.0176

4.4 CHARACTERIZATION OF VCO NANOEMULGEL

In the current study VCO nanoemulgel was subjected for measuring droplet size, poly dispersed index (PDI), viscosity, pH and accelerated stability test measurement to characterize the prepared formulation. Table 5 shows the results VCO nanoemulgel analysis based on the formulation and processing parameter described earlier. Based on the droplet size measurement, the size is approximately 164.3 nm and the PDI value of 0.211[12].

pH measurement was performed for VCO nanoemulgel sample to evaluate the behavior of the formulation at different temperatures of 4˚C, 25˚C and 45˚C and different period of time (0 hrs, 24 hrs and 48 hrs). At all conditions the VCO nanoemulgel formulations showed pH value not more than 6.0, which makes them a promising system for topical application, since the skin pH often range between 5.5 and 6.0 [13].

VCO nanoemulgel was sampled at four parameter of (100,150,200 and 250) rpm with four type of spindles number (61, 62, 63 and 64. The viscosity results decreased at higher rpm in different spindle number usage. The low viscosities of nano-sized particles below 200nm are one of the esthetic properties for topical applications [14]. Furthermore, oil type, surfactant concentrations and low viscosity are important parameter to be considered during the formulation.

In these sections, two different procedures of accelerated stability testing which consist of centrifugations and the freeze-thawing were performed on the VCO nanoemulgel. The centrifuge test is conducted to observe the phase separation of the sample at extreme conditions. The freeze-thaw test evaluated the colour and phase separation before and after the freeze-thawing processes

Table 5: Results of droplet size and PDI measurement of VCO Nanoemulgel formulation

Parameter of Analysis	Value	Control
Droplet size(nm)	164.5 ± 1.11	186.4±1.23
PDI	0.211 ± 0.0288	0.280 ±0.042

DISCUSSION

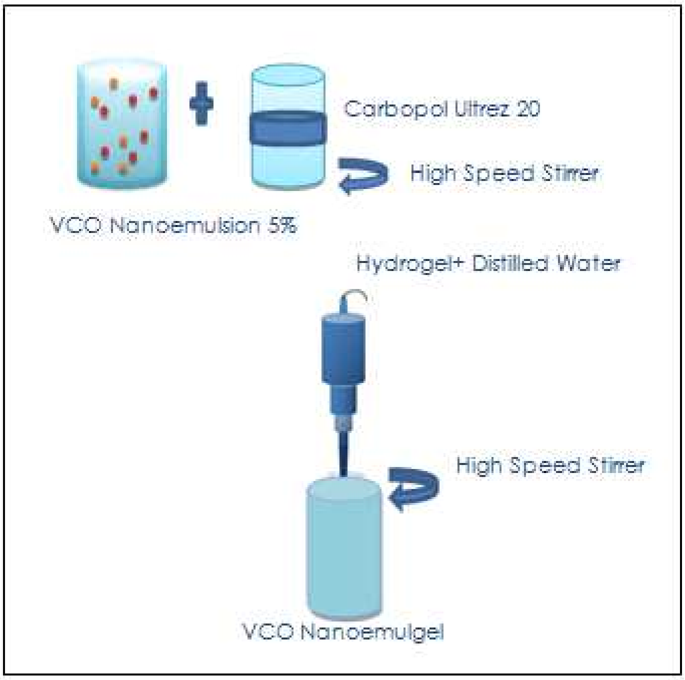

Figure 2: Graphical Abstract of Preparation of VCO Nanoemulgel

The preparation of VCO emulsion is adopted by the acceptable method which previously used to develop palm oil droplet size nanoemulsion [15]. Moreover, the PDI result showed minimum value which is relatively depends on the droplet size. The size distribution reflects the uniformity of droplet diameter [16],[17]. The visual observation indicates the phase separation on time. A good emulsion indicates droplets, which spread easily without separation to produce a fine milky emulsion. Nevertheless, the emulsifying techniques represent the phase separation on time [16].

The droplet size measurement of VCO nanoemulsion processed in HPH suggesting the nano-sized droplets (d<200nm) is obtained. From the analysis, smaller droplet size (153.8 nm) and PDI value (0.138) were obtained. The processed formulation in HPH showed a bluish/translucent appearance, which indicated that they contained nano- sized droplets. The result indicates the VCO emulsion preparation gives a macroemulsion and processed using HPH to give nanoemulsion. It is well suggested that, emulsion which undergo secondary homogenization usually produce smaller droplet size.

VCO nanoemulgel preparations were preduced by incorporating nanoemulsion and hydrogel matrix. The presence of hydrophilic functional groups at the polymeric backbone and the cross-linked between the network chains are behind the ability of the hydrogel to absorb water and be resistant to dissolution [18]. At different concentration of hydrogel (0.5 %, 1 % and 1.5 %) VCO nanoemulgel productions were studied. Based on the smallest droplet size and PDI value of 0.5% hydrogel concentrations were chosen.

Monitoring pH values is important for determining the stability of nanoemulsion because the change of pH values indicates the occurrence of chemical reactions that can compromise the quality of final product. The differentiation of storage condition might have destabilized the nanoemulgel because of fluctuation of pH values but still remain around 5.6 to 6.0 which is acceptable and non-skin irritating value [19]. The viscosity of the gel during handling, transport and storage is the important criteria. Viscosity is the measure of the internal friction of a fluid. This friction becomes apparent when a layer of fluid is made to move in relation to another layer. The greater the friction, the greater the amount of force required to cause this movement, which is called shear. Shearing occurs whenever the fluid is physically moved or distributed as in pouring, spreading, spraying, mixing, etc.

Stability is usually, observed by visual inspection that the emulsion would be creaming or flocculate. This analysis is necessary to show whether the active ingredient was degraded or uniformity distributed [20]. Stability test require a longer period of time, whereas a few companies can afford only limited time of three years testing before launch the new product.

CONCLUSION

To obtain the nanoemulsion in the range of 20-200nm secondary homogenization process is studied using high-pressure homogenizer, which leads to a substantial reduction of the particle size, and increase the stability of the formulation compared to conventional emulsion preparation techniques. The optimum formulation of VCO emulsion was selected to process in HPH with various pressures and passing time of homogenization. The prepared formulation with droplets size less than 200 nm and PDI value below 0.2, low viscosity, pH value at the range of 5.5 and 6.0 and no separation, which indicate that the formulation is stable.

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