



NANOEMULSION BASED PALM OLEIN AS VITAMIN E CARRIER

(Nanoemulsi Berasaskan Olein Sawit Sebagai Pembawa Vitamin E)

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Abstract

Aging process makes our skin getting thinner and drier. Antioxidant based on nanoemulsion system is favourable because of its effectiveness. This study was conducted to develop nanoemulsion system based on palm olein as a carrier of vitamin E and the physicochemical behaviours of the system were studied. Palm olein as oil phase, polyoxyethylene (4) lauryl ether (Brij 30) was used as a surfactant and vitamin E as an anti-aging agent. The mixtures of Brij 30 and palm olein at certain ratios were thoroughly mixed and titrated with 5 – 95% wt. of water and homogenized by using homogenizer. The Palm olein/Brij 30/Water mixtures that produced a clear solution and no birefringence after observation under polarized light microscopy are labelled as nanoemulsion. Nanoemulsion region was shown in ternary phase diagram. Based on the ternary diagram, these systems were formed at oil: surfactant (O/S) ratio 4:6, 5:5 and 6:4 and 20 – 50% wt of water. These systems were then characterized for stability test, particle size, electrical conductivity, viscosity and pH. Stability test was done at three temperatures, which were 4, 25 and 40 °C for one month and four cycles of freeze-thaw at storage temperature for 12 hours at each temperature. The systems were stable and no phase separation was observed. Particle size analysis showed that most systems have a particle size within the range of nanoemulsion (20 – 500 nm). Electrical conductivity tests showed that water-in-oil system formed at low water percentage (20 – 30% wt. of water) and bicontinuous at a higher percentage (30 – 50% wt. of water). Viscosity of both systems vitamin E-loaded and unloaded is very low. All systems have a pH value in a range of 6 – 7, which are suitable to be applied to human skin. Based on the physical characteristics of the system, it shows high potential as a vitamin E carrier.

Keywords: nanoemulsion, palm olein, vitamin E, ternary phase diagram, anti-aging agent

Abstrak

Proses penuaan menyebabkan kulit kita menjadi semakin nipis dan kering. Antioksidan yang berasaskan sistem nanoemulsi lebih digemari kerana kesannya yang lebih berkesan. Penyelidikan ini dijalankan untuk membangunkan sistem berasaskan olein sawit sebagai pembawa vitamin E dibangunkan dan sifat fizikokimianya dikaji. Olein sawit sebagai fasa minyak, poliolefin (4) lauril eter (Brij 30) digunakan sebagai surfaktan dan vitamin E sebagai bahan aktif. Campuran Brij 30 dan olein sawit dikacau sehingga sebati dan seterusnya dititratkan air sebanyak 5 – 95% (berat/berat) dan dihomogenkan menggunakan alat penghomogenan. Campuran Olein Sawit/Brij 30/Air yang menghasilkan larutan jernih dan tiada dwibiasan dilabelkan sebagai nanoemulsi selepas pemerhatian di bawah mikroskop cahaya terkutub. Rantau nanoemulsi ditunjukkan di dalam gambar rajah fasa ternari. Berdasarkan kepada gambar rajah fasa ternari, sistem ini terhasil pada nisbah minyak:surfaktan (M/S) sebanyak 4:6, 5:5 dan 6:4 dengan peratusan air sebanyak 20 – 50% (berat/berat). Seterusnya, sistem tersebut dicirikan melalui ujian kestabilan, saiz partikel, kekonduksian elektrik, kelikatan dan pH. Ujian kestabilan dilakukan pada setiap suhu berbeza iaitu 4 °C, 25 °C dan 40 °C selama sebulan dan empat kitaran pemanasan-penyejukan dengan suhu penyimpanan selama 12 jam pada setiap suhu. Sistem-sistem tersebut adalah stabil dan tidak berlaku perubahan fasa. Ujian pengukuran saiz partikel menunjukkan bahawa kebanyakan sistem bersaiz di dalam julat saiz nanoemulsi iaitu di antara 20 – 500 nm. Melalui ujian kekonduksian elektrik, dijangkakan sistem air-dalam-minyak pada 20% hingga 30% (berat/berat). Kelikatan nanoemulsi tanpa atau dengan bahan aktif

pula adalah sangat rendah. Semua sistem mempunyai nilai pH yang berada dalam julat 6 – 7, di mana ianya sesuai diaplikasi pada kulit. Berdasarkan kepada sifat fizikal sistem, ia sesuai diaplikasikan sebagai pembawa vitamin E.

Kata kunci: nanoemulsi, olein sawit, vitamin E, rajah fasa ternari, agen anti-penuaan

Introduction

Aging process is usually associated with changes in skin quality and appearance such as thinning, reduced elasticity and wrinkling of the skin [1]. The term cosmeceuticals was created by Dr. Albert Kligman who identified cosmeceuticals as a hybrid of drugs and cosmetics [2]. Cosmeceuticals are formulated with pharmaceutical-type ingredients that have special ability to treat and enhance the beauty of the skin from inside out [3]. Cosmeceuticals are currently very popular, especially for products that are designed and developed to help in prevention and treatment of aging skin. [4].

In the recent years, nanoemulsion has gain interest as a colloidal delivery system within the food, pharmaceuticals, cosmeceuticals, and other industries to encapsulate, protect and deliver bioactive lipophilic components, such as drugs, vitamins, nutraceuticals and antioxidants [5-7]. Nanoemulsion is defined as emulsion system having particle size ranging from 20 – 500 nm [8, 9]. The size range may vary depending on authors, in which some authors consider 500 nm as the upper limit [10]. Due to small particle size, nanoemulsion possess stability against sedimentation, flocculation or creaming [11]. Nanoemulsion is a non-equilibrium system and cannot be formed spontaneously. Nanoemulsion is only kinetically stable [12], in which it differs from microemulsion, which is also transparent or translucent but thermodynamically stable [13]. The attractions of nanoemulsion in personal care and cosmetics applications are due to the use of low surfactant concentration (3 – 10%) compared to microemulsion (usually 20% or higher) [14], small droplet size for uniform distribution on the skin [15] and efficient drug delivery of active ingredients through the skin [16]. Nowadays, nanoemulsion has been used as nanocarrier to deliver the active component in the product to its targeted cells more effectively due to advancement in nanotechnology [3]. Basically, nanoemulsion can be prepared using two methods: the high energy method that involves the use of mechanical devices (e.g. ultrasonicator, rotor/stator system and high pressure homogenizer) and low energy method in which the chemical potential of the component is used such as phase inversion temperature (PIT), phase inversion composition (PIC) and spontaneous emulsification method [6-7, 10].

Anti-aging agent such as antioxidant has been shown to decrease sunburn cells after UV exposure, neutralize free radicals and act as a humectant when applied on the skin [2]. Vitamin E is the most renowned type of antioxidant [3] and widely used in cosmeceuticals and pharmaceutical industries. It exists in eight different isomers including α , β , γ and δ derivatives of tocopherol and tocotrienol [17]. Among these isomers, α -tocopherol is the form of vitamin E with the highest abundance and biological activity that is important to protect cellular membranes from lipid peroxidation by free radicals [4]. Vitamin E is a lipid soluble antioxidant that scavenges free radicals and protects membrane lipids from peroxidation when taken orally [2]. However, the α -tocopherol form of vitamin E is highly unstable to oxidation due to its chemical degradation [18]. A system that can encapsulate these lipophilic nutraceuticals may able to protect from chemical degradation during storage and increase their bioavailability [19, 20]. For this reason, there has been increasing studies on nanoemulsion-based delivery system as vitamin E carrier [21].

Palm olein is the liquid fraction that is obtained from fractionation of palm oil. It is reported to have a higher content of monounsaturated fatty acid especially oleic acid (C 18:1; 39.8 – 43.9%) [22]. Palm olein has been considered to possess higher antioxidant activity due to presence of natural antioxidants such as α -tocopherol and α -tocotrienol [23, 24]. These natural antioxidants contains in palm olein are physiologically active as vitamin E [25, 26]. There was a research that proved its role as a natural antioxidant supplement that was capable in protecting the brain against oxidative damage [27]. Furthermore, it also possess free radical scavenger properties, which is to protect biological systems against oxidative and carcinogenic stress [28-30], in addition to inhibiting cholesterol biosynthesis and platelet aggregation [31, 32].

In this study, the formulation of nanoemulsions was developed by using palm olein as the oil phase (O), polyoxyethylene (4) lauryl ether (Brij 30) as surfactant (S) and vitamin E as anti-aging agent. This study was

conducted to formulate and improve the stability of nanoemulsion system based on palm olein as a carrier of vitamin E and the physicochemical behaviour of the system were studied.

Materials and Methods

Refined palm olein used in this study was obtained from Delima Oil Products Sdn. Bhd. Vitamin E was purchased from Sigma Aldrich (USA). Nonionic surfactant, polyoxyethylene (4) lauryl ether (Brij 30), $C_{20}H_{42}O_5$, was purchased from ACROS Organics (Singapore). Distilled water was used throughout the experiments. All the chemicals were analytical grade and directly used without further purification.

To determine the concentration range of components that can form nanoemulsions, pseudo-ternary phase diagrams were constructed using water titration method at 25 °C. Brij 30 was mixed with palm olein at oil: surfactant (O/S) ratio of 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2 and 9:1 [33, 34]. The mixtures of oil and surfactant at each specific weight ratios were thoroughly mixed and heated at 50 °C, and then cooled to ambient temperature before titrating with water drop wise from 5 – 95% wt. at 5% wt. of water intervals. The mixtures were then homogenized using homogenizer (IKA® T25 digital ULTRA-TURRAX®) for 3 minutes at 5000 rpm. The mixtures were assessed visually and the regions that formed clear mixtures were mapped on pseudo-ternary phase diagrams. Samples that did not show birefringence under polarized light microscopy were labelled as nanoemulsion. 1% wt. of vitamin E was added to selected nanoemulsion mixtures as shown in Table 1. The vitamin E-loaded and unloaded systems were characterized for stability, particle size, electrical conductivity, viscosity and pH [35, 36].

Table 1. List of the nanoemulsion systems

System	Details of the System
N1	Palm Olein/Brij 30/Water, ratio O/S=4:6
N2	Palm Olein/Brij 30/Water, ratio O/S=5:5
N3	Palm Olein/Brij 30/Water, ratio O/S=6:4
NE1	N1 with 1% wt. of vitamin E
NE2	N2 with 1% wt. of vitamin E
NE3	N3 with 1% wt. of vitamin E

The chemical and physical stability of nanoemulsions were studied via clarity and phase separation observation during a period of one month at different storage conditions at 4, 25 and 40 °C. Freeze-thaw cycle test was conducted for four cycles by keeping the nanoemulsion systems at different storage temperature of 12 hours at each temperature. The droplet sizes of nanoemulsions were determined by Particle Size Analyzer Zeta-Sizer (Malvern Instrument, England).

Conductivity measurements were performed at 25 ± 0.2 °C using a conductometer (inoLab cond. Germany). A solution of 0.01 mM sodium chloride (NaCl) was used in the preparation of the nanoemulsion samples instead of distilled water for the electrical conductivity [37]. The viscosity of various nanoemulsions was measured using Anton Paar Rheometer (Physica MCR 301, Austria) at 25 ± 0.2 °C. For pH values determination, the samples were calibrated with pH buffer before the measurements were done. The measurements were carried out triplicate using pH meter (Mettler Toledo MP 200, Switzerland) at 25 °C. All systems were tested and each measurement repeated three experiments.

Results and Discussion

Construction of ternary phase diagram

The region of nanoemulsion can be explained with the help of the pseudo-ternary phase diagrams. The phase diagram was constructed based on ‘water dilution lines’ which demonstrated an increase of water content while decreasing surfactant content that is represented by the arrow line as shown in Figure 1. The phase diagram of Palm Olein/Brij 30/Water nanoemulsion system which was prepared using homogenizer. Subsequently, the nanoemulsion

systems prepared were investigated with a polarizing light microscope. This was to identify the isotropic spherical structure of nanoemulsion (non-birefringent) from a liquid crystalline structures (birefringent) of non-nanoemulsion [33, 34, 38]. The shaded region in Figure 1 represents the nanoemulsion system of Palm Olein/Brij 30/Water with no birefringence under polarized light microscope. A successful pharmaceutical nanoemulsion system should not only have a stable and transparent, but also with a low content of surfactant to avoid skin irritation [14, 39]. Obtained nanoemulsion systems seem to meet these criteria, since it involved a low amount of surfactant as shown by the shaded region that located in the center of phase diagram. Therefore, six formulations N1, N2, N3, NE1, NE2 and NE3 were selected for further investigation (Table 1).

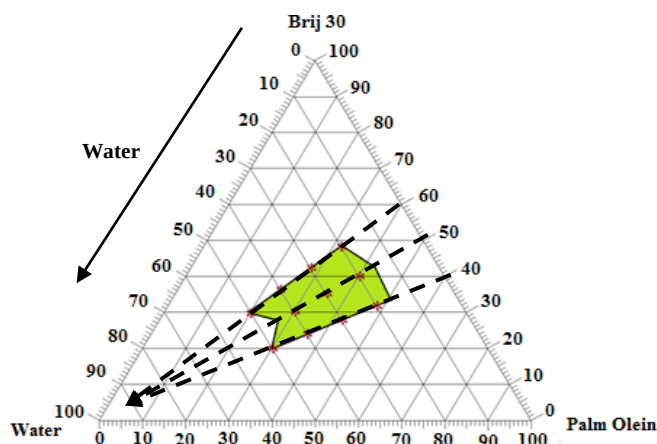


Figure 1. Pseudo-ternary phase diagram of the system Palm olein/Brij 30/Water. The shaded area represents the stable, clear and transparent formulations were produced. Dotted line arrow (- - ➔) shows the water dilution line that representing an increase of water content while decreasing surfactant levels

Particle size

The droplet size of the nanoemulsions were measured by assuming a monodispersed phase based on spherical globules. Table 2 show the mean droplet size of nanoemulsion systems. Addition of vitamin E into the system N1 and N2 did not significantly affect the particle size of the nanoemulsion system. However, it seems to significantly affect the particle size of system N3. Both NE1 and NE2 systems with vitamin E-loaded have a particle size in the range of nanoemulsion (20-500 nm). Meanwhile, the NE3 systems resulted on larger particle size (>500 nm). These can be explain that the component's composition may have a considerable effect in the droplet size of system [39]. The larger droplet size (>500 nm) were observed from these formulations might be due to the incompatibility or inhomogeneity of nanoemulsion's components that caused on particle coagulation [40]. Regarding to the smaller droplet size range as low as <100 nm, the NE1 and NE2 systems should be advantage in dermal and transdermal delivery system of vitamin E [41]. These nano-sized droplets system may have the potential to penetrate to virtually every organ of the body, including lymph nodes and the deep vasculature of solid tumors, yet too small to be readily removed by the liver [42].

Table 2. Particle diameter of Palm olein/Brij 30/Water for system N1, N2, N3, NE1, NE2 and NE3 along the water dilution line

Water Content (%)	Average Particle Size (nm)					
	N1	N2	N3	NE1	NE2	NE3
20	11.74±0.50	10.56±0.66	559.95±2.05	31.45±0.06	28.63±6.97	346.10±18.48
30	30.53±1.51	546.9±2.69	894.05±11.20	38.48±8.03	27.19±10.66	72.99 ± 0.19
40	14.51±0.08	42.23±0.06	868.15±3.32	42.58±24.93	47.50±6.84	550.70±17.50
50	134.50±2.97	625±34.51	791.35±16.41	46.50±3.83	42.66±7.25	560.10±19.56

Stability studies

Stability studies were imposed on nanoemulsion systems for a month at three different temperatures (4 °C, 25 °C and 40 °C) to observe for any phase separation, sedimentation, flocculation, coalescence or creaming during storage. Based on the observation, all systems are clear and stable at these temperatures with no phase separations as shown in Fig. 2. The samples are also subjected to four freeze-thaw cycle test at 4 °C, 25 °C and 40 °C for 12 hours storage at each temperature. The result shows that nanoemulsion systems with- and without addition of vitamin E are stable during the test cycle. This is due to higher content of oleic acid in palm olein that may have considerable stabilization effect against droplet coalescence [17].

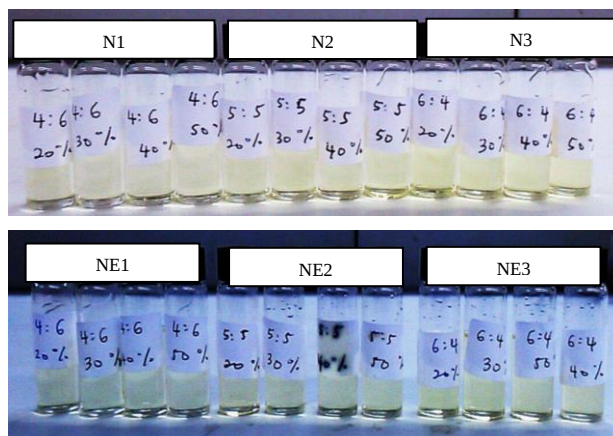


Figure 2. A transparent and stable nanoemulsion systems upon storage after a month

Conductivity

Electrical conductivity provide structural information and can be used to differentiate water-in-oil (w/o), bicontinuous (bc) and oil-in-water (o/w) types of nanoemulsion. The conductivity of nanoemulsion system tends to increase with an increase of water content, from (w/o) to (o/w) phase through bicontinuous phase [33-34, 43]. Fig. 3 shows the phase transitions of Palm olein/Brij 30/Water nanoemulsion along water dilution line. All systems shows slow incensement in conductivity from 20 to 30% wt. of water, but start to increase significantly up to 50% wt. of water. The low conductivity region with water content up to 30% wt. represented w/o region of nanoemulsion. At this stage, water droplets were discrete in an oil continuum and possess weak interactions [44, 45]. Subsequently, as the water content is raised up to 50% wt., the conductivity increasing due to increasing interactions between the aqueous domains forming interconnected conductive channels (bc structure). This is due to the attractive interaction between the spherical microdroplets of aqueous phase of w/o nanoemulsion [46, 47] as they changed from w/o structures to the bicontinuous region. Therefore, all systems can be classified as water-in-oil (w/o) at range 20 to 30% wt. of water and bicontinuous (bc) at >40% wt. of water.

The effect of solubilised vitamin E on the microstructure of the nanoemulsions only resulted on small increase in the electrical conductivity of the systems. However, these effect is not significant, especially when water is entrapped in the oil phase (w/o nanoemulsion), but it becomes larger once the continuous phase is water (>40% wt. of water) [48]. In conclusion, this can be considered that there is no major differences between the vitamin E-loaded and unloaded systems at which the phase transition occurs.

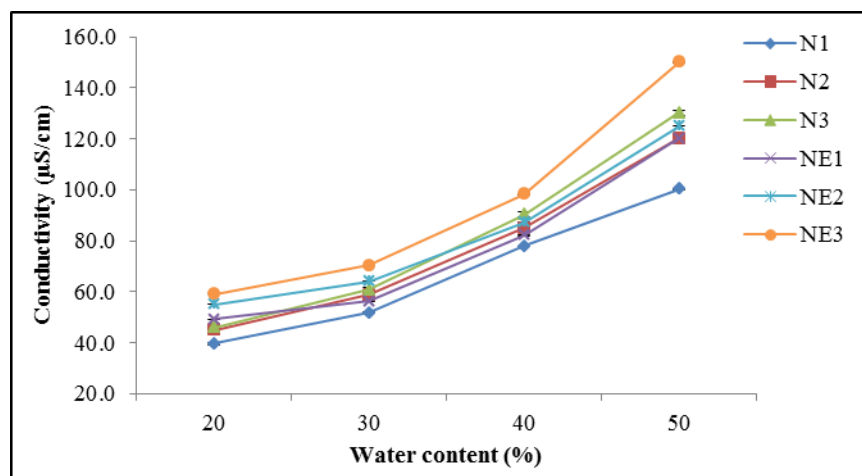


Figure 3. Electrical conductivity of Palm olein/Brij 30/Water nanoemulsion systems

Viscosity and pH

The values of pH and viscosity of the nanoemulsion system along water dilution line at 25 °C as shown in Table 2. Each data point is the mean ($\pm$ SD) of three experiments. Addition of vitamin E seem not significantly effect the viscosity of the nanoemulsion system. Plus, all systems show a very low viscosity that lies in the range of 0.04 to 0.07 Pa.S. These such low-viscosity colloidal dispersions consisting of micro domains of oil or water stabilized by an interfacial film of surfactant may be a good strategy to enhance the rate of drug delivery [49]. Most likely, the o/w nanoemulsion systems resulted on the lower viscosity compared to w/o nanoemulsion. The o/w nanoemulsion could act as drug reservoirs, where the loaded drug in the dispersed phase is released from the internal phase to the external phase and then improved the rate of drug delivery [50]. As an addition, all systems resulted on the pH values in the range of 6-7. These properties are acceptable in pharmaceutical application, for example, for the use as topical or transdermal drug delivery systems [51].

Table 2. Viscosity and pH of Palm olein/Brij 30/Water nanoemulsion systems

Formulations	Water Content (% wt.)	Viscosity (Pa.S)	pH
N1	20	0.0614 ± 0.0024	6.10 ± 0.01
	30	0.0702 ± 0.0064	6.30 ± 0.01
	40	0.0537 ± 0.0011	6.40 ± 0.01
	50	0.0460 ± 0.0000	6.40 ± 0.01
N2	20	0.0625 ± 0.0012	6.00 ± 0.01
	30	0.0451 ± 0.0001	6.20 ± 0.01
	40	0.0507 ± 0.0002	6.50 ± 0.01
	50	0.0447 ± 0.0005	6.80 ± 0.01

Table 2 (cont'd). Viscosity and pH of Palm olein/Brij 30/Water nanoemulsion systems

Formulations	Water Content (% wt.)	Viscosity (Pa.S)	pH
N3	20	0.0510 ± 0.0004	6.30 ± 0.01
	30	0.0502 ± 0.0003	6.40 ± 0.01
	40	0.0504 ± 0.0001	6.50 ± 0.01
	50	0.0496 ± 0.0002	6.80 ± 0.01
NE1	20	0.0453 ± 0.0001	6.20 ± 0.01
	30	0.0405 ± 0.0005	6.40 ± 0.01
	40	0.0461 ± 0.0009	6.50 ± 0.01
	50	0.0463 ± 0.0004	6.60 ± 0.01
NE2	20	0.0500 ± 0.0001	6.40 ± 0.01
	30	0.0506 ± 0.0002	6.50 ± 0.01
	40	0.0476 ± 0.0004	6.60 ± 0.01
	50	0.0488 ± 0.0003	6.80 ± 0.01
NE3	20	0.0525 ± 0.0001	6.30 ± 0.01
	30	0.0505 ± 0.0006	6.50 ± 0.01
	40	0.0509 ± 0.0006	6.70 ± 0.01
	50	0.0493 ± 0.0005	6.90 ± 0.01

Conclusion

Nanoemulsion with low content of surfactant were successfully formulated as a carrier of anti-aging agent. The pseudo-ternary phase diagram of nanoemulsion systems were constructed. All systems show high stability in various temperatures (4, 25 and 40 °C). Addition of vitamin E slightly changes the mean droplet size, electrical conductivity, viscosity and pH of system, however, these effects does not alter the microstructure of the system. The electrical conductivity measurement shows the transitions of these microemulsion systems from water-in-oil to bicontinuous phase without undergo phase separation. The antioxidant activity of palm olein together with vitamin E should enhanced the anti-aging activity of these nanoemulsion systems.

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