FORMULATION OF ANTI ACNE CREAM CONTAINING NATURAL ANTIMICROBIALS

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ABSTRACT

This study investigates about the production of nano-emulsion from coconut oil, lauric acid, and liposome containing lauric acid, xanthone, and nano gold using advanced technology. In addition, the antimicrobial effect of produced nano emulsion and nano gold was investigated. Using ultrasound it was possible to produce nano emulsion from lauric acid with oil droplet size of about 100 nm within 30 minutes at 45°C and 100 % amplitude. High pressure jet technique (at 3000 bars) was a suitable and fast method for production of nano emulsion using coconut oil. The droplet size of nano-emulsion was nearly independent of nozzle size (OD = 80 to 100 µm) and ranged from 185 to 240 nm. Production of gold nano-particles using Turkevich's method results in gold nano-particles of about 115 nm to 1385 nm dependent on concentration of gold solution. Atom Force Microscopy (AFM) confirmed the nano size of produced gold nano particles. In addition, the ultrasonic method was suitable to produce liposome from a mixture of lauric acid, xanthone and lecithin. The major parameter for droplet size of liposome was the concentration of lecithin in the sample. The optimum condition for liposome production using ultrasound was at 0.5 %, lecithin, 26 minutes and amplitude 90 % with average particle size of 116 nm. Xanthone concentration higher than 100 ppm was effective for the growth inhibition of *Propionibacterium acnes* (*Propionibacterium acne*). This showed that lauric acid has inhibition effect on *Propionibacterium acne* as well.

Keywords: anti acne, nano emulsion, liposome, nano-gold

INTRODUCTION

Acne is a skin disease that many people do not like. Although, acne is not a serious disease cause's mortality, it can lead to depression and lose of confidence¹. There are many causes of acne in skin such as hormone level, fat or oil in skin and a bacteria (*Propionibacterium acnes*) etc. People with acne have more *Propionibacterium acnes* in their follicles than people without acne. The control of acne could be achieved by cleaning the skin and reducing fat consumption. But the acne caused by bacteria is difficult to control. To reduce the microbial cause of acne some antimicrobial substance that inhibits the growth of microorganisms could be applied. Current treatment for acne is mostly based on antibiotics such as clindamycin and tetracycline derivatives². However, antibiotic-resistant *Propionibacterium acne* is widely spread and is a critical problem worldwide. Therefore, natural antimicrobials with anti- acne activity were of interest as an alternative treatment for acne. There are many natural antimicrobial substances such as lauric acid³ and xanthone⁴ from mangosteen peel that can inactive *Propionibacterium acne*. Many plants species including *Agelica anomala*, *Garcinia mangostana* (*G. mangostana*), *Eucommia ulmoides*, *Ailex parraguariensis* etc. have shown anti *Propionibacterium acne* effect and exhibit potential activity for acne treatment⁵⁻⁹. Recently, *G-mangostana* fruit extract is popularly used as a food supplement while the fruit rind extract has been used in herbal medicines, herbal cosmetics and pharmaceutical products for acne treatment¹⁰. *G. mangostana* and its xanthone compounds, α -mangostin, have intensive antimicrobial activity against *Propionibacterium acne*¹¹. The xanthone compounds have antibacterial activity against *S. aureus* both penicillin-resistant and methicillin-resistant strains^{12,13}. Another natural antimicrobial substance is coconut oil. Virgin coconut oil is the best natural ingredient for skin lotion available. It is skin moisturizer in cosmetic products. There is middle chain-free fatty acids (lauric acid) commonly found in coconut oil, that has an antimicrobial effect. The anti-bacterial and anti-inflammatory properties of

lauric and capric acid were reported in literature^{14,15}. Both capric and lauric acid are powerful bactericidal agents *in vitro*¹⁵. Capric acid exhibits antibacterial activity against several Gram-positive and Gram-negative bacteria¹⁶. Lauric acid exhibits significant antimicrobial and anti-inflammatory properties activities against *Propionibacterium acne*. Gold nano-particles have an effect on microorganisms because it makes the antibiotic more stable and could overcome some type of bacterial resistance¹⁷. Gold nano-particles are investigated as carriers for drugs such as Paclitaxel¹⁸. Liposomes have been extensively studied as a drug carrier since the early 1980s. They have shown great potential to act as a topical delivery system for carrying drugs and in skin care products. Liposomes can transport drugs to target sites and maintain a higher drug concentration than conventional dosage forms¹⁹. For production of high quality anti-acne cream, it may be necessary to apply new technology such as nanotechnology. There is a large amount of evidence confirming the positive effects of using nano-materials and nanotechnologies in health care product development. In this project we have applied nanotechnology for production of anti-acne cream and soap containing lauric acid nano-emulsion, gold nano-particle, liposome containing mangosteen peel extract and lauric acid. Furthermore the antimicrobial effect of developed products was examined.

MATERIAL AND METHODS

Materials

Xanthone was extracted from mangosteen peel powder using ethanol (95 %, 1 part mangosteen: 20 part ethanol). The concentration of xanthone in ethanolic extract was 6311 ppm. Lecithin used in this study was soya lecithin. Thin strip gold with purity of about 95 % was used for Nano-gold production. All other chemicals used in this study were analytical grade.

Equipment

Ultrasonic treatment of samples was by an ultrasound (Bandlin, Model HD 3200, Germany) with max. power of

200 W (at 100 % amplitude) and max. Amplitude of 137 μm was applied. The diameter of the sonotrode tip was 1.3 cm. The treatment chamber was equipped with a heating jacket to adjust temperatures during treatment.

Experimental Methods

For preparation of lauric acid nano emulsion was the mixture of lauric acid, Tween- 80 and water (0.5 %, 0.5 %, 99 % w/w respectively) homogenized by means of rotor-stator mixer (20,000 rpm, 5 minutes). After that, using Box Behnken design surface methodology the prepared mixture was treated with ultrasonic at different process conditions (Table 1) to determine the effect of process parameters on droplet size. For preparation of liposome containing lauric acid and xanthone, firstly 174 ml of ethanolic extract of mangosteen peel (6311 ppm xanthone concentration) was evaporated using a vacuum evaporator (175 mbar and 45°C) until removal of the ethanol. After that 2.2 g of melted lauric acid (about 50°C) was added to the xanthone and mixed. This mixture was used to prepare a solution containing 0.5 % w/w lauric acid containing xanthone, lecithin (0.5 % to 1.5 % w/w). Water (99.0 % to 98 % w/w) was added to the mixture. Finally the effect of ultrasonic conditions (amplitude and treatment time) and lecithin concentration (0.5; 1 and 1.5 %) on liposome production was investigated using Box Behnken surface methodology (Table 2). For preparation of HAuCl_3 using Turkevich method²⁰ the solid gold (purity 95 %) was dissolved in a mixture of nitric acid (10 ml) and concentrated hydrochloric acid (30 ml) and heated until no brown smock occurred. 10 ml of prepared HAuCl_3 solution was added into 50 ml boiling distilled water followed by adding 0.6 ml of

sodium citrate (1 % concentration). The boiling was continued until color changes (from yellow to purple and to red) occur. Finally the pH of sample was adjusted to pH 6.8 using 7.7 mM NaOH. Anti-acne cream and soap was formulated using recipes in Table 3 and 4. Finally the antimicrobial substances (xanthone, lauric acid nano-emulsion, gold nano-particle) were added into the cream and soap.

Analytical Methods

Measurement of droplet size in nano-emulsion and liposome was carried out using Nano-sizer equipment (Nano-zs, Malvern, UK). The samples were diluted up to 100 times (using bi-distilled water) before each measurement. The droplet size distribution as well as average droplet size was calculated. For measurement of particle size in gold nano-particle sample an Atom Forced Microscope (AFM) was applied. For Investigation of anti-acne activity of prepared nano-emulsion and liposome, agar nutrient containing Beef Extract:Peptone:Water: Agar (5 g : 3 g : 1000 ml : 15 g respectively) was prepared and then sterilized in an autoclave at 121°C for 15 minutes. After that the sterile nutrient was added to the sterile plates and stored for 1 day before incubation of bacteria (*Propionibacterium acne*) on the agar nutrient plate. After that about 100 μm of microorganism (*Propionibacterium acne*) was spread on the agar nutrient plate and then sterile filter paper containing test materials (nano-emulsion, xanthone, nano-gold) was placed on the agar media. Finally the plate was incubated at 37°C for about 2 to 3 days.

Table 1: The Box-Behnken Design for Ultrasonic Treatment of Lauric Acid Emulsion Containing 0.5 % Lauric Acid, 0.5 % Tween- 80 And 99 % Water

Number	X1 = Time (min)	X2 = Temperature (°C)	X3 = Amplitude (%)
1	5 (-1)	45 (-1)	60 (0)
2	15 (+1)	45 (-1)	60 (0)
3	5 (-1)	75 (+1)	60 (0)
4	15 (+1)	75 (+1)	60 (0)
5	5 (-1)	60 (0)	30 (-1)
6	15 (+1)	60 (0)	30 (-1)
7	5 (-1)	60 (0)	90 (+1)
8	15 (+1)	60 (0)	90 (+1)
9	10 (0)	45 (-1)	30 (-1)
10	10 (0)	75 (+1)	30 (-1)
11	10 (0)	45 (-1)	90 (+1)
12	10 (0)	75 (+1)	90 (+1)
13	10 (0)	60 (0)	60 (0)
14	10 (0)	60 (0)	60 (0)
15	10 (0)	60 (0)	60 (0)

Table 2: The Box-Behnken Experimental Design for Liposome Preparation using Ultrasonic (Treatment Temperature = 60°C Constant)

Number	X1 = Lecithin (% w/w)	X2 = Time (min)	X3 = Amplitude (%)
1	0.5 (-1)	10 (-1)	60 (0)
2	1.5 (+1)	10 (-1)	60 (0)
3	0.5 (-1)	30 (+1)	60 (0)
4	1.5 (+1)	30 (+1)	60 (0)
5	0.5 (-1)	20 (0)	30 (-1)
6	1.5 (+1)	20 (0)	30 (-1)
7	0.5 (-1)	20 (0)	90 (+1)
8	0.5 (-1)	20 (0)	90 (+1)
9	1.0 (0)	10 (-1)	30 (-1)
10	1.0 (0)	30 (+1)	30 (-1)
11	1.0 (0)	10 (-1)	90 (+1)
12	1.0 (0)	30 (+1)	90 (+1)
13	1.0 (0)	20 (0)	60 (0)
14	1.0 (0)	20 (0)	60 (0)
15	1.0 (0)	20 (0)	60 (0)

Table 3: Formulation of Anti Acne Soap

Chemical substance	Concentration (g)
Transparent bar soap	500
Amphitol 55 AB	100
Nicotinamide (vitamin B3)	0.5
DL-phenanthol (vitamin B5)	10
Glycerin	50
ISELUX LQ-CL RSB	12.5
Propylene glycol	50

Table 4: Formulation of Anti Acne Cream

Chemical substance	Concentration (%)
Cremophor A6	2.4
Cremophor A25	2.4
Triethanolamine	0.36
Water	80.34
Oil	6
Vitamin E	1.98
Vitamin B3	1.5
Vitamin B5	1.5
Vitamin A	0.025

Table 5: Effect of Gold Concentration on Particle Size of Nao-Gold Particle

Water (ml)	Na ₃ C ₆ H ₅ O ₇ (ml)	HAuCl ₄ (ml)	Concentration (ppm)	Particle size (nm)
				Average
50	16	30	112.5	115.3
25	16	30	152.11	213.85
12.5	16	30	184.61	238.3
-	16	30	234.78	1384.75

Figure 1: Atom Force Microscopy of gold nano-particle (data from phase at scan size 1 µm)

Figure 2: Effect of temperature and amplitude on lauric acid droplet size at constant treatment time of 15 minutes (concentration of lauric acid 0.5 % and Tween-80 0.5 %)

Figure 3: Effect of time and temperature on the oil droplet size at constant amplitude 90 %

Figure 4: Effect of time and amplitude on liposome droplet size at constant percent of lecithin concentration (0.5 %)

Figure 5: Effect of lecithin concentration on particle size of liposome (constant time 30 minutes)

Figure 6: Prepared soap containing gold nano-particle (top) and xanthone (bottom)

Figure 7: Effect of xanthone soap (298 ppm) and nano-gold soap (19 ppm) against *Propionibacterium acne* growth

Figure 8: Effect of anti-acne cream against *Propionibacterium acne* growth

RESULTS AND DISCUSSIONS

Effect of gold nanoparticles concentration on particles size

Preparation of nano gold from HAuCl_4 solution by adding sodium citrate was sensitive to the added water in the solution during reaction (Table 5). At higher amounts of added water (50 ml water, 30 ml HAuCl_4 solution and 16 ml sodium citrate solution) the particle size of produced nano-gold was very small (115 nm) and the color of prepared nano-gold solution was intensive red. Decreasing the amount of added water led to larger size of nano-gold (215 to 238 nm). In the case of not adding water to the reaction solution the nano-gold particle was very large (1384 nm) and the gold inside the solution tended to fast self assembling and formation of large and visible gold particles. From these results it is necessary to add at least 50 ml water in the reaction solution (30 ml and 16 ml sodium citrate) to ensure producing nano-gold particles with particle size about 115 nm. The color of the gold nano-particle solution was dependent on the size of the nano-gold particles and changed from red-purple (for 115 nm particle size) to purple-gray (for particle size larger than 200 nm). Figure 1 shows the atom forced microscopy of produced gold nano-particles.

Ultrasonic treatment of lauric acid for production of nano-emulsion

The effect of different process parameters (time, temperature and amplitude) on the droplet size of ultrasonically treated lauric acid was investigated. Increasing treatment time from 5 to 15 minutes and ultrasonic power from 30 to 90 % led to smaller oil droplet size. The effect of process temperature on droplet size was insignificant. After 15 minutes ultrasound treatment time at 90 % amplitude droplet size down to 80 nm was observed (Figure 2 and 3). From equation 1, the optimum condition for production of nano-emulsion from lauric acid using ultrasonic was time = 14 minutes, temperature = 45°C and Amplitude = 73 %

$$Y = 85.3 - 5.4t + 0.94T - 0.65A + 0.46t^2 - 0.0018T^2 + 0.03A^2 + 0.126t * T - 0.18t * A - 0.019T * A \quad (1)$$

t = time, T = Temperature, A = Amplitude and
Y = Responds (Oil droplet size)

Ultrasonic treatment for liposome nanoparticle emulsion

Process parameters (time and amplitude) as well as lecithin concentration (0.5 to 1.5 %) showed an effect on liposome droplet size. Increasing the treatment time and amplitude decreased the droplet size down to 112 nm (Figure 4). The effect of amplitude trends to the same direction. Low (0.5 %) or high concentration (1.5 %) lecithin showed a positive effect on droplet size reduction during ultrasound treatment. In contrast at lecithin concentration of 1 % and moderate amplitude a reverse effect of lecithin concentration on droplet size was observed (Figure 5). From the equation, the optimum condition for liposome production was 0.5 % lecithin, time = 26 minute and amplitude = 90 %

$$Y = 18.08 + 281L - 1.8t + 0.27A - 155.11L^2 + 0.038t^2 - 0.0026A^2 + 1.13L * t + 0.4L * A - 0.0082t * A \quad (2)$$

L = Lecithin, t = time, A = Amplitude and
Y = Responds (liposome droplet size)

Anti-acne cream and soap

The prepared anti acne soap (Figure 6 and 7) containing xanthone (298 ppm in soap) and gold nano-particle (19 ppm in soap) have shown antimicrobial effect against *Propionibacterium acne*. Whereas the anti acne effect of soap

containing xanthone was obvious, the anti acne effect of nano-gold soap was weak (Figure 7). This may be because of low concentration of nano-gold particles in the soap. Interestingly, the prepared anti-acne creams (receipt 2 and 3) were suitable for inhibition of *Propionibacterium acne* microorganisms. Increasing lauric acid-xanthone concentrations in cream from 2 to 5 % obviously increased the anti acne effect of this cream (Figure 8).

CONCLUSION

The results of this study have indicated that it is possible to produce nano emulsion from lauric acid using ultrasound technique. The droplet size of emulsion was dependent on the process parameters (amplitude, time, temperature). Increasing the amplitude, as well as time led to a decrease in droplet size. Preparation of liposome containing lauric acid and xanthone using ultrasonic have shown that by increasing the treatment time and amplitude the droplet size will be drastically decreased. The lecithin concentration of 0.5 % was optimal for production of liposome with particle size of about 116 nm. Using the Turkevich method it was possible to prepare nano-gold solution. Nano-gold particle size down to about 120 nm was produced by means of this method. The formulated anti acne soap (containing nano-gold, Xanthone) and cream (containing 2 and 5 % lauric acid-xanthone) was effective in inhibiting the growth of *Propionibacterium acne* microorganisms on an agar plate.

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