FORMULATION AND OPTIMIZATION OF CAFFEINE NANOEMULSION USING FACTORIAL DESIGN STUDY

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INTRODUCTION

Caffeine was an alkaloid commonly used as active ingredient in cosmetics due to its high biological activity for skin health as anticelulit, sunscreen, antioxidant, and photoaging inhibitor [6]. Caffeine had potential antioxidant properties that could protect cells against the UV radiation induce carcinogenesis with stimulatory effect on apoptosis in the UVB-treated epidermis cell in mice skin [8]. High hidrophilic properties of caffeine (log P = -0.07) caused difficulties to penetrate through the stratum corneum of human skin [9]. Nanoemulsion was a drug delivery system that had good stability and could potentially increase penetration of the drug through the skin that had particle size approximately 1 – 100 nm. Oil phase, aqueous phases, and surfactant are basic component of nanoemulsion [11]. Surfactant was the important compound in preparation of nanoemulsion. Type, amount of surfactant, and HLB value of surfactant can influence nanoemulsion stability [1]. Isopropyl miristate (IPM) was chemical penetration enhancer (CPE), was added in skin drug formulation to incrase the penetration ability of the drug [3]. This study aimed to investigate the effect of hydrophile lipophile balance (HLB) value of surfactant combination (tween 80 and span 80) and IPM concentration on the stability and penetration ability of nanoemulsion using factorial design method and obtain the optimum formula of caffeine nanoemulsion using Design Expert 9.0 analysis.

MATERIAL AND METHODS

Material

Caffeine (PT. Bratachem), tween 80 (PT. Bratachem), span 80 (PT. Bratachem), benzil alkohol (Sigma Aldrich), aquadest, and buffer saline phospat pH 7,4 ± 0,05.

Design Factorial

This study uses two factor and two level factorial design. The factors were HLB value of combination surfactant and IPM concentration in high and low level. Respon that was obseved in this resarch was stability and penetration ability of cafeine nanoemulsion. Level of the factor was determined by a preliminary test and literature studies, shown in Table 1.

Table 1. Design of factor and level of nanoemulsion formula

Factor	Low level (-1)	High level (+1)
HLB value	12	15
IPM consentration	1 %	10 %

Formula and Preparation Caffeine Nanoemulsion

Four formula caffeine nanoemulsion were shown in Table 2.

Table 2. Formulas of caffeine nanoemulsion

Ingredient	Function	Formula of Nanoemulsion			
		1	A	B	AB
Caffeine	Active ingredien t	1 g	1g	1g	1g
Benzyl Alkohol	Oil phase	10 mL	10 mL	10 ml	10 mL
Tween 80	Surfactan	25,2 mL	35 mL	25,2 mL	35 mL
Span 80	Surfactan	9,8 mL	-	9,8 mL	-
Isopropyl Myristate	CPE	1 mL	1 mL	10 mL	10 mL
Aquadest	Water phase	Ad 100 mL			

Caffeine nanoemulsion was prepared by dissolving the caffeine into benzyl alcohol, stirred using a magnetic stirrer on a hot plate at 50° C, then adding a surfactant mixture tween 80 and span 80, stirred until homogeneous, then added IPM and recently added distilled water. The process of stirring for 30 minutes to produce a clear and homogeneous nanoemulsion.

Stability Testing

Stability test was conducted on four formula nanoemulsion with the heating cooling cylce method. Caffeine nanoemulsion was stored in 40° C oven for 24 hours and then stored in the closet pendingn 4° C for 24 hours (1 cycle). The tests was performed of 6 cycles (12 days). the stability was determined by measuring pH and viscosity friction before and after the test using pH meter and viscometer.

In Vitro Skin Penetration

In vitro skin penetration studies across rat skin were performed using Franz diffusion cell. Abdominal skins were obtained from male albino wistar rats aged 2-3 months weighing 150 ± 20 g. Acceptor compartment was fulfilled with buffer saline phosphate pH 7.4±0.05 and the donor compartment was filled with 3 ml of caffeine nanoemulsion. Franz diffusion cell was placed on a hot plate at 37° C. Samples were taken at minute 0, 15.30, 25, 60, 90, 120, 180, 240, 300, 360, 420, and 480 about 3 mL and replaced with 3 mL of buffer saline phosphate pH 7.4 ± 0, 05. Caffeine content in the samples was determined using UV-Vis spectrophotometer instrument at a wavelength of 273 nm. Penetration flux was slope values of cumulative level of caffeine which penetrated to time.

Data Analysis

Flux penetration, pH friction, and viscosity friction datas were analyzed by using Design Expert 9.0 softwere to determine the effect of HLB value of surfactant combination and IPM concentration against flux pentration, viscosity and pH that was shown by the general equation Y = b0 + b1XA + b2XB +b12XAXB. Design Expert 9.0 also generate recommendations optimum formula of caffeine nanoemulsion that has good pentration ability with high flux penetration and good stability with smallest friction of viscosity and pH.

Characteristic Nanoemulsion

Organoleptic properties of caffeine nanoemulsion were observed visually in color, odor, homogenity and clarity. Characterization of nanoemulsion used PSA (Particle Size Analyzer) SZ- 100 to measure zeta potential, particle size and distribution of particles nanoemulsion. Morphology of droplet nanoemulsion was observed by using TEM (Tramspission Electron Microscope).

RESULT AND DISCUSSION

Preparation Caffeine Nanoemulsion

Four formula of caffeine nanoemulsions produce transparant and homogenous pale yellow solution with pungent odor that was shown in Figure 1.

The result of analysis were general equation and two demension contour plot for every respon. Contour plot show the effect of HLB value of combination surfactant and IPM consentration in respon pH friction, viscosity friction, and flux penetration with different colour, red area showed maximum effect prediction and blue area showed minimum effect prediction.

Figure 1. Caffeine nanoemulsion had pale yellow color, transparant and homogenous. F(1) HLB 11, IPM 1%; F(A) HLB 15, IPM 1 %; F(B) HLB 11, IPM 10%; F(AB) HLB 15, IPM 10%.

Analysis with Design Expert 9.0

Viscosity and pH friction datas were obtained from stability testing and flux penetration datas were obtained from in vitro penetration testing were analyzed using Design Expert 9.0. The result of stability and in vitro penetration testing were shown in Table 2.

Table 2. Result of stability and in vitro penetration testing

F	R	Factor		Respon		
		A	B	Viscosity friction	pH friction	Penetration Flux (μg/cm².menit)
F (1)	1	-1	-1	1,10	0,08	3,801
	2			1,40	0,23	3,659
	3			1,10	0,27	3,179
F (A)	1	+1	-1	0,70	0,06	3,960
	2			0,30	0,12	3,650
	3			0,20	0,06	4,059
F (B)	1	-1	+1	1,50	0,14	7,674
	2			0,70	0,04	7,737
	3			0,80	0,06	7,941
F (AB)	1	+1	+1	0,30	0,01	4,994
	2			0,20	0,02	5,234
	3			0,20	0,01	5,234

F: formula, R: replication, A: HLB value of surfactant combination factor, and B: IPM consentration factor.

Viscosity and pH friction showed stability of nanoemulsion. Caffeine nanoemulsions with the lowest pH and viscosity friction has good stability. pH friction respon had general equation Y = 0,092 – 0,045 XA -0,045 XB+ 0,012 XAXB and contour plot that was shown in Figure 2.

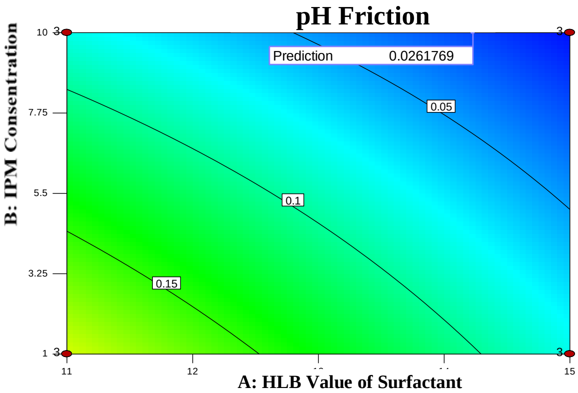


Figure 2. Two dimensions contour plot of pH friction respon. The blue area showed low pH friction and the yellow area showed high pH friction.

Viscosity friction respon had general equation Y = 0,70 – 0,40 XA -0,079 XB+ 0,013 XAXB and contour plot that was shown ini Figure 3.

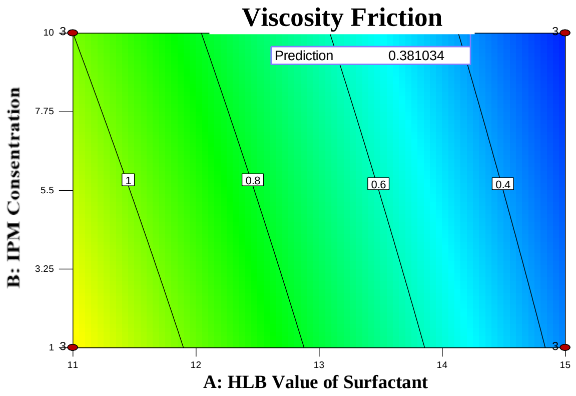


Figure 2. Two demensions contour plot of viscosity friction respon. The blue area showed low viscosity friction and the yellow area showed high viscosity friction.

Penetration flux respon had general equation Y = 5,02 – 0,61 XA +1,52XB -0,65 XAXB and contour plot that was shown in Figure 4.

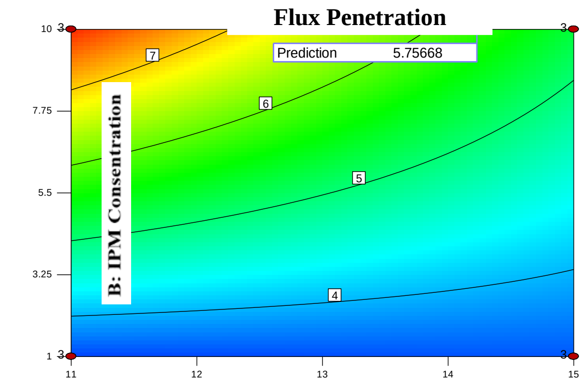


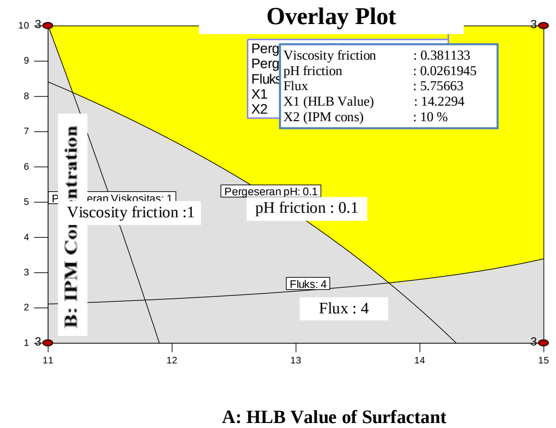
Figure 2. Two de[...] of flux penetration respon. The blue area showed low flux penetration and the yellow area showed high flux penetration.

Higher HLB value of surfactant combination could decrease pH and viscosity friction, decrease flux penetration, that produced more stabil caffeine nanoemulsion but had low penetration ability. Higher IPM consetration could increase flux penetration, pH friction and viscosity friction, produced low stability caffeine nanoemulsion, but had good penetration ability. Intraction of HLB value and IPM cosntration could increase pH and viscosity friction, and decrease flux penetration.

HLB value of surfactant combination tween 80 and span 80 had higher effect to increase stability of caffeine nanoemulsion becasue surfactant had important role to form stabil nanoemulsion by lowering surface tension between oil and aqueous phases, so the oil droplet could disperse uniformly in aqueous phase. Higher HLB value of surfactant combination cause impairment of flux penetration, caused by surfactant tween 80 which has hydrophilic properties that would reduce the ability to penetrate the skin barrier membrane [2]

High consentration of IPM generate high penetration flux that showed good penetration ability of caffeine in nanoemulsion. IPM was an chemical penetration enhancer that could increase the penetration ability of drugs to penetrate through the barrier stratum corneum. IPM's mode of action as penetration promoter was presumably based on incorporation into the stratum corneum lipid matrix, extraction of certain stratum corneum lipids into a separate phase and perturbation of the multilamellar lipid assembly [4].

The analysis using Design Expert 9.0 resulted 19 recommendations optimum formula of caffeine nanoemulsion that was in the yellow area in the overlay plot, shown in Figure 5.



The optimum formula was determined based on the predicted value of viscosity friction, pH friction, and flux penetration. Formula optimum had lowest viscosity and pH friction showed good stability of nanoemulsions, and highest flux penetration showed good penetration ability. Optimum caffeine nanoemulsion formula had an HLB value of surfactant combination of 14.22 and 10% IPM concentration with highest desirability values of 0.760. This optimum formula was predicted to have a viscosity friction of 0.03811, pH friction of 0.02619, and flux penetration of 5.75663 g/ cm^2.menit.

Characteristic Nanoemulsion

Optimum formula of caffeine nanoemulsion was prepared and charactherized to get physical and chemical properties of caffeine nanoemulsion that were shown in table 4.

Caffeine nanoemulsion had particle size 43 nm and had good homogenity of particle size. The droplet surface of nanoemulsion had same electrical charge, called zeta potential. Zeta potential value of the caffeine nanoemulsion was influenced by the type and amount of surfactant .The combination of nonionic surfactant could cover the surface of nanoemulsion droplets caused lower charges of nanoemulsion droplet surface and increased attraction between particles. Index polydispers (IP) showed the distribution of particle size. there were two type of particle size distribution, monodispers (IP= 0,01-0,7) and polydispers (IP= above 0,7). Caffeine nanoemuslion had monodispers type (IP= 0,572), showed that the droplet size of nanoemulsion was homogent [10].

Physicochemical Properties	Result
Type of nanoemulsion	Oil in water (O/W)
pH	5,84 ± 0,04
Viscosity	1,07 ± 0,12 dPas
Spesific gravity	1,030 ± 0,005 g/mL
Caffeine content	105,93% ± 2,79 %
Particle siz	43 nm
Particle distribution	Monodispers (IP = 0,572)
Zeta potential	- 0,2 mV
Particle morphology	Spheris

Th result of morphology analysis using TEM showed that nanoemulsion has spherical droplet. Based on the characteristic obeservation used TEM, showed that the center of droplet nanoemulsion was filled by caffeine and was covered by oil phase that was dispersed in aquouse phase. Spherical droplet shape is the most stable form because they has low surface interactions between droplets that could prevent aggregation of droplet [7]. Particles with spherical shape was able to control the release of active substances, prolonged the action of drugs, and reduced the side effects of drugs [6]. Morphology on nanoemulsion was shown in Figure 5.

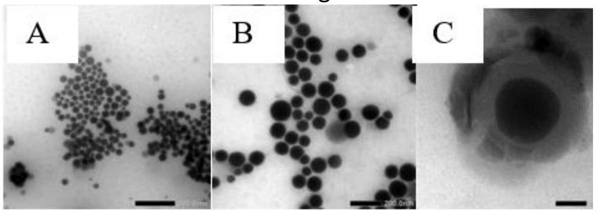


Figure 6. The droplet morphology of nanomulsion with a magnification of (A) 12.000X , (B) 25.000x, and (C) 80.000x. Droplet nanoemulsion had a spherical shape (round).

CONCLUSIONS

HLB value of surfactant combination and IPM concentration had a significant influence on viscosity friction, pH friction, and flux penetration that involved stability and penetration ability of nanoeulsion. Optimum formula of caffeine nanoemulsion had HLB value of 14.22 and IPM concentration of 10%. Caffeine nanoemulsion had a spherical droplet, pH of 5.84, specific gravity of 1.030 g/ml, viscosity of 1.07 dPas, particle size of 43 nm, monodisperse particle distribution with polydisperse index (PI) of 0.572, and the zeta potential of -0.2 mV.

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