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PRAFORMULATION STUDY OF *p*-METHOXYCINNAMIC ACID (PMCA) NANOEMULSION USING VEGETABLE OILS (SOYBEAN OIL, CORN OIL, VCO)

Tristiana Erawati M., Esti Hendradi, Widji Soeratri

Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

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Abstract

Objective: This study aims were to obtain the praformulation data of *p*-methoxycinnamic acid (PMCA) (solubility in buffer; apparent partition coefficient between buffer and vegetable oils), to determine the fatty acids content and screening HLB needs of each oils as well as to determine the composition of nanoemulsion using these oils, surfactants (Tween 80 and Span 80) and ethanol 96% as cosurfactant.

Methods: Solubility test of PMCA in acetate buffer pH 4.2±0.2 were conducted with shakes and followed by measuring PMCA amount dissolved by spectrophotometer. To determine the PMCA apparent partition coefficients between buffer pH 4.2±0.2 and soybean oil, corn oil, VCO, respectively, PMCA dissolved in buffer then oil that has been saturated with buffer were added. Firstly by shaking followed by centrifugations than PMCA remained amount in the buffer was measured by spectrophotometer. Determination of fatty acid contents in the oils was used GC-MS while screening HLB was carried by various compositions of surfactants and cosurfactant to obtain a clear emulsion (nanoemulsion).

Results: From the results of this study, the solubility of PMCA in acetate buffer pH 4.2±0.2 was 70.04±0.66 mg/L. PMCA apparent partition coefficient in buffer pH 4.2±0.2 and soybean oil, corn oil, and VCO, respectively were 2.39; 2.38 and 2.41. The main contents of soybean oil fatty acids were 19.57% palmitic acid, 45.2% linoleic acid, 25.36% elaidic acid, and 7.07% stearic acid. The main contents of corn oil fatty acids were 26.86% palmitic acid, 31.52% oleic acid, 31.30% elaidic acid and 4.68% stearic acid. The main contents of VCO fatty acids were 32.41% lauric acid, 24.15% myristic acid, 15.68% palmitic acid, 2.29% linoleic acid, 11.06% elaidic acid and 5.22% stearic acid. These oils HLB needs was 14 and the ratio of surfactant - cosurfactant which can formed a clear emulsion (nanoemulsion) was 6:1

Conclusion: 1) To produce nanoemulsion with soybean oil, corn oil, and VCO as oil phase HLB needs was 14 and the ratio of surfactant and cosurfactant was 6:1. 2) Nanomulsions with soybean oil and corn oil more turbid than nanoemulsion with VCO.

Key words: PMCA, solubility, apparent partition coefficient, fatty acids, soybean oil, corn oil, VCO, HLB needs.

INTRODUCTION

One of the drug delivery system that has been known to increase the solubility and penetration of the drug is nanoemulsion system. Nanoemulsion system consists of a water phase, oil phase, surfactant and cosurfactant [1]. Increased penetration of the system is due to the increasing amount of material in the form of molecular medicine so that differences in the concentration of the drug

outside and inside skin which can be greater as the driver of the penetration process, in addition to the amount of surfactant and cosurfactant high enough to serve as an enhancer [2]. Another advantage of this system is that the spontaneous creation without the need for heating and vigorous stirring. To increase the use of natural resources and improve the products of traditional medicine into modern medicine, the nanoemulsion delivery system is applied to the p-methoxycinnamate acid (PMCA). In order to attempt the development of PMCA as topical anti-inflammatory preparations on nanoemulsion system utilizing plant oils (soybean oil, corn oil, VCO). PMCA is a component of the *Kaempferia galangal* rhizome, widely used as a traditional medicine (Jamu) [3]. Kaempferia galangal rhizome by Javanese traditional herb used in a formula named "bobok" blend with oryza sativa. The formula function removes the body pain due to sprains, exercise or heavy work. To formulate a topical preparation is needed praformulasion data, then in this research study involves determining solubility and partition coefficient of PMCA, determine the fatty acid content and screening HLB need of vegetable oil and to get a comparison of surfactant-cosurfactant which can produce a clear (translucent) emulsion.

METHODS

These research started by qualitative analysis of PMCA by *IR JASCO FT/IR-5300 Instrument* and *Differential Thermal Analysis* (DTA), than determination of λ maximum and PMCA standard curve in acetate buffer pH 4.2±0.2 followed by solubility test of PMCA in acetate buffer pH 4.2±0.2. Solubility test of PMCA in acetate buffer pH 4.2±0.2 were conducted by shaking

for 4 hours at 150 rpm followed by measuring PMCA amount dissolved by spectrophotometer. To determine the PMCA apparent partition coefficients between buffer pH 4.2±0.2 and soybean oil, corn oil, VCO, respectively, PMCA (50 ppm and 60 ppm) was dissolved in 10.0 mL acetate buffer pH 4.2±0.2 then oil (0.5; 1.0 and 2.0 mL) that has been saturated with acetate buffer pH 4.2±0.2 were added. Firstly by shaking for 2 hours at 150 rpm, followed by centrifugations for 15 minutes at 2200 rpm than PMCA remained amount in the buffer was measured by spectrophotometer. Determination of fatty acid contents in the oils was used GC-MS while screening HLB was carried by various combinations of surfactants (Tween 80 and Span 80 to produce HLB 10,11,12,13 and 14). To obtain a clear emulsion one portion of oil added with nine portion of combination of surfactant and cosurfactant (1:1 ; 2:1; 3:1 etc. …. 9:1), than added slowly with acetate buffer pH 4.2±0.2.

RESULTS AND DISCUSSIONS

The result of PMCA qualitative test by *IR JASCO FT/IR-5300 Instrument* between wave's numbers 450 – 4000 cm^{-1} that found the infrared spectra as showed Figure 1 and PMCA melting temperature (174.4^{o}C) by *Differential Thermal Analysis* (DTA) showed in Figure 2.

Results of the determination of the maximum wavelength of PMCA in acetate buffer solution of pH 4.2±0.2; the PMCA maximum wavelength obtained in acetate buffer pH 4.2±0.2 was 301 nm. Regression equation of the relationship curve between the absorbance vs. PMCA concentration obtained y = 0.1063x - 0.0014 with a correlation coefficient r = 0.9999.

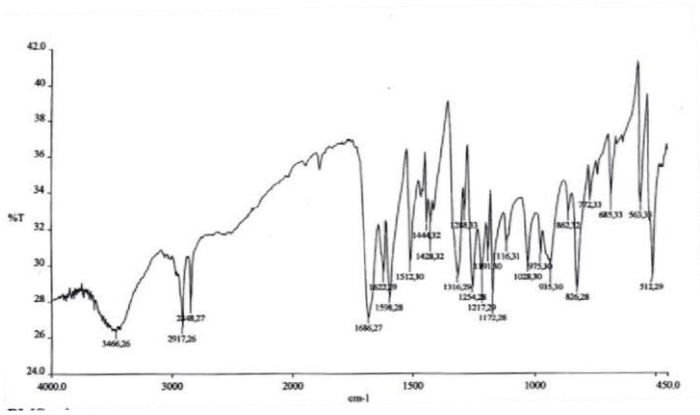

Fig.1; PMCA infrared spectra between waves number 450 – 4000 cm^{-1} by *IR JASCO FT/IR-5300 Instrument.*

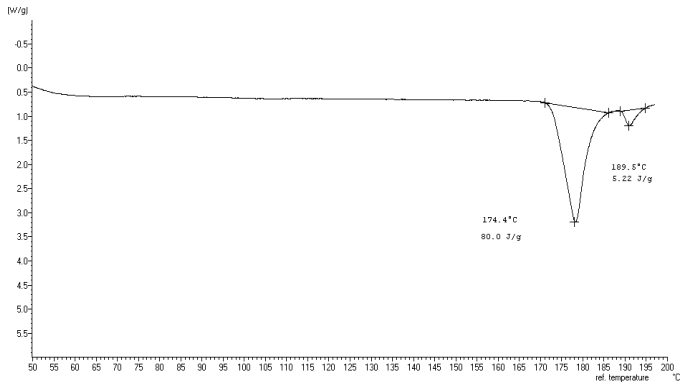

Fig.2; PMCA melting temperature (174.4°C) by *Differential Thermal Analysis* (DTA)

PMCA solubility determination in acetate buffer solution of pH 4.2±0.2 begins with the timing of PMCA saturated solubility in acetate buffer solution of pH 4.2±0.2 can be seen in Figure 3. and the results of ANOVA test is known all PMCA concentration at 4, 5, 6, 7 and 8 hour did not differ significantly so that it can be concluded time its saturation solubility at 4th hour. While the results of the determination of the PMCA solubility in acetate buffer pH 4.2±0.2 during 4th hour was 70.04±0.66 mg/L can be seen in Table 1.

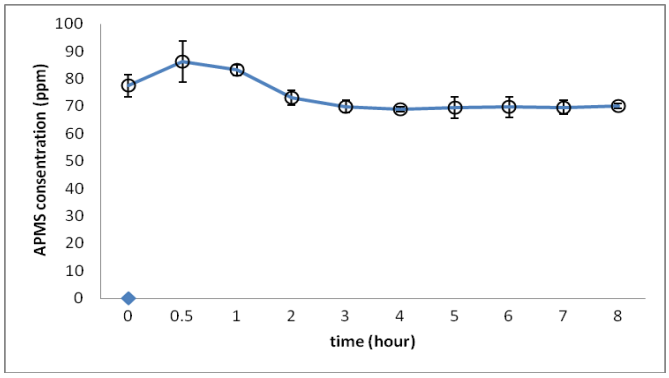

Fig.3; Correlation curve between PMCA concentrations (ppm) in acetate buffer pH 4.2 ± 0.2 vs. time (hour)

Table 1; The PMCA solubility in acetate buffer pH 4.2±0.2

SOLUBILITY OF PMCA IN ACETATE BUFFER pH 4.2 ±0.2	
Replicate	CONSENTRATION (mg/L)
1	69.69
2	69.44
3	70.54
4	68.99
5	71.10
6	70.45
mean ± SD	**70.04 ± 0.66**

The determination results apparent partition coefficient of PMCA in acetate buffer pH 4.2±0.2 and each oil (soybean oil, corn oil and VCO) with a ratio of 10:0.5 ; 10:1.0 and 10:2.0 and the concentration of the acetate PMCA buffer pH 4.2±0.2 at 50 and 60 ppm can be seen in Table 2. From Table 2 known apparent partition coefficients of PMCA in acetate buffer pH 4.2±0.2 and soybean oil, corn oil, VCO were 2.39±0.11; 2.38±0.12 and 2.41±0.13 respectively.

Table 2; PMCA apparent partition coefficient in acetate buffer pH 4.2±0.2 and each oil (soybean oil, corn oil, and VCO)

OIL	BUFFER : OIL RATIO (mL)	PMCA APPARENT PARTITION COEFFICIENT CONSENTRATION IN BUFFER						MEAN ± SD
		50 ppm			60 ppm			
		1	2	3	1	2	3	
SOYBEAN OIL	10 : 0.5	2.53	2.52	2.51	2.57	2.55	2.56	2.54
	10 : 1.0	2.36	2.33	2.34	2.38	2.39	2.37	2.36 2.39±0.11
	10 : 2.0	2.24	2.29	2.26	2.21	2.33	2.31	2.27
CORN OIL	10 : 0.5	2.52	2.54	2.56	2.55	2.55	2.54	2.54
	10 : 1.0	2.38	2.36	2.37	2.38	2.39	2.36	2.37 2.38±0.12
	10 : 2.0	2.29	2.28	2.29	2.16	2.16	2.16	2.22
VCO	10 : 0.5	2.51	2.48	2.46	2.69	2.64	2.62	2.57
	10 : 1.0	2.39	2.39	2.42	2.48	2.49	2.51	2.45 2.41±0.13
	10 : 2.0	2.25	2.26	2.29	2.33	2.28	2.29	2.28

Results of the determination of fatty acid content of soybean oil, corn oil and VCO using GC-MS can be seen in Table 3. From Table 3 it can be seen that the highest levels of soybean oil fatty acids was 45.20% linoleic acid (C18:2), the highest levels of corn oil fatty acids was 31.52% oleic acid (C18:1/-*cis*) and 31.30% elaidic acid (18:1/-*trans*), while the highest fatty acid levels VCO is 32.41% lauric acid (C12).

Table 3; The main contents of soybean oil, corn oil, and VCO fatty acids.

FATTY ACIDS MAIN CONTENTS OF VEGETABLE OIL					
SOYBEAN OIL		CORN OIL		VCO	
FATTY ACID	CONC. (%)	FATTY ACID	CONC. (%)	FATTY ACID	CONC. (%)
palmitic acid (C16)	19.57	palmitic acid (C16)	26.86	lauric acid (C12)	32.41
linoleic acid (C18:2)	45.20	oleic acid (C18:1) *cis*	31.52	myristic acid (C14)	24.15
elaidic acid (C18:1) *trans*	25.36	elaidic acid (C18:1) *trans*	31.30	palmitic acid (C16)	15.68
stearic acid (C18)	7.07	stearic acid (C18)	4.68	linoleic acid (C18:2)	2.29
				elaidic acid (C18:1) *trans*	11.06
				stearic acid (C18)	5.22

Screening of oils HLB needs and determination of surfactants (combination surfactants Tween 80 - Span 80):cosurfactant (ethanol) ratios were done by ratio 1:1 until 9:1 and combination surfactants Tween 80-Span 80 at HLB 10, 11, 12, 13 and 14. That result known at surfactant combination with HLB 10, 11 and 12 the emulsion using all oils turbid, at surfactants combination with HLB 13 emulsion using soybean oil and corn oil were turbid, while emulsion using VCO start from surfactant and co-surfactant ratio 3:1 produced clear emulsion. At surfactants combination with HLB 14 emulsion using soybean oil and corn oil appear translucent start from surfactant and cosurfactant ratio 5:1, while emulsion that used VCO start from surfactant and cosurfactant ratio 3:1. So that for application suggest use surfactant and cosurfactant ratio 6:1 at combination surfactants with HLB 14 to produce more stable clear emulsion. The example of emulsion can see in Figure 4, 5, 6 and 7. From Figure 7 was shown that emulsion with VCO more clearly than emulsion with soybean oil and corn oil. It was causes atom C chain of soybean oil and corn oil fatty acid mean content longer than VCO, so that emulsion droplet will be bigger and transparency was decrease [4].

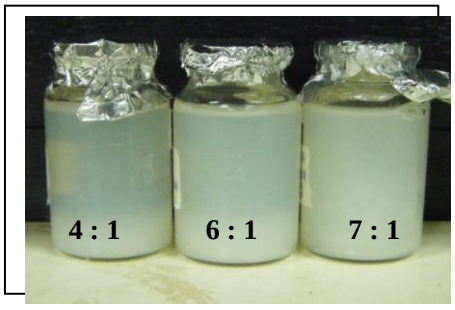

Fig.4; Emulsion used VCO with surfactants combination to produced HLB 12

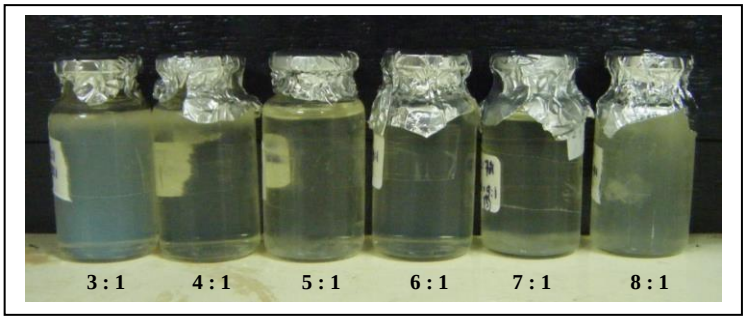

Fig.5; Emulsions used VCO with surfactants combination to produced HLB 13

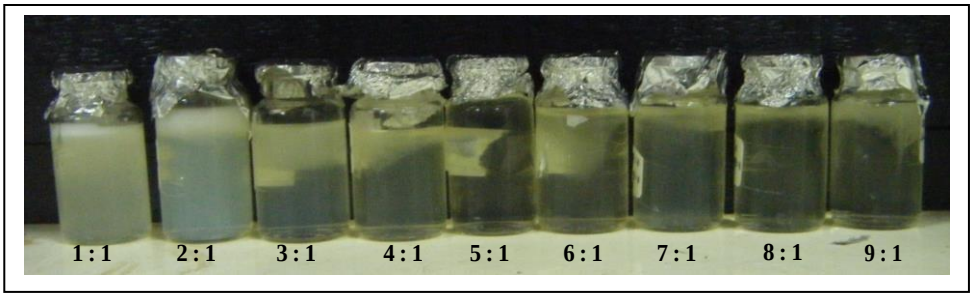

Fig.6; Emulsions used VCO with surfactants combination to produced HLB 14

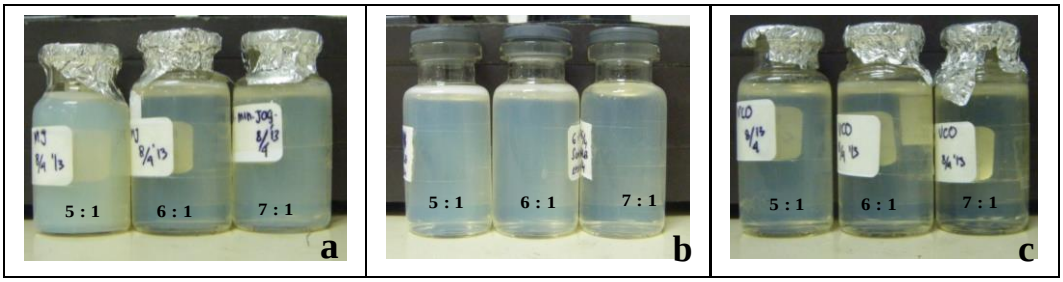

Fig.7; Emulsions used soybean oil (a), corn oil (b) and VCO (c) with surfactants combination to produced HLB 14.

CONCLUSION

From the results of this study concluded:

1. The solubility of PMCA in acetate buffer pH 4.2±0.2 was 70.04±0.66 mg/L.

2. PMCA apparent partition coefficient in acetate buffer pH 4.2±0.2 and soybean oil, corn oil, and VCO, were 2.39±0.11; 2.38±0.12 and 2.41±0.13 respectively.
3. The main contents of soybean oil fatty acids were 19.57% palmitic acid, 45.2% linoleic acid, 25.36% elaidic acid, and 7.07% stearic acid. The main contents of corn oil fatty acids were 26.86% palmitic acid, 31.52% oleic acid, 31.30% elaidic acid and 4.68% stearic acid. The main contents of VCO fatty acids were 32.41% lauric acid, 24.15% myristic acid, 15.68% palmitic acid, 2.29% linoleic acid, 11.06% elaidic acid and 5.22% stearic acid.
4. Soybean oil, corn oil, and VCO HLB needs was 14 and the ratio of surfactant and cosurfactant which can formed a clear emulsion (nanoemulsion) was 6:1.
5. Nanomulsions with soybean oil and corn oil more turbid than nanoemulsion with VCO.

Acknowledgment

The authors want to thank Indonesia Government who give research fund through Rector of Airlangga University (through LPPM-UA), also to my students; Akhmadi Aslakh**,** Laurin Anastasia Winarso, Rizka Arifa Rahmawati, Silvia Noor Hidayah and Widya Nur Pratama whose supported this research.

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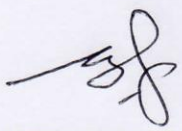

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Manuscript Title: PRAFORMULATION STUDY OF *p*-METHOXYCINNAMIC ACID (PMCA) NANOEMULSION USING VEGETABLE OILS (SOYBEAN OIL, CORN OIL, VCO)

NO	Correction
1	**Section** : ABSTRACT **Page No** 1 **Line No** 12 **Column** 1 **Uncorrected Text**: oleic acid **Correction** : linoleic acid
2	**Section** : Table 1 **Page No** 1 **Line No** 1 **Column** 2 **Uncorrected Text**: ph **Correction** : pH
3	**Section** : text under Fig 3 **Page No** 2 **Line No** : 1-5 **Column** 2 **Uncorrected Text**: The determination results apparent partition coefficient of PMCA in acetate buffer pH 4.2±0.2 and each oil (soybean oil, corn oil and VCO) with a ratio of 10:0.5 ; 10:1.0 and 10:2.0 and the concentration of the acetate PMCA buffer pH 4.2±0.2 at 50 and 60 ppm can be seen in Table 2. **Correction** : The apparent partition coefficient of PMCA (concentration of 50 and 60 ppm) in acetate buffer pH 4.2 ± 0.2 and each oil (soybean oil/corn oil/VCO) with ratio of 10:0.5; 10:1.0; 10:2.0 are presented in Table 2.

4

Section : Table 2

Page No 2

Line No 2

Column 1

Uncorrected Text: CONSENTRATION IN BUFFER

Correction : CONCENTRATION PMCA IN BUFFER

5

Section : Table 3

Page No 2

Line No 2

Column 1

Uncorrected Text: position of **Corn oil** above Conc.(%) in column 2 and position of **Vco** above Conc.(%) in column 4

Correction : position of **Corn oil** above Fatty acid in column 3 and position of **VCO** above Fatty acid in column 5

FATTY ACIDS MAIN CONTENTS OF VEGETABLE OIL

SOYBEAN OIL		CORN OIL		VCO	
FATTY ACID	CONC. (%)	FATTY ACID	CONC. (%)	FATTY ACID	CONC. (%)
palmitic acid (C16)	19.57	palmitic acid (C16)	26.86	lauric acid (C12)	32.41
linoleic acid (C18:2)	45.20	linoleic acid (C18:2)	31.52	myristic acid (C14)	24.15
elaidic acid (C18:1) *trans*	25.36	elaidic acid (C18:1) *trans*	31.30	palmitic acid (C16)	15.68
stearic acid (C18)	7.07	stearic acid (C18)	4.68	linoleic acid (C18:2)	2.29
				elaidic acid (C18:1) *trans*	11.06
				stearic acid (C18)	5.22

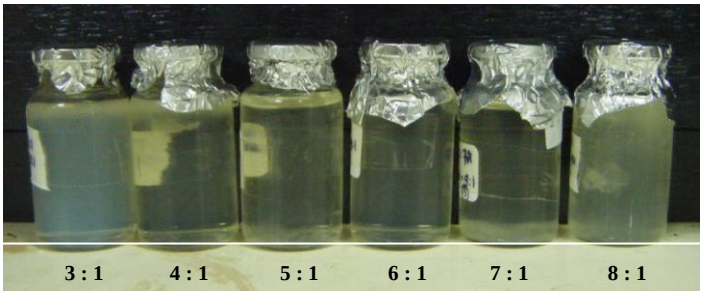
6

Section : Table 3

Page No 2

Line No 3

Column 1

	Uncorrected Text: Z **Correction** : Fatty acid
7	**Section** : Table 3 **Page No** 2 **Line No** : 6 and 7 **Column** 3 **Uncorrected Text**: oleic acid (C18:1) *cis* **Correction** : linoleic acid (C18:2)
8	**Section** : text under Table 3 **Page No** 2 **Line No** 1 **Column** 2 **Uncorrected Text**: oleic acid (C18:1/- *cis*) **Correction** : linoleic acid (C18:2)
9	**Section** : Fig 4 and 5 **Page No** 3 **Line No** : - **Column** 1 **Uncorrected Text**: Fig 4; (three bottles) was accidentaly replace by Fig 5; (five bottles)  **Fig.4;** Emulsion used VCO with surfactants combination to produced HLB 12

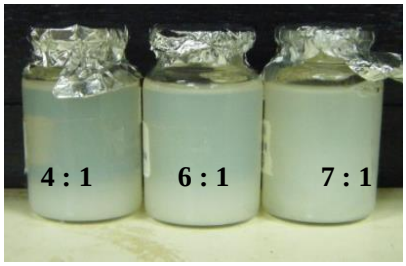


Fig.5; Emulsions used VCO with surfactants combination to produced HLB 13

Correction : Fig 4; three bottles than Fig 5; five bottles

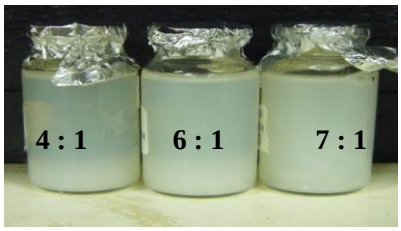


Fig.4; Emulsion used VCO with surfactants-cosurfactants ratio of 4:1; 6:1; 7:1 and combination surfactants to produced HLB 12

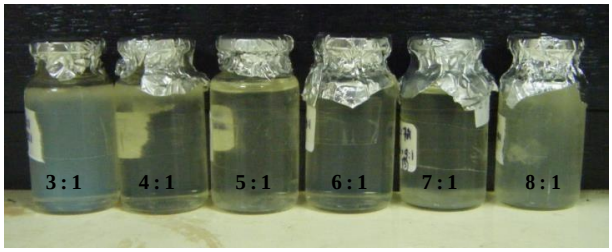


Fig.5; Emulsions used VCO with surfactants-cosurfactants ratio of 3:1; 4:1; 5:1; 6:1; 7:1; 8:1 and surfactants combination to produced HLB 13

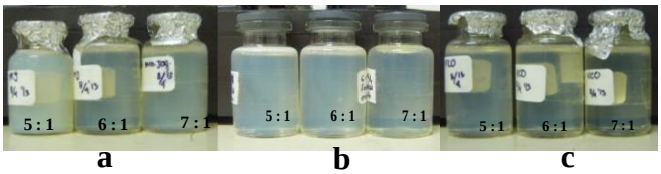
10 **Section** : Fig 6 and 7

Page No 3

Line No : -

Column : 1 and 2

Uncorrected Text: Fig 6; (nine bottles in one frame) was accidentaly replace by Fig 7; (nine bottle in three frames)



a b c

Fig.6; Emulsions used VCO with surfactants combination to produced HLB 14

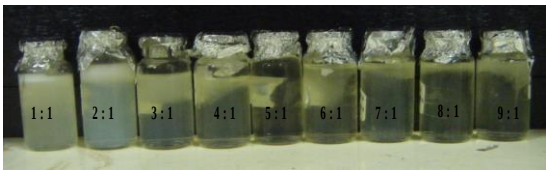


Fig.7; Emulsions used soybean oil (a), corn oil (b) and VCO (c) with surfactants combination to produced HLB 14.

Correction: Fig 6; (nine bottle in one frame) than Fig 7; (nine bottles in three frames)

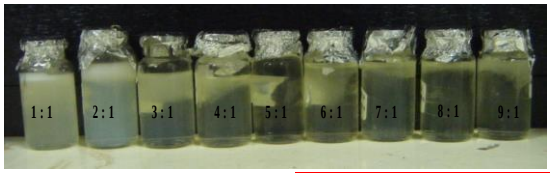
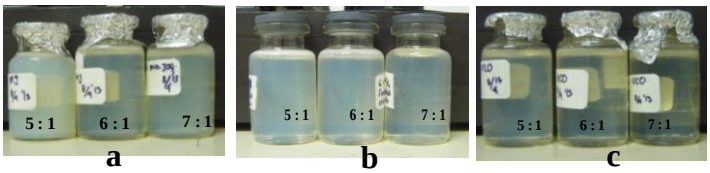


Fig.6; Emulsions used VCO with surfactants-cosurfactants ratio of 1:1; 2:1; 3:1; 4:1; 5:1; 6:1; 7:1; 8:1; 9:1 and surfactants combination to produced HLB 14



a b c

Fig.7; Emulsions used soybean oil (a), corn oil (b) and VCO (c) with surfactants-cosurfactants ratio of 5:1; 6:1; 7:1 and surfactants combination to produced HLB 14.

11	**Section** : CONCLUSION **Page No** 3 **Line No** : point 3 line 4 **Column** 2 **Uncorrected Text**: oleic acid **Correction** : linoleic acid

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Research Article

PRAFORMULATION STUDY OF *P*-METHOXYCINNAMIC ACID (PMCA) NANOEMULSION USING VEGETABLE OILS (SOYBEAN OIL, CORN OIL, VCO)

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Received: 04 Dec 2013, Revised and Accepted: 07 Feb 2014

ABSTRACT

Objective: This study aims were to obtain the praformulation data of *p*-methoxycinnamic acid (PMCA) (solubility in buffer; apparent partition coefficient between buffer and vegetable oils), to determine the fatty acids content and screening HLB needs of each oils as well as to determine the composition of nanoemulsion using these oils, surfactants (Tween 80 and Span 80) and ethanol 96% as cosurfactant.

Methods: Solubility test of PMCA in acetate buffer pH 4.2±0.2 were conducted with shakes and followed by measuring PMCA amount dissolved by spectrophotometer. To determine the PMCA apparent partition coefficients between buffer pH 4.2±0.2 and soybean oil, corn oil, VCO, respectively, PMCA dissolved in buffer then oil that has been saturated with buffer were added. Firstly by shaking followed by centrifugations than PMCA remained amount in the buffer was measured by spectrophotometer. Determination of fatty acid contents in the oils was used GC-MS while screening HLB was carried by various compositions of surfactants and cosurfactant to obtain a clear emulsion (nanoemulsion).

Results: From the results of this study, the solubility of PMCA in acetate buffer pH 4.2±0.2 was 70.04±0.66 mg/L. PMCA apparent partition coefficient in buffer pH 4.2±0.2 and soybean oil, corn oil, and VCO, respectively were 2.39; 2.38 and 2.41. The main contents of soybean oil fatty acids were 19.57% palmitic acid, 45.2% linoleic acid, 25.36% elaidic acid, and 7.07% stearic acid. The main contents of corn oil fatty acids were 26.86% palmitic acid, 31.52% linoleic acid, 31.30% elaidic acid and 4.68% stearic acid. The main contents of VCO fatty acids were 32.41% lauric acid, 24.15% myristic acid, 15.68% palmitic acid, 2.29% linoleic acid, 11.06% elaidic acid and 5.22% stearic acid. These oils HLB needs was 14 and the ratio of surfactant - cosurfactant which can formed a clear emulsion (nanoemulsion) was 6:1

Conclusion: 1) To produce nanoemulsion with soybean oil, corn oil, and VCO as oil phase HLB needs was 14 and the ratio of surfactant and cosurfactant was 6:1. 2) Nanomulsions with soybean oil and corn oil more turbid than nanoemulsion with VCO.

Keywords: PMCA, Solubility, Apparent partition coefficient, Fatty acids, Soybean oil, Corn oil, VCO, HLB needs.

INTRODUCTION

One of the drug delivery system that has been known to increase the solubility and penetration of the drug is nanoemulsion system. Nanoemulsion system consists of a water phase, oil phase, surfactant and cosurfactant [1]. Increased penetration of the system is due to the increasing amount of material in the form of molecular medicine so that differences in the concentration of the drug outside and inside skin which can be greater as the driver of the penetration process, in addition to the amount of surfactant and cosurfactant high enough to serve as an enhancer [2]. Another advantage of this system is that the spontaneous creation without the need for heating and vigorous stirring. To increase the use of natural resources and improve the products of traditional medicine into modern medicine, the nanoemulsion delivery system is applied to the p- methoxycinnamate acid (PMCA). In order to attempt the development of PMCA as topical anti-inflammatory preparations on nanoemulsion system utilizing plant oils (soybean oil, corn oil, VCO). PMCA is a component of the *Kaempferia galangal* rhizome, widely used as a traditional medicine (Jamu) [3]. Kaempferia galangal rhizome by Javanese traditional herb used in a formula named "bobok" blend with oryza sativa. The formula function removes the body pain due to sprains, exercise or heavy work. To formulate a topical preparation is needed praformulasion data, then in this research study involves determining solubility and partition coefficient of PMCA, determine the fatty acid content and screening HLB need of vegetable oil and to get a comparison of surfactant-cosurfactant which can produce a clear (translucent) emulsion.

MATERIALS AND METHODS

These research started by qualitative analysis of PMCA by *IR JASCO FT/IR-5300 Instrument* and *Differential Thermal Analysis* (DTA), than determination of λ maximum and PMCA standard curve in acetate buffer pH 4.2±0.2 followed by solubility test of PMCA in acetate buffer pH 4.2±0.2. Solubility test of PMCA in acetate buffer pH 4.2±0.2 were conducted by shaking for 4 hours at 150 rpm followed by measuring PMCA amount dissolved by spectrophotometer. To determine the PMCA apparent partition coefficients between buffer pH 4.2±0.2 and soybean oil, corn oil, VCO, respectively, PMCA (50 ppm and 60 ppm) was dissolved in 10.0 mL acetate buffer pH 4.2±0.2 then oil (0.5; 1.0 and 2.0 mL) that has been saturated with acetate buffer pH 4.2±0.2 were added. Firstly by shaking for 2 hours at 150 rpm, followed by centrifugations for 15 minutes at 2200 rpm than PMCA remained amount in the buffer was measured by spectrophotometer.

Determination of fatty acid contents in the oils was used GC-MS while screening HLB was carried by various combinations of surfactants (Tween 80 and Span 80 to produce HLB 10,11,12,13 and 14). To obtain a clear emulsion one portion of oil added with nine portion of combination of surfactant and cosurfactant (1:1 ; 2:1; 3:1 etc. .... 9:1), than added slowly with acetate buffer pH 4.2±0.2.

RESULTS AND DISCUSSION

The result of PMCA qualitative test by *IR JASCO FT/IR-5300 Instrument* between wave's numbers 450 – 4000 cm^{-1} that found the infrared spectra as showed Figure 1 and PMCA melting temperature (174.4^{0}C) by *Differential Thermal Analysis* (DTA) showed in Figure 2.

Table 1: The PMCA solubility in acetate buffer pH 4.2±0.2

Solubility of PMCA in ACETATE buffer pH 4.2 ±0.2	
Replicate	**Concentration (mg/L)**
1	69.69
2	69.44
3	70.54
4	68.99
5	71.10
6	70.45
mean ± SD	**70.04 ± 0.66**

Results of the determination of the maximum wavelength of PMCA in acetate buffer solution of pH 4.2±0.2; the PMCA maximum wavelength obtained in acetate buffer pH 4.2±0.2 was 301 nm.

Regression equation of the relationship curve between the absorbance vs. PMCA concentration obtained y = 0.1063x - 0.0014 with a correlation coefficient r = 0.9999.

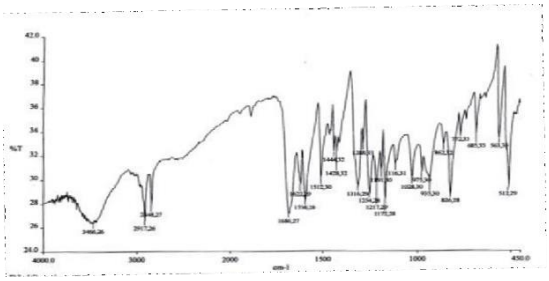

Fig. 1: PMCA infrared spectra between waves number 450 – 4000 cm^{-1} by *IR JASCO FT/IR-5300 Instrument.*

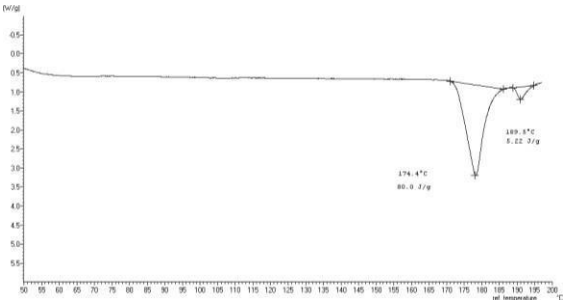

Fig. 2: PMCA melting temperature (174.4^{0}C) by Differential *Thermal Analysis* (DTA)

PMCA solubility determination in acetate buffer solution of pH 4.2±0.2 begins with the timing of PMCA saturated solubility in acetate buffer solution of pH 4.2±0.2 can be seen in Figure 3. and the results of ANOVA test is known all PMCA concentration at 4, 5, 6, 7 and 8 hour did not differ significantly so that it can be concluded time its saturation solubility at 4th hour.

While the results of the determination of the PMCA solubility in acetate buffer pH 4.2±0.2 during 4th hour was 70.04±0.66 mg/L can be seen in Table 1.

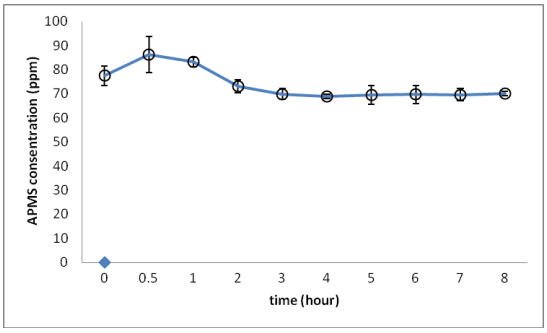

Fig. 3: Correlation curve between PMCA concentrations (ppm) in acetate buffer pH 4.2 + 0.2 vs. time (hour)

The apparent partition coefficient of PMCA (concentration of 50 and 60 ppm) in acetate buffer pH 4.2 ± 0.2 and each oil (soybean oil/corn oil/VCO) with ratio of 10:0.5; 10:1.0; 10:2.0 are presented in Table 2. From Table 2 known apparent partition coefficients of PMCA in acetate buffer pH 4.2±0.2 and soybean oil, corn oil, VCO were 2.39±0.11; 2.38±0.12 and 2.41±0.13 respectively.

Table 2: PMCA apparent partition coefficient in acetate buffer pH 4.2±0.2 and each oil (soybean oil, corn oil, and VCO)

Oil	Buffer : Oil Ratio (mL)	PMCA Apparent partition coefficient Concentration PMCA in buffer						Mean ± SD	
		50 ppm			60 ppm				
		1	2	3	1	2	3		
Soybean oil	10 : 0.5	2.53	2.52	2.51	2.57	2.55	2.56	2.54	2.39±0.11
	10 : 1.0	2.36	2.33	2.34	2.38	2.39	2.37	2.36	
	10 : 2.0	2.24	2.29	2.26	2.21	2.33	2.31	2.27	
Corn oil	10 : 0.5	2.52	2.54	2.56	2.55	2.55	2.54	2.54	2.38±0.12
	10 : 1.0	2.38	2.36	2.37	2.38	2.39	2.36	2.37	
	10 : 2.0	2.29	2.28	2.29	2.16	2.16	2.16	2.22	
VCO	10 : 0.5	2.51	2.48	2.46	2.69	2.64	2.62	2.57	2.41±0.13
	10 : 1.0	2.39	2.39	2.42	2.48	2.49	2.51	2.45	
	10 : 2.0	2.25	2.26	2.29	2.33	2.28	2.29	2.28	

Table 3: The main contents of soybean oil, corn oil, and VCO fatty acids

Fatty acids main contents of vegetable oil					
Soybean oil	Corn oil		VCO		
Fatty acid	Conc. (%)	Fatty acid	Conc. (%)	Fatty acid	Conc. (%)
palmitic acid (C16)	19.57	palmitic acid (C16)	26.86	lauric acid (C12)	32.41
linoleic acid (C18:2)	45.20	linoleic acid (C18:2)	31.52	myristic acid (C14)	24.15
elaidic acid (C18:1) *trans*	25.36	elaidic acid (C18:1) *trans*	31.30	palmitic acid (C16)	15.68
stearic acid (C18)	7.07	stearic acid (C18)	4.68	linoleic acid (C18:2)	2.29
				elaidic acid (C18:1) *trans*	11.06
				stearic acid (C18)	5.22

Results of the determination of fatty acid content of soybean oil, corn oil and VCO using GC-MS can be seen in Table 3. From Table 3 it can be seen that the highest levels of soybean oil fatty acids was 45.20% linoleic acid (C18:2), the highest levels of corn oil fatty acids was 31.52% oleic acid (C18:1/-*cis*) and 31.30% elaidic acid (18:1/- *trans*), while the highest fatty acid levels VCO is 32.41% lauric acid

(C12). Screening of oils HLB needs and determination of surfactants (combination surfactants Tween 80 - Span 80): cosurfactant

(ethanol) ratios were done by ratio 1:1 until 9:1 and combination surfactants Tween 80-Span 80 at HLB 10, 11, 12, 13 and 14.

That result known at surfactant combination with HLB 10, 11 and 12 the emulsion using all oils turbid, at surfactants combination with HLB 13 emulsion using soybean oil and corn oil were turbid, while emulsion using VCO start from surfactant and co-surfactant ratio 3:1 produced clear emulsion. At surfactants combination with HLB 14 emulsion using soybean oil and corn oil appear translucent start from surfactant and cosurfactant ratio 5:1, while emulsion that used VCO start from surfactant and cosurfactant ratio 3:1. So that for application suggest use surfactant and cosurfactant ratio 6:1 at combination surfactants with HLB 14 to produce more stable clear emulsion. The example of emulsion can see in Figure 4, 5, 6 and 7. From Figure 7 was shown that emulsion with VCO more clearly than emulsion with soybean oil and corn oil. It was causes atom C chain of soybean oil and corn oil fatty acid mean content longer than VCO, so that emulsion droplet will be bigger and transparency was decrease[4].

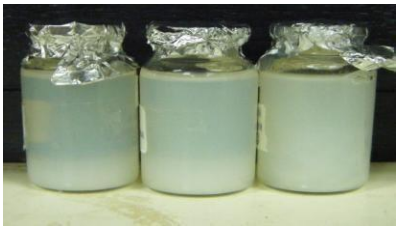

Fig. 4: **Emulsion used VCO with surfactants-cosurfactants ratio of 4:1; 6:1; 7:1 and combination surfactants to produced HLB 12**

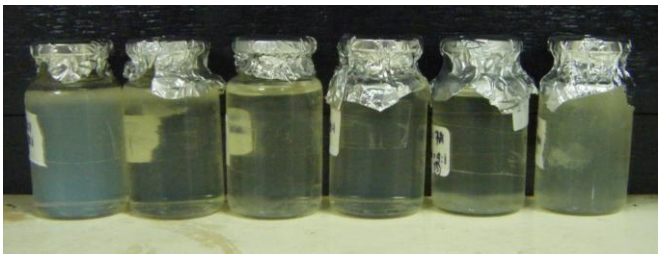

Fig. 5: Emulsions used VCO with surfactants-cosurfactants ratio of 3:1; 4:1; 5:1; 6:1; 7:1; 8:1 and surfactants combination to produced HLB 13

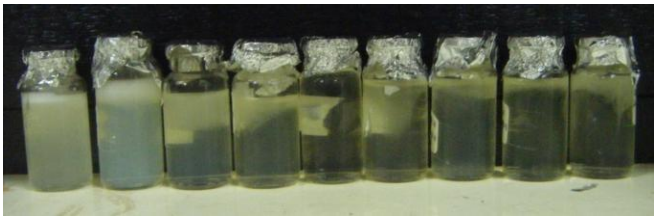

Fig. 6: Emulsions used VCO with surfactants-cosurfactants ratio of 1:1; 2:1; 3:1; 4:1; 5:1; 6:1; 7:1; 8:1; 9:1 and surfactants combination to produced HLB 14

Fig.7: Emulsions used soybean oil (a), corn oil (b) and VCO (c) with surfactants-cosurfactants ratio of 5:1; 6:1; 7:1 and surfactants combination to produced HLB 14.

CONCLUSION

From the results of this study concluded:

1. The solubility of PMCA in acetate buffer pH 4.2±0.2 was 70.04±0.66 mg/L.

2. PMCA apparent partition coefficient in acetate buffer pH 4.2±0.2 and soybean oil, corn oil, and VCO, were 2.39±0.11; 2.38±0.12 and 2.41±0.13 respectively.

3. The main contents of soybean oil fatty acids were 19.57% palmitic acid, 45.2% linoleic acid, 25.36% elaidic acid, and 7.07% stearic acid. The main contents of corn oil fatty acids were 26.86% palmitic acid, 31.52% linoleic acid, 31.30% elaidic acid and 4.68% stearic acid. The main contents of VCO fatty acids were 32.41% lauric acid, 24.15% myristic acid, 15.68% palmitic acid, 2.29% linoleic acid, 11.06% elaidic acid and 5.22% stearic acid.

4. Soybean oil, corn oil, and VCO HLB needs was 14 and the ratio of surfactant and cosurfactant which can formed a clear emulsion (nanoemulsion) was 6:1.

5. Nanomulsions with soybean oil and corn oil more turbid than nanoemulsion with VCO.

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