CURRENT ADVANCES IN NANODIAGNOSTIC AND NANOTHERAPEUTIC

Proceeding Book
The 2nd International Conference on Pharmaceutical Nanotechnology/Nanomedicine

Jakarta, June 6th 2015

Faculty of Pharmacy, Pancasila University
Srengseng Sawah, Jagakarsa, South Jakarta, 12640
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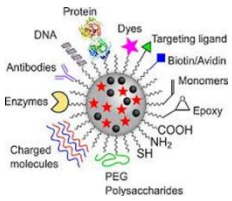

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PREFACE

The second International Conference on Pharmaceutical Nanotechnology/Nanomedicine has been scheduled to take place at Faculty of Pharmacy, Pancasila University (FFUP), Jakarta, Indonesia, on 5-6th of June 2015. The program had a theme: *"Current Advances in Nanodiagnostics and Nanotheurapetic"*.

The aim of the conference is to share the recent development in pharmaceutical nano-technology/nanomedicine. Hence, the conference could be an educational conference for development of pharmaceutical nanotechnology/nanomedicine especially in universities, research institutions and pharmaceutical industries in Indonesia.

The total poster participant in this conference was 31 from FFUP and other institutions (Bandung Institute of Technology, Sekolah Tinggi Farmasi Perintis Padang, Universitas Jenderal Soedirman, BPPT, National institute of Science and Technology, LIPI, UHAMKA) with various fields including nanotechnology, pharmaceutical technology, clinical pharmacy, chemistry, pharmacology and biotechnology.

Finally, on behalf of Organizing Committee, we would like to express our appreciation to Bank Nasional Indonesia (BNI) and alumni FFUP as sponsors for their help and support.

Jakarta, June 3, 2015

Chairman of The Organizing Committee,
Dr.rer.nat. Deni Rahmat, M.Si., Apt.

TABLE OF CONTENT

FORMULATION AND PHYSICAL STABILITY NITROGLIYCERINE MICROEMULTION WITH TWEEN 80 AS SURFACTANT

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Abstract

Nitroglycerin is a drug of choice in the treatment of angina pectoris. Commonly used as buccal, sublingual and injection. Sublingual treatment cause discomfort and sensation burning on its use. In this research used forms microemulsion transdermal. Transdermal offers a way alternatives for the delivery of nitroglycerin without passing through the intestines, so it is more convenient and safe for long term use. Microemulsion is expected to increase the penetration of the drug because contains high concentrations of surfactants. This research aims to determine the effect of the active agent nitroglycerin into microemulsion system and see its physical stability and to obtain the best formulation. Used three variations of the concentration of tween 80, there is 40%, 42.5% and 45%. Stability testing of physical properties during 8 weeks. From the results of evaluation data showed that Formula 2 is the best formula with the result, among others, as The following pH of 5.69 ± 0.01, the viscosity of 1480.26 ± 2.83 Cps, Specific Gravity of 1.0723 ± 0.00011 g / mL, Surface Tension of 38.52 ± 0.037 dyne / cm and particle size of 77.95 nm.

Keywords: Tween 80, Mikroemulsi, Nitroglycerine, Transdermal

INTRODUCTION

Angina pectoris is discomfort in the chest as a result of myocardial ischemia without infarction. Symptoms of angina pectoris basically arise due to acute ischemic not settled as a result of an imbalance between demand and supply of myocardial O2. Angina syndrome has long been known as the early symptoms of acute myocardial infarction (IMA). Many studies report that angina is a risk for the occurrence of IMA and death. Several retrospective studies indicate that 60-70% of patients with IMA and 60% of patients suddenly die in the history of the disease have symptoms of angina prodoma. While the long-term research to get the IMA occurs in 5-20% of patients with angina with 14-80% mortality rate (Bahir 2004).

nitroglycerin ussualy used by sublingual, but sublingual administration of drugs has several disadvantages including patients will experience difficulty and discomfort in sublingual drug use and in the long term can irritate the mucosa of the mouth and cause a burning sensation in their use. Such inconveniences can be overcome by transdermal administration. Transdermal administration has several advantages, including not through first-pass metabolism in the liver, the drug is not broken down by enzymes in the digestive tract. Microemulsion is an alternative dosage can penetrate transdermally.

Microemulsion is a transparent system that is isotropic, thermodynamically stable consisting of oil, water and surfactant. All three are combined with a co-surfactant. Microemulsion has a droplet size range of 20 to 200 nm. Microemulsion can be classified

as an oil-in-water (o / w), water-in-oil (w / o) or bicontinuous system depending on their structure (Lawrence 2000).

One component of the microemulsion is surfactants. Surfactants are surface active agent used for dispersing insoluble drugs as a colloidal dispersion. Surfactants are used as wetting and prevent crystallization physicochemical of drugs such as hydrophilicity / lipophilicity, pKa and polarity (Date AA, 2008)

Kori et al. (2011) conducted a research using a microemulsion system making VCO as the oil phase and tween 80 as surfactant. The results showed that microemulsion Kori et al. (2011) conducted a research using a microemulsion system making VCO as the oil phase and tween 80 as surfactant. The results showed that microemulsion stable and accept pharmaceutical requirements on the use of a number of tween 80 45%.

From the results of this research can be developed further by the addition of active substances nitroglycerin into the microemulsion system. Tween 80 as surfactant concentration in the microemulsion formulation, made in three concentrations, namely, 40, 42.5 and 45%. Expected with the addition of nitroglycerin as the active ingredient in the microemulsion system still generates a microemulsion that accept pharmaceutical requirements are the same.

METHODS

Materials

Materials used in this research was 10% Diluted Nitroglisein in Propilenglikol obtained from Umang Phrama Ltd, India. Virgin coconut oil (Virgin Coconut Oil) obtained from LIPI Cibinong, Bogor. Tween 80 was obtained from PT. KAO Chemical. Nipagin (methyl paraben), Nipasol (propyl paraben) from PT. Clariant

Tools

The tools used in this research include: analytical balance, glassware , pycnometer, pH meter (Hanna), Brookfield viscometer type LVDV-E (Brookfield, USA), Du Nouy tensiometer (Cole Parmer), water bath, weighing bottle, oven (Binder), refrigerator (Memmert Chiller), sentrifugator, heating magnetic stirrer (SSM 79-1) and Nanosizer (Beckman Coulter).

Making the microemulsion

Microemulsion formulation of nitroglycerin made in three formulas. Formula can be seen in Table 1. Tween 80 dissolved in aqua fervida and the mixture then stirred until

homogeneous. Combine virgin coconut oil into the water phase, mix well . Dissolve nipagin and nipasol into 10% Diluted Nitroglycerin in Propilenglikol to dissolve and enter into the oil and water phase, mix well until form a microemulsion is clear and transparent.

Table 1. Microemulsion Formula

Component	F1	F2	F3	Function
Diluted 10% Nitroglycerin in Propylenglycol	20	20	20	Active Content Co Surfactant
Virgin Coconut Oil (%)	5	5	5	Oil Phase
Tween 80 (%)	40	42,5	45	Surfactant
Nipagin (%)	0.18	0.18	0.18	Preservative
Nipasol (%)	0.02	0.02	0.02	Preservative
Aqua destillata ad (%)	100	100	100	Aqueos Phase

Evaluation microemulsion

Microemulsion which has been finished then performed a series of tests, among others: Measurement of particles carried by 1 times of the formula that showed most excellent physical stability. Organoleptic test, the measurement of pH, Specific Gravity measurement, measurement of surface tension and viscosity tests performed for 8 weeks and testing performed at week 0 to week 8.

1. Organoleptic

   Includes the observation of the shape, color and odor at room temperature
2. Measurement of pH (Department of Health 1995)

   pH measurement by using a pH meter and checks carried out for 8 weeks at room temperature
3. Test the viscosity

   Measurement by using a Brookfield viscometer LVDV-E spindle 63 and a speed of 60 rpm for 8 weeks, ie at week 0 to week 8 at room temperature.
4. Specific Gravity (Department of Health 1995)

   Specific Gravity was measured using a pycnometer and examinations carried out for 8 weeks.
5. Measurement of Surface Tension (Voigt 1994)

   Measurement of surface tension is done by using a tensiometer with Du Nouy ring method and examinations carried out for 8 weeks at room temperature
6. Phase separation test(Martin et al. 1993)

   a. Centrifugation

Microemulsion included in centrifugation tube then conducted agitation or centrifugation in speed of 3750 rpm for 5 hours, observe the changes that occur.

b. freeze thaw

Cycle phase separation with a freeze thaw was done by first storage microemulsion in 40C followed by storage at a temperature of 450C. Observe organoleptic changes, for 6 cycles.

7. Particle size (Martin et al. 1993 and Voight 1994)

The particle size was measured at room temperature using the Nano Particle Size Analyzer. Samples to be measured is a microemulsion which is still fresh or fresh. The solution is taken and put in a cuvette, which has been filled sample cuvette is inserted into the sample holder.

Analysis

The data were statistically tested included with two-way analysis of variance of the formula and the time. Among the data of viscosity measurements, pH, Specific Gravity and surface tension every week

RESULTS AND DISCUSSION

From the results of the examination, the Specific Gravity obtained fulfill the standards because in between 0.9150 to 0.9200 g / ml. Acid number indicates the amount of free fatty acids contained in the oil while the saponification showed heavy oil molecules are composed by fatty acids. Number of free fatty acids resulting from the inspection fulfill the requirements because it is below 0.5%. The refractive index produced f the requirements of being between 1.4480 - 1.4492. The results obtained from the examination of the characteristics of virgin coconut oil being tested fulfill the standards so that it can be concluded that the tested virgin coconut oil of good quality so that the fatty acids contained in virgin coconut oil is beneficial in maintaining skin moisture still contained in the microemulsion.

Table 2. Results of examination of VCO characterization

Evaluation	Result	Standard
Organoleptic test	a. Form : Viscous Liquid	Viscous Liquid
	b. Colour : Clear	Clear
	c. Smell : Characteristic Odor	Characteristic Odor
Specific Gravity	0.918 g/mL	0,9150 - 0,9200 g/mL
Free Fatty Acid Value	0.316 %	≤ 0,5%.
Refractive Index	1.4488	1.4480 – 1.4492

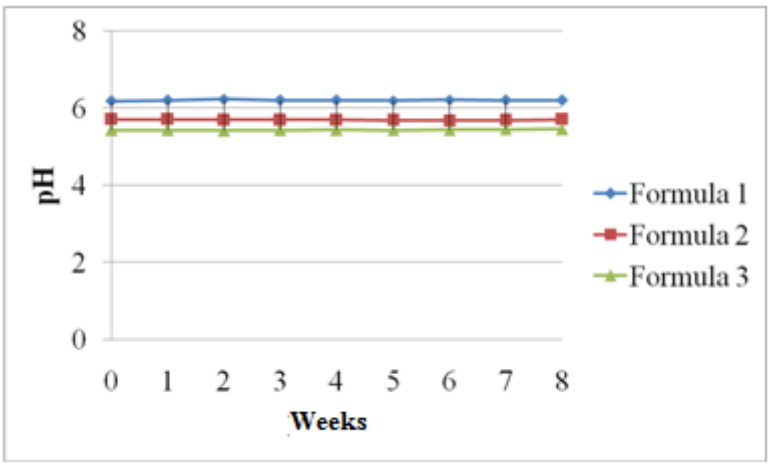
Based on the above table it can be seen that the three formulas are no changes in terms of shape, color and odor during storage. These formula remains in a liquid state, clear yellow and a characteristic odor. This showed that the three formulas have reasonably good physical stability during storage time. Microemulsion storage at room temperature and preparations which remain stored in a tightly closed container, so as to make the microemulsion was not influenced by environmental factors.

Tabel 3. Organoleptic of Nitroglycerin Microemulsion

Organoleptic Evaluation	**Formula**	**Time (Week)**								
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Bentuk	**1**	L	L	L	L	L	L	L	L	L
	2	L	L	L	L	L	L	L	L	L
	3	L	L	L	L	L	L	L	L	L
Warna	**1**	S	S	S	S	S	S	S	S	S
	2	S	S	S	S	S	S	S	S	S
	3	S	S	S	S	S	S	S	S	S
Bau	**1**	C	C	C	C	C	C	C	C	C
	2	C	C	C	C	C	C	C	C	C
	3	C	C	C	C	C	C	C	C	C

Note : F1 : Tween 80 : 40% L : Liquid
F2 : Tween 80 : 42,5% S : Spesific
F3 : Tween 80 : 45% C : Clear Yellow

pH measurement results of the three formulas are still fulfill the requirements of pH skin in the range 4.5 to 6.5. Results of pH measurement is in the range of 5.42 -6.24 so it still qualifies for use on the skin. From the picture above it can be seen that the F2 formula is a formula that has the most stable pH conditions among other formulas.



Picture 1. pH Measurement

Statistical analysis of pH, note that the data are normally distributed with significance value > 0.05 either to the formula or the time. Results of analysis of variance test of the pH is performed to determine whether or not the difference of the pH of the formula and to the storage time. Obtained results value sig. for the formulation is 0.000 (<0.05) and value sig. for the week was 0.783 (> 0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects the pH of the preparation, but the storage time does not affect the pH of the microemulsion each week.

The next separation test is freeze thaw, freeze thaw is done by storage at two different temperatures, namely storage at 4oC followed by storage at a temperature of 45oC. Observations microemulsion with the freeze-thaw method performed for 6 cycles. In the 3rd cycle have started the change of shape. At $4^{o}C$ respectively - each formula start murky and embossed white colour at bottom. At $4^{o}C$ the oil phase tends to freeze at low temperatures and after being put into the oven temperature $45^{o}C$ it back to normal with regular agitation (reversible).

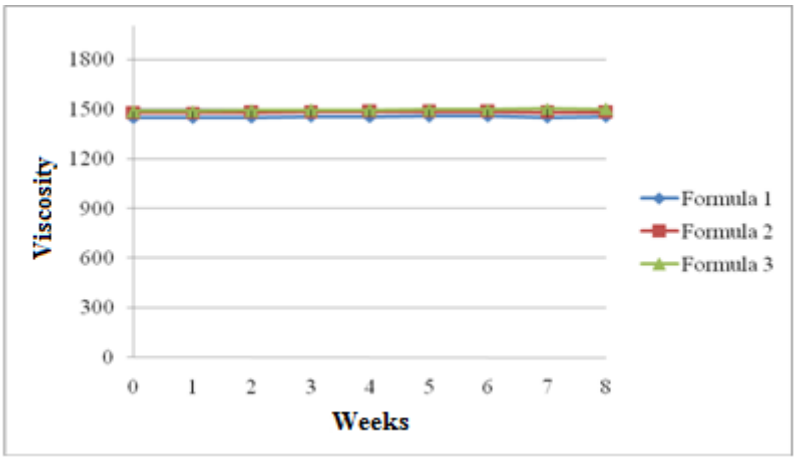
Table 4. Results of freeze thaw test

Formula		Cycle 1		Cycle 2		Cycle 3		Cycle 4		Cycle 5		Cycle 6	
		4° C	45° C	4° C	45° C	4° C	45° C	4° C	45° C	4° C	45° C	4° C	45° C
F1	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
F2	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-
F3	1	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-	-	-	-	-
	3	-	-	-	-	-	-	-	-	-	-	-	-

Viscosity measurement results of all formulas microemulsion for 8 weeks by using Brookfield viscometer type LVDV-E using 63 spindle at 60 rpm were performed at week 0 to week 8 showed that the greater the concentration of tween 80 as surfactant can increase the viscosity of the preparations microemulsion. Formula F1 to F3 has a viscosity that is likely to increase, but the increase is not significant. Results of viscosity measurements indicate that the F1, F2 and F3 for 8 weeks viscosity values obtained ranged between 1446.33 Cps - 1501 Cps.

This can be caused by the differences or increase in the concentration of tween 80 as surfactant in each formula so as to increase the viscosity of the microemulsion. So also the same as the emulsion, it is because viscosity generally increases with increasing age miroemulsi preparations (Lachman 1994). From the picture above we can see that the formula F2 shows the results of viscosity measurement values tend to be more stable than other formulas. The higher the viscosity of a preparation, the preparation is more stable due to the movement of the particles tend to be difficult, so the rate of creaming decreased (Viyoch 2003)

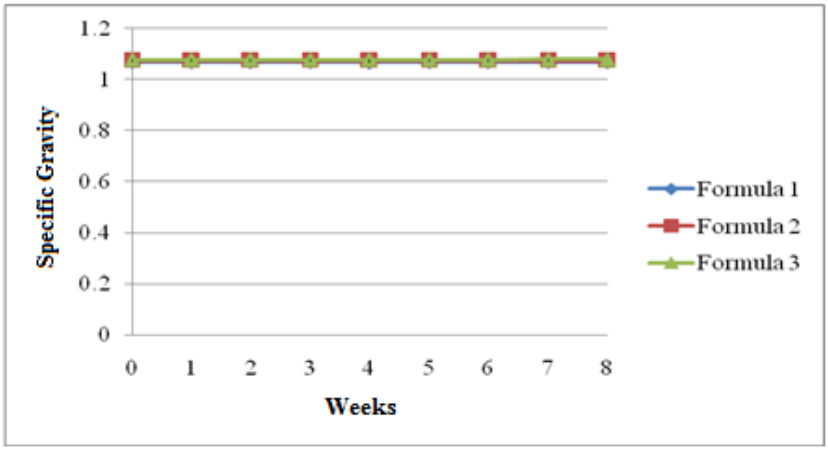
Statistical analysis of viscosity, it is known that the data are normally distributed with significance value> 0.05 either the formula or with respect to time. Results of analysis of variance to test the viscosity is made to determine whether or not the difference of the viscosity of the formula and to the storage time. Obtained results sig. for the formulation is 0.000 (<0.05) and sig. for the week was 0.001 (<0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects the viscosity of the preparations, as well as storage time affects the viscosity of the microemulsion each week.



Picture 2. Viscosity measurement

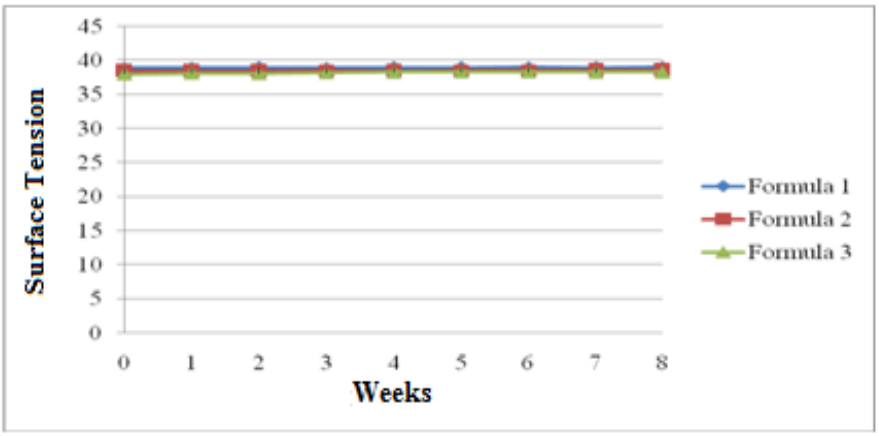
On results of measurements of Specific Gravity microemulsion nitroglycerin showed that the increasing concentration of surfactant (tween 80), the weight of the type of preparation will tend to rise. It is linear with viscosity measurements before.

Results of statistical analysis specific gravity, note that the data are normally distributed with significance value> 0.05 either the formula or with respect to time. The result of variance analysis against specific gravity conducted to determine whether or not the difference of specific gravity of the formula and to the storage time. Obtained results sig. for the formulation is 0.000 (<0.05) and sig. for the week was 0.112 (> 0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects specific gravity preparations, but does not affect the storage time specific gravity microemulsion each week.



Picture 3. Specific Gravity Measurement

On the surface tension measurement results microemulsion nitroglycerine show that increasing the concentration of surfactant (tween 80), the surface tension becomes increasingly declining microemulsion. This is consistent with the theory that the addition of surfactant in the solution would cause a decline in surface tension, at a certain concentration the surface tension will be constant even if the concentration of surfactant enhanced.Cosurfactant added to help lower the interfacial tension of oil phase and water phase. Cosurfactant will form a microemulsion droplets thereby increasing the solubility of non-polar groups.



Picture 4. Surface tension measurement

Statistical analysis of surface tension, it is known that the data are normally distributed with significance value> 0.05 either the formula or with respect to time. Results of analysis of variance test of the surface tension is performed to determine whether or not the difference of the surface tension of the formula and to the storage time. Obtained results sig. for the formulation is 0.000 (<0.05) and sig. for the week was 0.020 (<0.05). It can be concluded that the ratio of the concentration of tween 80 different in each formula affects the surface tension of the preparation, as well as storage time affect the surface tension of the microemulsion each week.

Determination of particle size using F2 preparations. Selection of F2 was based on earlier observations of the physical evaluation and F2 obtained that has a most excellent physical stability among others, of the results of the examination, the microemulsion at F2 has a particle size of 77.95 nm. The test results on the particle size F2 meets the requirements of a microemulsion particle size is 6-100 nm. (Bowman 2000)

CONCLUSION

Based on the results of the Research showed that there was an effect when the addition of the active substance into the microemulsion system. This Research shows that the microemulsion is physically stable and meet pharmaceutical requirements with the use of surfactant concentration tween 80 at 42.5%

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CERTIFICATE

This is to certify that

Kori Yati

as

Participant

in

The 2nd International Conference on Pharmaceutical Nanotechnology/Nanomedicine 2015

"Current Advances in Nanodiagnostic and Nanotherapeutic"

at Faculty of Pharmacy, Pancasila University

Jakarta, 6 June 2015

Dean

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Dr. rer. nat. Deni Rahmat, M.Si., Apt.

Based on decree of Indonesian Pharmacist Association (IAI) : 251/SKEP-47/PD-IAI/VIII/2015

Participant: 10 SKP, Speaker/Presenter: 5 SKP, Moderator/facilitator: 5 SKP, Committee: 1 SKP