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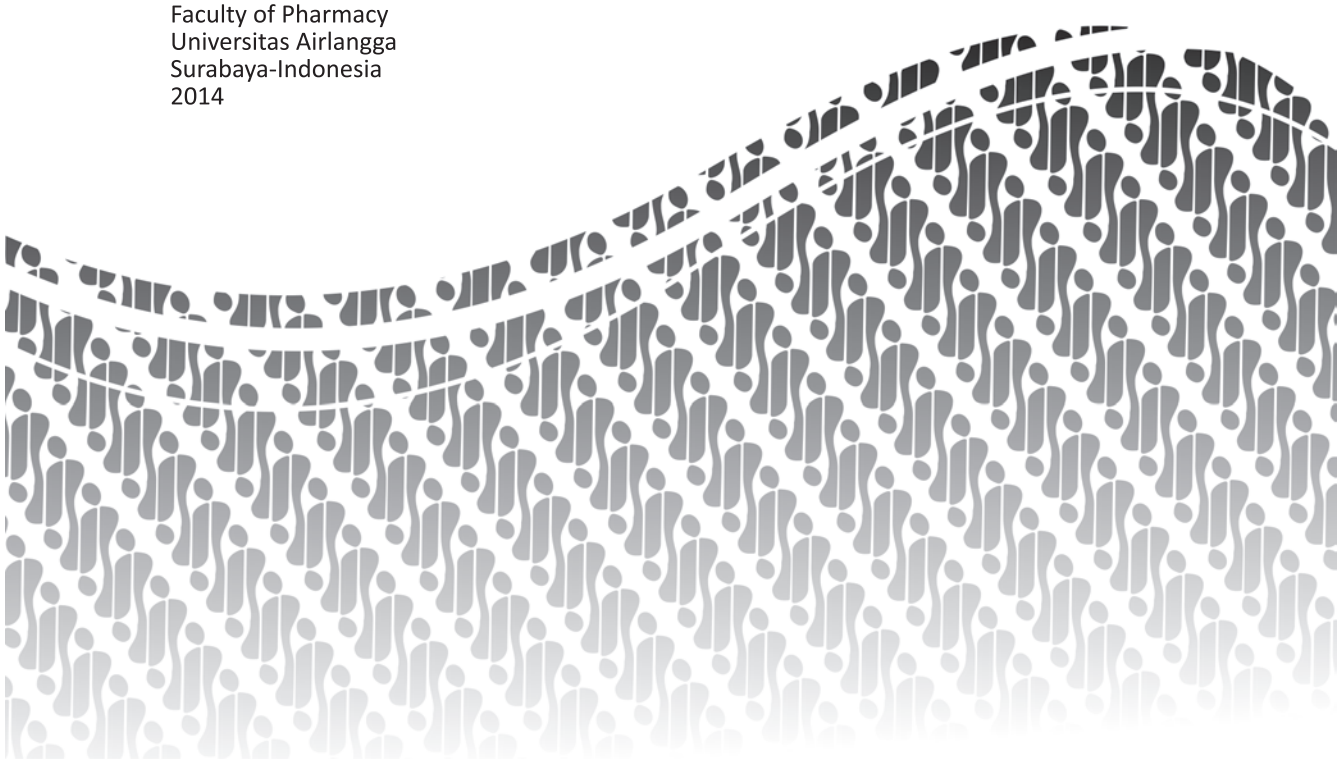

The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences Proceedings

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PREFACE From Chairman

It is our pleasure to present you the proceedings of The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS) organized by The Faculty of Pharmacy Universitas Airlangga Surabaya Indonesia.

The proceeding was produced based on papers and posters presented at The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS), held in Surabaya, Indonesia, 14-15 November 2014.

The proceeding clearly reflects broad interest, from the participants that coming from all around the world.

The papers presented were pharmaceutics and biopharmaceutics; requirements on how to evaluate molecules in discovery and their appropriateness for selection as potential candidate; their development in context of challenges and benefits, together with associated time and cost implications and also requirements to progress through pre-clinical and clinical.

In this an opportunity, I would like to express my appreciation to the editorial team of the proceeding who have been working hard to review manuscripts, and making the first edition of this proceeding be possible.

I would like also to thanks to all invited speakers and presenters who participated in The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS) and your contribution to this proceeding.

Finally, I hope this proceeding will give contribution to the Pharmaceutics and Pharmaceutical Sciences research.

Chairman,

Dra. Esti Hendradi, MSI., Ph.D., Apt

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ANTIOXIDANT STABILITY ASSAY OF ALPHA TOCOPHERYL ACETATE IN SOLID LIPID NANOPARTICLE SYSTEM (LIPID BASE BEESWAX AND MONOSTEARIC GLISERYL 50:50)

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ABSTRACT

Introduction : As an antioxidant, alpha tocopheryl acetate is easily degraded by light and free radical in air. Solid Lipid Nanoparticle (SLN) is a system that can provide protection of active ingredient because of its drug entrapment ability and physical protective from UV light due to its nano-sized. The aim of this study is for investigated the ability of SLN that could increase stability of antioxidant potency from alpha tocopheryl acetate. SLN is expected to have higher stability of antioxidant potency compared with simple cream.

Methods : SLN and simple cream was made SLN was made using lipid beeswax (BW) and monostearic glyceryl (GMS) as base and Tween 80 as stabilizer. SLN was produced using high shear homogenization method and simple cream was produced using hot plate magnetic stirrer. Antioxidant potency was measured by DPPH method. Sample was radiated by UV C light as free radical initiator.

Result and Discussion : Alpha tocopheryl acetate loaded in SLN system has higher stability of antioxidant potency compared with simple cream that shown with k value as constanta of antioxidant potency degradation between time. This could be due to its physical blocker of UV light and drug entrapment properties.

Conclusion : SLN was selected as antioxidant carrier due to its ability to increase antioxidant stability of alpha tocopheryl acetate.

Keywords : antioxidant, alpha tocopheryl acetate, DPPH, stability, SLN

INTRODUCTION

Solid Lipid Nanoparticle (SLN) is a dispersion system with nano-sized particles in range 40-1000 with spherical shape (Muller, 2009). SLN has widely used in cosmetic formulation because of its advantages due to its nano-sized particle such as UV protective effect and enhance emmolient (Souto, 2008; Wissing, 2002).

Alpha tocopheryl acetate is derivate from tocopherol that commonly used as an antioxidant in cosmetic due to its properties of photo protective for skin and could decrease skin's damage of free radical (Nam, 2012; Tsai, 2012). As an antioxidant, alpha tocopheryl acetate is easily degraded by light and free radical in air. SLN is one of the system that could prevent the antioxidant degradation of alpha tocopheryl acetate especially because the presence of light. It is result from SLN ability to scattering light with its nano-sized particles and produce physical UV protective effect (Golmohammadzadeh, 2012). The lipid matriks that contained in SLN also has the protective effect for active ingredient (Souto, 2008).

The aim for this study was for investigated the impact of SLN system with binary lipid beeswax and monostearic glyceryl (50:50) on antioxidant stability of alpha tocopheryl acetate compared with simple cream. Antioxidant activity is measured by DPPH method. Combination 50:50 of lipid component beeswax and monostearic glyceryl was selected due to the combination has higher physical stability and drug entrapment (Jenning, 2000; Rosita, 2013).

protein expression. This data might show that it takes time for the cell to synthesize the protein of interest, in this case rhEPO.

Day	Transfected cells /ml media	Untransfected /ml media
0	50.000	50.000
1	827.500	1.100.000
2	1.010.000	1.162.500
3	1.130.000	1.130.000
4	1.030.000	1.050.000
5	942.500	360.000

Table 1. Cell density of cultured cells

To study and compare on how transfection affect cell growth in CHO-K1, CHO-K1 cells were cultured on the same media and time. The data showed that, on day 5, the density of untransfected cells was still high (9.4 x 105 cells/ml media), while the transfected one was about 3.6 x 105 cells/ml media). On day 1, 2, 3, and 4 the density of the cells was comparable. It is very possible that overproduction of specific protein like heterologous recombinant protein production affect the growth of the cell itself.

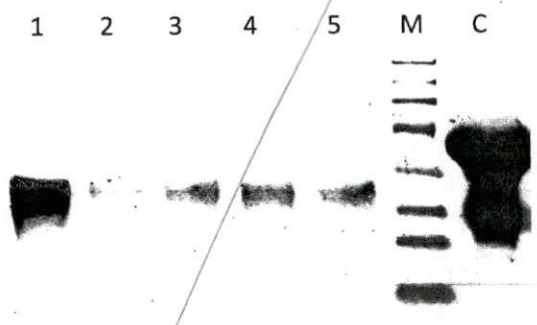

Figure 2. Western blot analysis of rhEPO protein expression. 1-5: day 1 to day 5 (molecular weight: approximately 47 kDa), M: protein marker, C: Positive control (molecular weight: approximately 30 kDa).

CONCLUSION

The data of this research showed that the connection between cell growth, cell density and peak of the interested protein being expressed was complex and not linear. As different cell line and protein may have their own complexity, the best way of finding the right timing, cell density and cell growth to obtain the highest protein production was to perform cell growth and protein expression experiment of that specific protein.

ACKNOWLEDGEMENT

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MATERIALS AND METHOD

Materials:

Tocopheryl acetate, DPPH (Sigma Aldrich), beeswax, monostearic glyceryl, tween 80, (PT. Bratako). Dapar component: acetic acid and sodium citrate (E. Merck) , aquadest (PT. Jawisesa).

Instrument:

Double beam UV Spectrophotometer UV-1800 Shimadzu, One Fourier Transform Infrared (FTIR) Spectrophotometer, pH meter Eutech Instruments pH 700 , Differential Thermal Analyzer (DTA), DelsaTMNano C Particle Analyzer, JEOL JEM-1400 Electron Microscope, Ultra Turrax IKA® T25 Digital High Shear Homogenizer, Sentrifuge Hettich Rotofix 32, Ultrasonic LC 60h Elma, , Hot plate, UV C lamp

Preparation method of SLN

SLN formed by beeswax and monostearic glyceryl (GMS) as lipid core 50: were prepared by high shear homogenization method . 10% of tween 80 was used as surfactant and 20% of propylene glycol was used as co-surfactant. Acetic buffer pH 4.2, μ=0,5 was used as aqueous phase (table 1). They were stirred at 24,000 rpm for 8 minutes, with 30 seconds intervals every two minutes, using an Ultra Turrax homogenizer T-25. The hot dispersion were cooled keep in stirring decreased speed gradually.

Ingredient	Function	Concentration
Beeswax	Lipid	10 %
Monostearic glyceryl	Lipid	
Alpha tocopheryl acetate	Active ingredient	10%
Tween 80	surfactant	10%
Propilen glycol	Co-surfactatnt	20%
Acetic buffer pH 4,2 , μ= 0,5	Aqueous phase	Ad 100%

Table 1. Formula of SLN and simple cream tocopheryl acetate

Characterization of SLN

Measurement of Particle Size

Each sample was diluted with water before measurement. The particle sizes were analyzed by Delsa Nano Particle Size Analyzer at 25°C. Each measurement was performed in triplicates and the particle average diameter and polydispersity index (PI) was determined.

Observation of SLN morphology

The morphology of SLNs was observed by Transmission Electron Microscope (TEM). Either SLN blanks or SLN-PMCA were stained with phosphotungstic acid 2% w/v and placed on copper grids with former film for viewing at 120 kV (JEOL JEM-1400) and operated using software. The shape of SLN observed with Thermal Electron Microscope (TEM) and Drug entrapment of PMCA was measured by centrifugation method.

Measurement IC 50 Value of Alpha Tocopheryl Acetate

IC 50 value of alpha tocopheryl acetate was measured by DPPH method. The series of concentration of alpha tocopheryl acetate was prepared. 2 ml of sample in ethanol solution was centrifuged with 2 ml of DPPH solution. After 30 minutes absorbance of sample was measured by spectrophotometer in 517 nm.

Measurement of Antioxidant stability of Alpha tocopheryl acetate in SLN and simple cream

Each sample of SLN and simple cream alpha tocopheryl acetate was radiated by UV C light as free radical initiator. 1 gram of each sample was taken and diluted in ethanol and then centrifuged at 2500 rpm for 20 minutes. Supernatant was taken 2 ml and then mixed with 2 ml of DPPH. The reduction in DPPH radical was measured by spectrophotometer at 517 nm.

$$\%\ reduction = \left\{\frac{A_{control} - A_{sample}}{A_{control}}\right\} \times 100\%$$

RESULT AND DISCUSSION

SLN-alpha tocopheryl acetate had most spherical shape (fig 1,). Range of particle size, average of particle size and their Polydispersity Index (PI) can be seen in table 2.

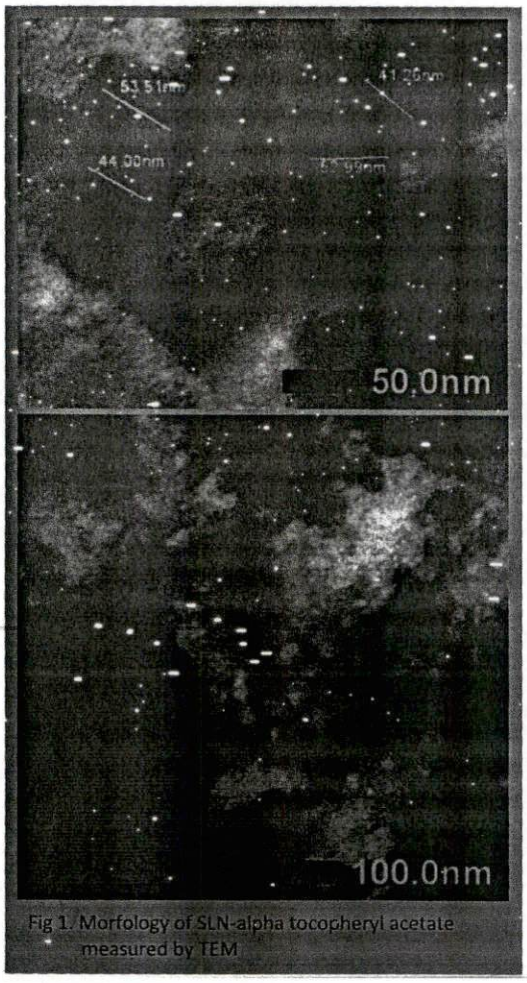

Fig 1. Morfology of SLN-alpha tocopheryl acetate measured by TEM

The existence of the particle size of SLN alpha tocopheryl acetate that are outside the range because the weakness of the manufacturing method of SLN with high shear homogenizer / high speed homogenizer which can result in the size of the microparticles in the SLN (Lason, 2011).

From table 3 it shown the IC 50 value of alpha tocopheryl acetate. The mean value of IC 50 alpha tocopheryl acetate is 2,56% ± 0,0850.

Replication	IC 50 Value (%)	Mean of IC 50 value ± SD (%)
1	2,66	
2	2,53	2,56±0,0850
3	2,50	

Table 3. IC 50 value of alpha tocopheryl acetate

The antioxidant stability of SLN and simple cream alpha tocopheryl acetate was shown in table 4 and fig.2.

System	Replication	Diameter range (nm)	Poly –dispersity Index (PI)	Diameter (nm)	Mean of Particle size ± SD (nm))
SLN alpha tocopheryl acetate	1	252,2 – 2544,9	0,345	900,7	1087,03 ± 256,28
	2	353,5 – 1764,3	0,386	981,1	
	3	338,4 – 6184,0	0,530	1379,3	
Simple cream alpha tocopheryl acetate	1	727,6 – 34129,2	0,766	2302,5	1957,9 ± 321,95
	2	414,7 – 213904,4	0,623	1664,8	
	3	417,9 – 95650,4	0,642	1906,4	

Table 2. Range of particle size, average of particle size and their Polydispersity Index (PI)

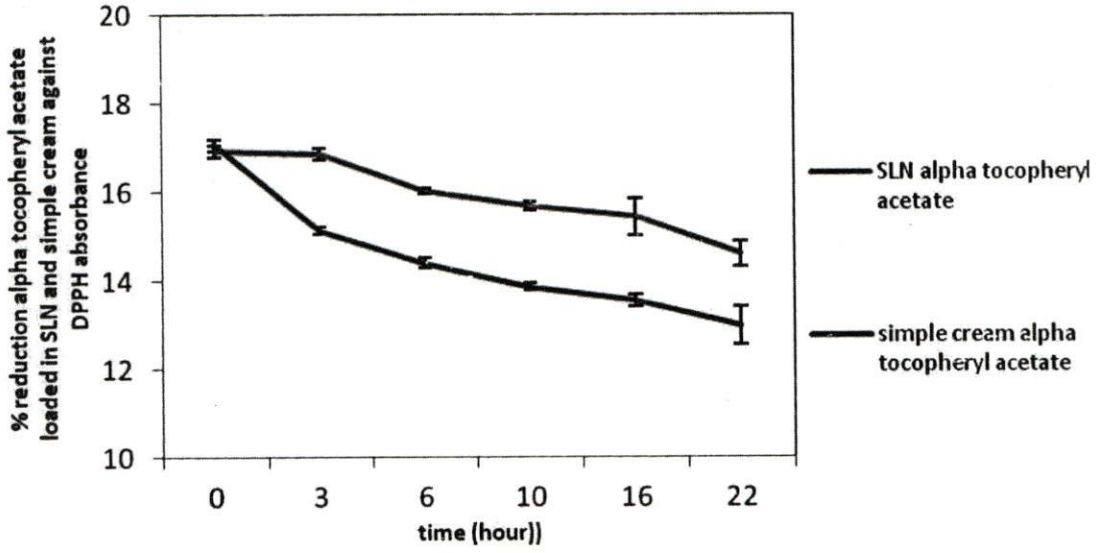

Fig. 2. Graphic % reduction alpha tocopheryl acetate loaded in SLN and simple cream against DPPH absorbance between time

System	Replication	constanta of antioxidant potency degradation between time (/hour)	Mean of constanta of antioxidant potency degradation between time±SD
SLN alpha tocopheryl acetate	1	0,0913	0,1030±0,0175
	2	0,0946	
	3	0,1232	
Simple cream konvensional alpha tocopheryl acetate	1	0,1714	0,1558±0,0169
	2	0,1379	
	3	0,1580	

Table 4. Constanta value of antioxidant potency degradation between time

From graphic in fig.2 it can be determined that SLN alpha tocopheryl acetate shown less sloping than simple cream alpha tocopheryl acetate. It means that decrease of antioxidant stability of alpha tocopheryl acetate loaded in SLN over the time is smaller than loaded in simple cream. This result was confirmed with constanta value of antioxidant potency degradation between time among SLN and simple cream loaded alpha tocopheryl acetate. SLN alpha tocopheryl acetate has smaller value of constanta of antioxidant potency degradation between time than simple cream alpha tocopheryl acetate. This phenomenon may occur as a result of UV blocker by SLN and drug entrapment effect of SLN that could protecting active ingredient.

CONCLUSION

SLN was selected as antioxidant carrier due to its ability to increase antioxidant stability of alpha tocopheryl acetate.

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