Proceeding

The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences

Published and Organized by Faculty of Pharmacy Universitas Airlangga Surabaya-Indonesia 2014

The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences Proceedings

ISBN: 978-602-72333-0-0

(Letter of National ISBN Agency No. 4127/E.8/p/03.2015 Date 18 March 2015)

1st edition Proceeding Published by:

Faculty of Pharmacy Universitas Airlangga Surabaya, Indonesia

Address:

Kampus B Jl. Dharmawangsa Dalam Surabaya 60286 Phone +62 31 5033710 Fax +62 31 5020514

Website: www.icpps2014.com or www.ff.unair.ac.id

Email: icppinfo@gmail.com

ISBN 978-602-72333-0-0

9 7 8 6 0 2 7 2 3 3 3 0 0

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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EFFECT OF TREHALOSE ON THERMAL PROPERTIES OF PHOSPHOLIPID-DDA AND TPGS MIXTURES

Helmy Yusuf, Department of Pharmaceutics, Airlangga University. Jl. Dharmawangsa DAlam, Surabaya 60286, Indonesia. helmy-yusuf@ff.unair.ac.id

INTRODUCTION

The freeze-dried liposome as immunological adjuvant/vaccine delivery systems is one of recent particular interest in the area. DDA is one of the particular interests in immunological adjuvant materials. DDA is a quarterammonium compound with long chain alkyl groups that contribute to its lipophilic properties. It also comprises dimethylammounium headgroup (positively charged) that is attached to the two carbon alkyl chains and contributes to its hydrophilic properties. Both properties of DDA make it suitable for such application in the preparation of cationic liposomes [1-3]. However, DDA liposomes have some drawbacks such as physical instability, as they are easily aggregate in presence of small amounts of salt or even in pure water [7, 8]. As water has been considered as an ideal dispersion medium, then improving DDA formulations is still challenging. This project focuses on the development of potential new formulations of lyophilized liposomes as immunological adjuvant in vaccine delivery system and investigations based on their physicochemical characteristics. Lyoprotectant, i.e. trehalose and membrane stabilizer, i.e. TPGS (D-alpha tocopherol polyethylene glycol 1000 succinate) were used in the freeze-dried liposomes formulations.

EXPERIMENTAL METHODS

Materials

TPGS, DDA and BSA were purchased from Sigma (UK), soyphosphatidylcholines (SPC) was purchased from Lipoid (Germany) and trehalose was purchased from Ferro Pfanstiehl (USA).

Sample Preparation of Phospholipid-DDA-

TPGS mixture

SPC, DDA and TPGS were dissolved and mixed in chloroform/methanol (9:1) solvent system. After solvent was removed, the resulted lipid film was then hydrated with pre-heated (60°C) 10 mM tris buffer (pH 7.4). Trehalose solution at concentration 0.02 M in this buffer were also used as hydration solutions. The ratio of DDA and SPC (1:1) was chosen for practical reason, regarding that these two lipids have same length of acyl chains. Samples were stirred for 30 min (at 60°C) and then sonicated for 2 min (probe sonicator, Fisher Scientific, USA) with pulsative mode. Single lipid components were also prepared in the same way with concentration 50 mg/ml. All samples were then freeze-dried (Advantage, VirTis, USA) for 36 h to obtain solid cakes.

Dry Product Characterization

Water Content. Water content in freeze dried cakes was measured by using thermogravimetric analysis (TGA) at 10°C/min to 200°C. Differential Scanning Calorimetry (DSC). DSC instrument (Q-100, TA Instruments, New Castle, DE USA) was used to determine the phase transition temperature (Tm) of the dried samples. Tm was measured as endothermic peak minimum for the lipid gel-to-liquid crystalline phase transition during the heating scan. Samples were scanned from -20°C to 200°C at 10°C/min. Annealing technique was applied with heating rate 10°C/min in range of 60° -150°C for further investigation. All samples were measured in three replicates using aluminum hermetic. An empty pan was used as reference. Data were analyzed using Universal Analysis Software.

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RESULTS AND DISCUSSIONS

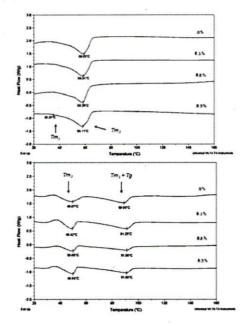


Figure 1. DSC thermograms of freeze-dried DDA-SPC (1:1 molar ratio) mixtures containing different concentration of TPGS as indicated; (A) without trehalose, and (B) with 0.02 M trehalose.

A small concentration of trehalose (0.02 M) was applied to the lipid mixture to make 1:4 molar ratios of sugar towards total lipid concentration of 0.08 M (Table 2.6.). In this particular experiment, the effect of trehalose was examined in the lipid mixtures with TPGS concentration of 0; 0.1; 0.3 and 0.5 mol %. The results showed that trehalose decreased the Tm of the lipid mixtures by approximately 10°C from 58° or 60°C to 49°C, regardless of the concentration of TPGS (Figure 1 and Table 1). The explanation of Tm depression by addition of trehalose can be attributed to the capability of trehalose to restrict or prevent lipid contraction during dehydration processes that would occur in the absence of trehalose (Ohtake, Schebor et al. 2005). This restriction that is achieved by the direct interaction of trehalose with phospholipids has also been reported in another study (Pereira, Lins et al. 2004). It has been proposed previously that the Tm depression of lipid by the presence of trehalose occurs through a 'water replacement' theory (Crowe, Crowe et al. 1985).

Phospholipid	TPGS (mol %)	Trehalose (M)	Tm (°C)		?H (KJ/mol)#		Water Content
			1	2	1	2	(%)
DDA – SPC	0	0	-	60.2 ± 1.2	-	39.1 ± 4.0	3.9 ± 0.2
1:1	0.1		-	58.6 ± 0.2	-	34.9 ± 1.0	3.0 ± 0.4
	0.3		-	58.7 ± 0.4	-%	34.1 ± 6.2	3.1 ± 0.6
	0.5		36.3 ± 1.2	59.7 ± 1.5	0.35 ± 0.3	31.9 ± 1.7	3.1 ± 0.7
DDA - SPC	0	0.02	-	49.2 ± 0.2	-	8.1 ± 1.2**	2.1 ± 0.1
1:1	0.1			49.1 ± 1.5	_	$8.0 \pm 2.3*$	2.2 ± 1.1
	0.3		•	48.7 ± 1.2	- 2	$8.1 \pm 1.8*$	2.8 ± 0.6
	0.5		-	50.2 ± 0.9	-	9.0 ± 0.9	2.3 ± 0.7

*p<0.05, **p<0.01 as compared to the corresponding lipid mixture without trehalose. #the enthalpy values (ΔH of Tm2) of lipid mixtures are presented in units of KJ/mol where the mol was calculated as total of mol fraction of every component in the mixtures.

Table 1. Transition temperature and the melting enthalpy of freeze-dried lipid mixture with and without the presence of trehalose 0.02 M ($n = 3 \pm represents SD$).



CONCLUSION

Trehalose as a lyoprotectant interacts well with the phospholipid-DDA-TPGS mixture through water replacement mechanism is indicated by the decreased Tm of the lipid mixtures in the presence of 0.02 M trehalose.

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