

**Journal of
Biomimetics, Biomaterials
and Biomedical Engineering**

TTP TRANS TECH PUBLICATIONS

[./\(\)](#)[./Payment/Cart](#)LOG IN
(/ACCOUNT/LOG
RETURNURL=%21

Search

Periodicals

Journal of Biomimetics, Biomaterials and Biomedical Engineering

[\(/JBI](#)**ISSN: 2296-9845**[Volumes \(/JBBBE\)](#)[My eBooks \(/JBBBE/ebooks\)](#)[Details \(/JBBBE/Details\)](#)[Editorial Board \(/JBBBE/Editors\)](#)

Editor(s) in Chief

Dr. Sooraj Hussain Nandyala[ORCID \(https://orcid.org/0000-0002-2736-5224\)](https://orcid.org/0000-0002-2736-5224)

Universidade do Porto, Departamento de Física, Faculdade de Ciências; Rua do Campo Alegre 687, Porto, 4169-007, Portugal;

Senior Editors

Dr. Stanislav Kolisnychenko

Editorial Board

Mr. Souvik Das

SHEIKHPARA A. R. M. POLYTECHNIC, GOVT. OF WEST BENGAL, MEDICAL LABORATORY TECHNOLOGY; Sheikhpura, Murshidabad, Nadia, India, 741235;

Prof. Qing Ling Feng

Tsinghua University, School of Materials Science and Engineering; Beijing, China, 100084;

Dr. Yao Dong Gu

Ningbo University; No.818, Fenghua Road, Ningbo, China, 315211;

Prof. Mohammad Abu Jafar Mazumder

King Fahd University of Petroleum and Minerals, Chemistry Department; P. O. Box # 1683, Dhahran, Saudi Arabia, 31261;

Dr. Krishna Madhavan

University of Colorado at Denver, Health Sciences Center; Denver, USA;

Prof. F.N. Oktar

Marmara University, Department of Bioengineering, Faculty of Engineering; Goztepe Campus, Engineering D Building, Kuyubasi, 34722, Turkey;

Prof. Murugan Ramalingam

Centre for Stem Cell Research (a Unit of inStem, Bengaluru), Christian Medical College and Hospital Campus; Bagayam, India, 632002;

Prof. Débora dos Santos Tavares

Federal University of Sergipe, Health Campus, Education in Health Department, Colégio Estadual Prof. Abelardo Romero Dantas; Rua Padre Alvares Pitangueira, n. 248., Centro Lagarto – SE, Brazil, 49.400-000;

DISTRIBUTION & ACCESS (/DISTRIBUTOR)

FOR PUBLICATION (/FORPUBLICATION/CONFERENCE)

SUPPLEMENTS (/SUPPLEMENTS)

ABOUT US (/HOME/ABOUTUS)

POLICY & ETHICS (/HOME/MISCONDUCT)

CONTACT US (/HOME/CONTACTS)

IMPRINT & PRIVACY POLICY (/HOME/IMPRINTANDPRIVACYPOLICY)

SITEMAP (/HOME/SITEMAP)

Scientific.Net is a registered brand of Trans Tech Publications Inc
© 2019 by Trans Tech Publications Inc. All Rights Reserved

[\(/\)](#)中文 (<https://zh.scientific.net/JBBBE.33>)[\(/Payment/Cart\)](#)LOG IN
(/ACCOUNT/LOG
RETURNURL=%2F

Search

Main Themes

Journal of Biomimetics, Biomaterials and Biomedical Engineering Vol. 33

Volume 33

doi: <https://doi.org/10.4028/www.scientific.net/JBBBE.33> (<https://doi.org/10.4028/www.scientific.net/JBBBE.33>)[Papers \(/JBBBE.33\)](#)[Book \(/JBBBE.33/book\)](#)

Search

Paper Title

Page

[Finite Element Analysis for the Estimation of the Ground Reaction Force and Pressure Beneath the Foot Prosthesis during the Gait of Transfemoral Patients \(/JBBBE.33.1\)](#)

1

Authors: Le Van Tuan, Kengo Ohnishi, Hiroshi Otsuka, Yukio Agarie, Shinichiro Yamamoto, Akihiko Hanafusa

Abstract: Ground reaction forces (GRF) and pressure beneath the foot prosthesis are the main parameters used in biomechanical analysis to estimate the joint load
[...more](#)

[Study and Analysis of Mechanical Behavior between Rigid and Dynamic Fixation Systems Analyzed by the Finite Element Method \(/JBBBE.33.12\)](#)

12

Authors: Samir Zahaf, Said Kebdani

Abstract: Orthopedic fixation devices are widely used in treatment of spinal diseases. It is expected that application of dynamic stabilization confers valuable
[...more](#)

- 3D Finite Elements Modeling of the Interfacial Stresses Bone/Dental Implant - Effects of the Geometric Parameters (/JBBBE.33.32)** 32
Authors: Saida Ghoggali, Outtas Toufik, Saber Latrèche
Abstract: A successful osseointegration involves the simultaneous optimization of the primary stability of the implant and the minimization of interfacial stresses bone
[...more](#)
-
- Simulation of *In Vitro*-Like Electrical Activities in Urinary Bladder Smooth Muscle Cells (/JBBBE.33.45)** 45
Authors: Chitaranjan Mahapatra, Rohit Manchanda
Abstract: Urinary bladder smooth muscle (UBSM) generates spontaneous electrical activities due to stochastic nature of purinergic neurotransmitter release from the
[...more](#)
-
- Adaptive Epileptic Seizure Prediction Based on EEG Synchronization (/JBBBE.33.52)** 52
Authors: Sutrisno Ibrahim, Sohaib Majzoub
Abstract: Epilepsy is type of neurological disorder characterized by recurrent seizures that may cause injury to self and others. The ability to predict seizure
[...more](#)
-
- Investigating a Denoising Approach to an Improved Otsu Segmentation on Cell Images (/JBBBE.33.59)** 59
Authors: Kazeem Oyeyemi Oyebode
Abstract: Image denoising provides an opportunity to minimize unwanted signal from any image in order to improve its interpretation by either human or machine.
[...more](#)
-
- An Efficient Computational Approach for the Detection of MR Brain Tissues in the Presence of Noise and Intensity Inhomogeneity. (/JBBBE.33.65)** 65
Authors: G. Sandhya, Giri Babu Kande, T. Satya Savithri
Abstract: The automatic detection of brain tissues such as White Matter (WM), Gray Matter (GM), and Cerebrospinal Fluid (CSF) from the MR images of the brain using
[...more](#)
-
- Effects of Collagen Concentration Variation toward Characteristics of Bacterial Cellulose-Collagen Biocomposites as Candidate of Artificial Dura Mater (/JBBBE.33.80)** 80
Authors: Adanti Wido Paramadini, Prihartini Widiyanti, Djoni Izak Rudyardjo
Abstract: Traffic accident is the highest cause of head injury. In the field of neurosurgery, it is closely related to the defect of duramater (outer layer of the
[...more](#)

Physical Characterization of Liposomes Formulation Lyophilized in the Presence of Disaccharide and HPMC as Dispersed Matrix (/JBBBE.33.88)

88

Authors: Nur Aini Mulyadi, Noorma Rosita, **Helmy Yusuf**

Abstract: The present study focuses on characterization the physical properties of liposome formulation which was dispersed in HPMC matrix and lyophilized in the [...more](#)

Immunological Status of Children Infected with *Helicobacter pylori* (/JBBBE.33.95)

95

Authors: Y.G. Romanenko

Abstract: Chronic gastritis and duodenitis at the children, associated with *Helicobacter pylori* infection, carried out by violations in a cellular link of immunity: [...more](#)

Showing 1 to 10 of 10 Paper Titles

DISTRIBUTION & ACCESS (/DISTRIBUTOR)

FOR PUBLICATION (/FORPUBLICATION/CONFERENCE)

SUPPLEMENTS (/SUPPLEMENTS)

ABOUT US (/HOME/ABOUTUS)

POLICY & ETHICS (/HOME/MISCONDUCT)

CONTACT US (/HOME/CONTACTS)

IMPRINT & PRIVACY POLICY (/HOME/IMPRINTANDPRIVACYPOLICY)

SITEMAP (/HOME/SITEMAP)

Scientific.Net is a registered brand of Trans Tech Publications Inc
© 2019 by Trans Tech Publications Inc. All Rights Reserved

Physical Characterization of Liposomes Formulation Lyophilized in the Presence of Disaccharide and HPMC as Dispersed Matrix

Nur Aini Mulyadi^{1,a}, Noorma Rosita^{1,b} and Helmy Yusuf^{1,c*}

¹Department of Pharmaceutics, Faculty of Pharmacy, University of Airlangga, Indonesia

^anurainimulyadi.2612@gmail.com, ^bitanr@yahoo.com, ^chelmy-yusuf@ff.unair.ac.id

*Corresponding author: helmy-yusuf@ff.unair.ac.id

Keywords: Lyophilized Liposome, Sucrose, Lactose, HPMC, Phase Separation

Abstract. The present study focuses on characterization the physical properties of liposome formulation which was dispersed in HPMC matrix and lyophilized in the presence of disaccharides. The lyophilized formulations featured cationic dimethyldioctadecylammonium (DDA) to produce dry solid and overcome limitations in terms of detrimental phase separation in phospholipid membranes during production process. Disaccharides, such as sucrose and lactose, have been reported to protect phospholipid membranes during drying, while HPMC was used as dispersed matrix to inhibit recrystallization of disaccharide. Their physical properties were characterized including their morphology using scanning electron microscopy (SEM), crystallinity using x-ray diffractometry (XRD), and solid phase separation using differential scanning calorimetry (DSC). On the basis of these evaluations it was found that the presence of sucrose and HPMC in the formulation showed a miscible mixture and relatively less crystalline-forming properties compared to those using lactose, thus potentially construct a stable dried liposomal formulation. The present study reveals prospective advantages of using combination of sucrose and HPMC in development of dried-DDA liposomal formulation.

1. Introduction

Liposome is termed as microscopic spherical vesicles with bilayer membranes which is formed when the phospholipids are exposed to water-rich environments, due to their amphipathic nature [1]. Liposomes have been widely used in drug formulation because of their advantageous features including liposomes are good carriers for both hydrophilic and lipophilic drugs, liposome can decrease toxicity by ensuring target organ specificity, that might reduce the effective dose of the drug [2]. In addition, the use of liposome may reduce the risk exposure to other organs that may experience adverse effects [3]. Despite promising potential as a pharmaceutical carrier, the drawback of phospholipid membranes during production restrict liposomal application [4,5].

One of the most efficient ways to produce dry liposomes is by lyophilization [6]. The removal of water from liposomal system prevents the degradation of phospholipid caused by hydrolysis. Moreover, solid product may reduce movement of molecules and led to overcome limitations in terms of delicate compared to those liquid product [7,8,9].

Preservation of dried liposome has been achieved by the discovery that sugars can protect membranes during dehydration [10,11]. Sugar molecules act to replace water molecules bound to lipid headgroups and maintain headgroup spacing in the dry state similar to that of the hydrated lipid [12,13].

A cationic lipid dimethyl-dioctadecylammonium (DDA), a cationic surfactant from quaternary (tetraalkyl) ammonium group has been attracted attention and was selected as component of the developed formulation. DDA has been widely reported to carry an adjuvant effect to elicit humoral and cell-mediated immune responses [14,15].

In the present study, the new strategies in enhancing preservation of dried liposomal formulation were employed. This include the use of disaccharides (sucrose and lactose) as lyoprotectant and hydroxypropylmethylcellulose (HPMC) as dispersed matrix to inhibit crystallization of components [16]. Therefore, the effect of disaccharides and

hydroxypropylmethylcellulose (HPMC) on the physical properties of the developed formulations were characterized. Their physical properties including morphology, crystallinity, and solid phase separation were evaluated. Information on such characteristics may offer future perspective into development of potential formulations of dehydrated liposomes.

2. Research Methods

2.1 Instruments and Materials

The instruments were used for liposome synthesis included rotary evaporator (Buchi Rotavapor R-215, Swiss), waterbath sonicator (Sonica Ultrasonic, Swiss), lyophilizer (VirTis 2KBTXL-75 Benchtop SLC Freeze Dryer). The test instruments used comprised scanning electron microscope (Hitachi TM 3000 Tabletop Microscope, Japan), differential scanning calorimeter (Mettler Toledo SP 85, Swiss), x-ray diffractometer (X'PertPRO PANalytical, Netherlands), and double beam spectrophotometer UV-Vis (Shimadzu UV-1800).

The materials were used as liposomal former included soybean phosphatidylcholine/SPC (Lipoid GmbH, Germany) and dimethyl-dioctadecylammoniumbromide/DDAB (Sigma Aldrich, Singapore), while cholesterol (Sigma-Aldrich, Singapore) was used as a liposomal membrane stabilizer. Hydroxypropylmethylcellulose (HPMC) (Shin-Etsu, Japan) was used as a dispersing matrix and increase the mass of compact matrix for liposomes, while sucrose and lactose (Sigma-Aldrich, Singapore) were used as lyoprotectant. Methanol (E. Merck) was used of analytical grade as a solvent to facilitate the mixing of liposomal ingredients.

2.2 Preparation of Dried Liposomes

Liposomes were prepared by thin film hydration technique. Briefly, all weighed amount lipids in mol ratio of 1:1:0,1 (DDA:SPC:Cholesterol) were dissolved in methanol. Solvent was evaporated under vacuum for an hour. The lipid film was hydrated with pre-warmed (50°C) disaccharide aqueous solution (5%w/v or 10%w/v) under consistent stirring for 10 min. The formed liposomes were sonicated using waterbath sonicator for 5 min, then dispersed into a pre-formed HPMC gel (2.5%w/v or 7.5%w/v). The liposomal formulation was subsequently lyophilized at -20°C for 48 hours to obtain solid product. All prepared formulations can be seen in Table 1.

Table 1. Prepared Formulation of Dried Liposome.

Formulation	Sucrose Concentration	Lactose Concentration	HPMC Concentration
FS1	5%	-	2.5%
FS2	10%	-	2.5%
FS3	5%	-	7.5%
FS4	10%	-	7.5%
FL1	-	5%	2.5%
FL2	-	10%	2.5%
FL3	-	5%	7.5%
FL4	-	10%	7.5%

2.3 Morphology Test using Scanning Electron Microscope (SEM)

The morphology of the entrapped liposomes as well as the solid matrix was investigated by SEM. Portions of the dried product were scattered and glued onto 25 mm diameter plates, which were attached to SEM specimen mounts. The specimens were sputter coated with a layer of gold approximately 5 nm in thickness for 180 seconds and specimens were examined on magnification 5000x.

2.4 Thermal Analysis using Differential Scanning Calorimeter (DSC)

Thermal properties of liposome formulation formed were examined by using the DSC (differential scanning calorimeter) instrument. Heating rate is 10°C per minute at a temperature range of 30-250°C. The transition temperature of the sample is observed through a generated

thermogram and measured as endothermic peak minimum of phase transition (T_m) during the heating scan. Formation of a new phase which does not undergo phase separation (miscible) and high phase transition temperature may good signs that liposomes can be stored in relatively higher temperature.

2.5 Crystallinity Analysis using X-Ray Diffractometer (XRD)

Crystallinity of the dry product were examined by XRD (X-ray diffractometer). Samples were weighed and inserted into the holder and the surface leveled. Furthermore, the holder is inserted into the instrument with the following conditions: Cu metal target, voltage of 40 kV, current of 30mA, slit width of 0.2 inch. Data is collected by scanning mode 0,2-0,5° per minute with a scanning distance $2\theta = 5-40^\circ$.

3. Result and Discussion

3.1 Scanning Electron Microscopy Data (SEM)

The morphology of liposomes and the solid matrix were imaged by SEM (figure 1 and 2). The micrographies showed that the liposomes were intactly observed in all formulations. FS4 and FL4 which contained sucrose and lactose, respectively at concentration of 10% and also HPMC at concentration of 7.5% showed relatively larger and aggregated vesicles trapped in the solid matrices (figure 1b and figure 2b). In contrast, FS3 and FL3 with HPMC at concentration of 2.5% showed more porous matrices than FS4 and FL4 (figure 1a and figure 2a).

Furthermore, all liposomes were spherical shaped with smooth surface and intact in porous matrices structure. Such porous matrices are beneficial in terms of a more rapid reconstitution [17].

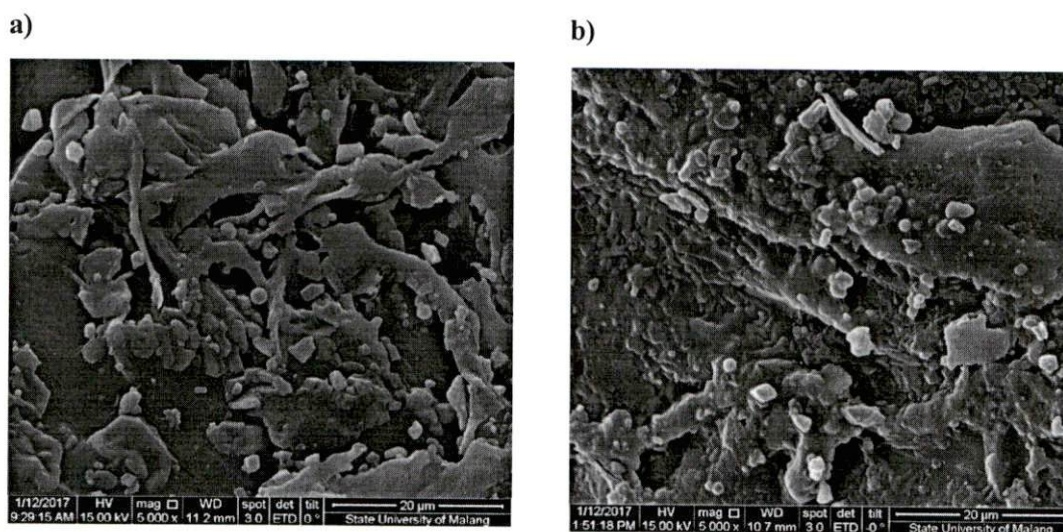


Fig. 1. Scanning electron microscope image of lyophilized liposomal powders containing sucrose with magnification of 5000x: (a) FS3 and (b) FS4.

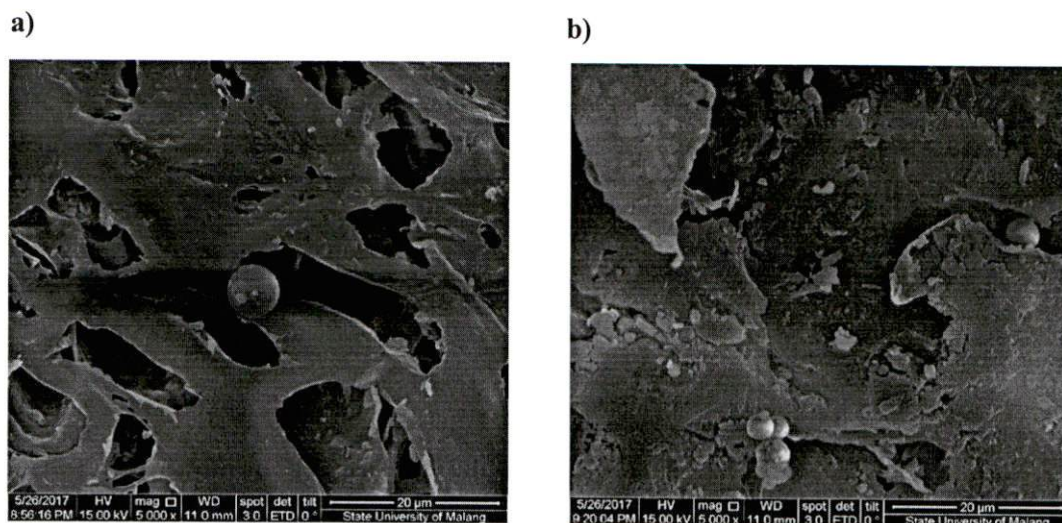


Fig. 2. Scanning electron microscope image of lyophilized liposomal powders containing lactose with magnification of 5000x: (a) FL3 and (b) FL4.

3.2 Differential Scanning Calorimetry Data (DSC)

Phase separation in liposomal mixtures can give disadvantage for their application such as make liposomal membrane collapse [18,19]. To explore this phenomenon thoroughly, phase separation of phospholipids mixtures was investigated using DSC. The DSC thermograms showed the effect of disaccharides on the phase behavior (i.e. phase separation) of dehydrated phospholipids mixtures.

First, as can be seen from figure 3 and 4, the splitting endothermic peaks were observed for both sucrose and lactose formulations. In case of sucrose-containing formulas, FS2, FS3, and FS4 showed splitting endothermic peaks were observed at 190°C and 222°C. These peaks were phase separated sucrose as they were confirmed with its single thermogram that showed peaks at 193°C and 220°C (data not shown). Moreover, FS2 and FS3 exhibited exothermic peaks respectively at 148.25°C and 153.54°C that indicated a re-crystallization was occurred. The obtained solid product from FS2 and FS3 might be metastable as they underwent recrystallization upon heating. However, FS1 did not show an exothermic peaks and exhibited slightly broad distribution, indicating more homogenous mixtures were achieved.

The splitting endothermic peaks were also observed in the lactose-containing formulation at 143°C and 220°C. They were further confirmed to be phase separated lactose for their identical profile to single lactose (data not shown). Moreover, FL1; FL2 and FL3 exhibited exothermic peaks respectively at 176.53°C, 181.36°C, and 168.73°C, indicating that they were metastable. This event was not observed in FL 4, indicating that the mixture was relatively more stable.

Sucrose or lactose at concentrations of 5%w/v with HPMC at concentrations of 2.5%w/v did not lead to phase separation during freeze drying, indicating a good miscibility of all components. Moreover, the HPMC matrix at concentration 2.5%w/v might be sufficient in inhibiting recrystallization of all components upon manufacturing processes. We suggested that neither sucrose or lactose molecules interact to other sucrose or lactose molecules as the function of HPMC matrix that act as barrier and preventing crystallization.

Furthermore, the presence of disaccharides between the lipid head molecules might increase attraction between the head groups of phospholipids packing, therefore increasing the integrity of the liposomal membrane [20,21]. In this case, we suggested that sucrose or lactose are potentially fulfilling the purpose of preserving liposomal membrane as function of concentration.

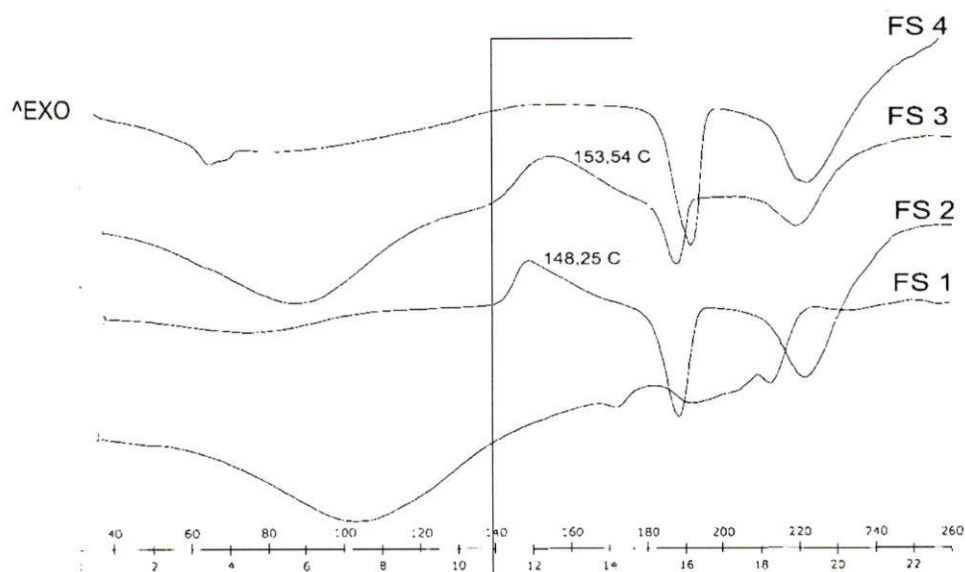


Fig. 3. DSC thermogram of sucrose-containing formulations (FS1-FS4).

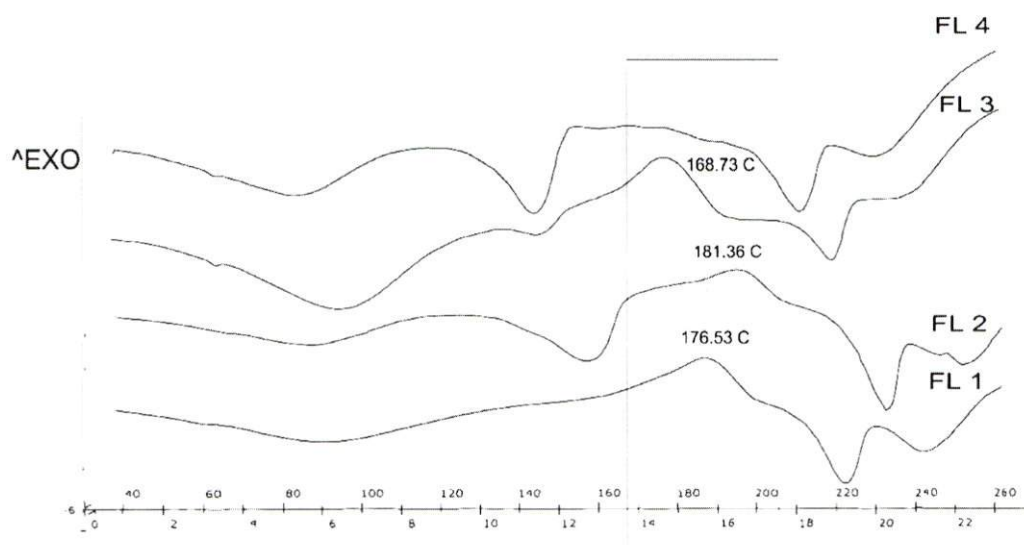


Fig. 4. DSC thermogram of lactose-containing formulations (FL1-FL4).

3.3 X-Ray Diffraction Analysis Data (XRD)

The resulting XRD diffractograms were shown in figure 5 and 6. The formulations of FS1 and FL1 showed much less intensive peaks, indicating that both formulas exhibited less crystalline-forming properties.

Nevertheless, FS2 and FL2 with higher concentration of sucrose or lactose showed sharper peaks. These reveal that there might be portion of disaccharide still present in crystal form. There was possibility that some sucrose or lactose molecules interact each other causing the molecules to recrystallize. Thus, the present of HPMC matrix at concentration of 2.5%w/v was not able to act as barrier to prevent recrystallization of sucrose or lactose at concentration of 10%w/v.

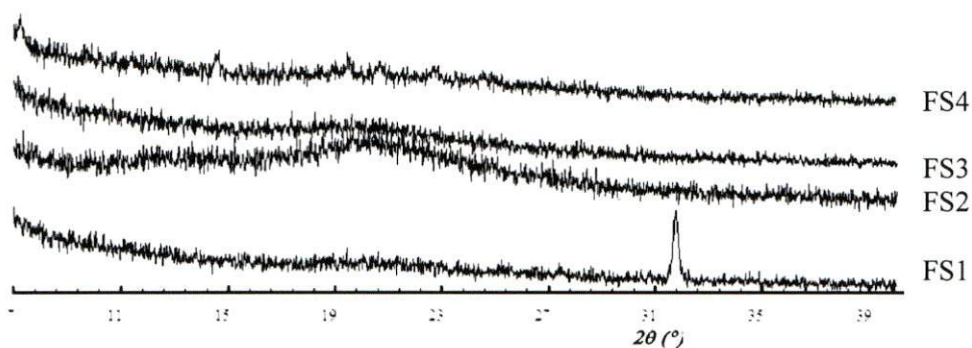


Fig. 5. X-ray diffractogram of sucrose-containing formulations (FS1-FS4).

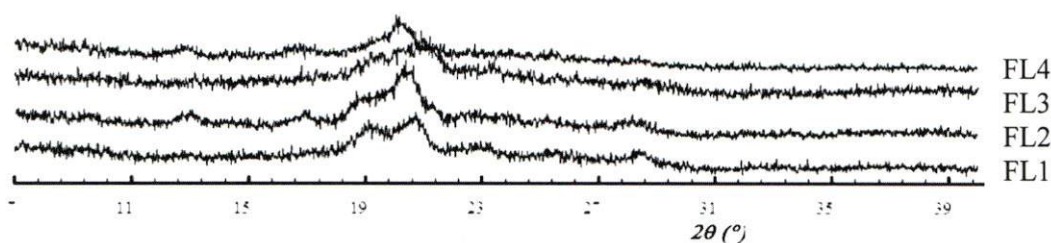


Fig. 6. X-ray diffractogram of lactose-containing formulations (FL1-FL4).

4. Conclusions

The present study demonstrates the potential application of sucrose or lactose as lyoprotectant together with HPMC as dispersed matrix for the development of lyophilized DDA liposomal formulation. The amount of disaccharide (sucrose or lactose) and HPMC in formulation significantly affect the phase behavior of lipid mixtures. The addition of sucrose or lactose at concentration of 5%w/v and HPMC at concentration of 2.5%w/v in liposomal formulation showed more miscible mixtures which preserved intact liposomes on amorphous and porous matrix. The developed liposomal formulation in form of dry solid may be potential for pharmaceutical application.

5. Acknowledgements

This research received funding form the Directorate General of Higher Education, The Republic of Indonesia.

References

- [1] G. Bozzuto and A. Molinari, Liposomes as nanomedical devices, *Int. J. Nanomedicine*, Feb.2015, p. 975.
- [2] W. Andreas., V.U. Karola, Liposome Technology for Industrial Purposes. *J. Drug Deliv.* (2011) 1–9.
- [3] A. Akbarzadeh, R.R. Sadabady, S. Davaran, S.W. Joo, N. Zarghami, Y. Hanifehpour, M. Samiei, M. Kouhi, K.N. Koshki, Liposome: classification, preparation, and applications, *Nanoscale Res. Lett.*, 8, 1 (2013) 102.
- [4] A. Samad, Y. Sultana, M.Aqil, Liposomal drug delivery systems: An Update Review, *Current Drug Delivery*. 4 (2007) 297-305.
- [5] A. R. Mohammed, V. W. Bramwell, A. G. A. Coombes, and Y. Perrie, Lyophilization and sterilization of liposomal vaccines to produce stable and sterile products, *Methods*, 40, 1, (2006) 30–38.

- [6] A.Samad, Y.Sultana, M.Aqil, Liposomal drug delivery systems: An Update Review, *Current Drug Delivery*, 4 (2007) 297-305.
- [7] O. S. Kumru, S. B. Joshi, D. E. Smith, C. R. Middaugh, T. Prusik, and D. B. Volkin, Vaccine instability in the cold chain: Mechanisms, analysis and formulation strategies, *Biologicals*, vol. 42, 5 (2014) 237–259.
- [8] D. M. Matthias, J. Robertson, M. M. Garrison, S. Newland, and C. Nelson, Freezing temperatures in the vaccine cold chain: A systematic literature review, *Vaccine*, vol. 25, no. 20, May 2007, 3980–3986.
- [9] N. Vijaykumar, V. Natarajan, B. Guru V, Proliposomes for Oral Delivery: Progress and Challenges. *Curr. Pharm. Biotechnol.* 16 (2015) 303–31
- [10] P. Cristina, L. Roberto, C. Indira, F. Luis Carlos, H. Philippe, Interaction of the Disaccharide Trehalose with a Phospholipid Bilayer: A Molecular Dynamics Study, *Biophys. J.* 86 (2004) 2273–2285.
- [11] E.A. Disalvo, F. Lairion, F. Martini, E. Tymczyszyn, M. Frascas, H. Almaleck, G.J. Gordillo, Structural and functional properties of hydration and confined water in membrane interfaces. *Biochim. Biophys. Acta BBA - Biomembr.* 1778 (2008) 2655–2670.
- [12] O. Satoshi, S. Carolina, P. Sean P., de Pablo, Juan J, Phase behavior of freeze-dried phospholipid–cholesterol mixtures stabilized with trehalose. *Biochim. Biophys. Acta BBA - Biomembr.* 1713 (2005) 57–64.
- [13] I. Pall Thor, Y. Mingshi, N. Hanne Morck, R. Jukka, and F. Camilla, Stabilization of liposomes during drying. *Expert Opin. Drug Deliv.* 8 (2011) 375–388.
- [14] D. Christensen, M.Henriksen-Lacey, A.T.Kamath, T.Lindenstrøm, K.S.Korsholm, J.P.Christensen, A.F.Rochat, P.H.Lambert, P.Andersen, C.A.Siegrist, Y.Perrie, E.M.Agger, A cationic vaccine adjuvant based on a saturated quaternary ammonium lipid have different in vivo distribution kinetics and display a distinct CD4 T cell-inducing capacity compared to its unsaturated analog, *J. Controlled Release.* 160, 3 (2012) 468–476.
- [15] L. Holten-Andersen, T. M. Doherty, K. S. Korsholm, and P. Andersen, Combination of the Cationic Surfactant Dimethyl Dioctadecyl Ammonium Bromide and Synthetic Mycobacterial Cord Factor as an Efficient Adjuvant for Tuberculosis Subunit Vaccines, *Infect. Immun.* 72, 3 (2004) 1608–1617.
- [16] S.B.Tejaa, S.P.Patila, G.Shetea, S.Patelb, A.K.Bansal, Drug-excipient behavior in polymeric amorphous solid dispersions, *J. Excip. Food Chem*, 4 (2013) 70–94.
- [17] Y. Helmy, N. Raditya, M. Nur Aini, S. Dwi, R. Noorma, Phase Behavior of Dried – DDA Liposomal Formulation Dispersed in HPMC Matrix in the presence of Saccharides, *Int. J. PharmTech Res.* 10, 1 (2017) 50-56.
- [18] E. M. Curtis, X. Xiao, S. Sofou, and C. K. Hall, Phase Separation Behavior of Mixed Lipid Systems at Neutral and Low pH: Coarse-Grained Simulations with DMD/LIME, *Langmuir.* 31, 3 (2015) 1086–1094.
- [19] S. Salmaso and P. Caliceti, Stealth Properties to Improve Therapeutic Efficacy of Drug Nanocarriers, *J. Drug Deliv.* 2013, 1–19.
- [20] S. Ohtake, C. Schebor, S. P. Palecek, and J. J. de Pablo, Phase behavior of freeze-dried phospholipid–cholesterol mixtures stabilized with trehalose, *Biochim. Biophys. Acta BBA - Biomembr.* 1713, 1, (2005) 57–64.
- [21] G. van den Bogaart, N. Hermans, V. Krasnikov, A. H. de Vries, and B. Poolman, “On the Decrease in Lateral Mobility of Phospholipids by Sugars,” *Biophys. J.* 92, 5, (2007) 1598–1605.