

Dynamics insights into aggregation of phospholipid species with cholesterol and vitamin C

Dwi Hudyanti¹, Sherllyn Meida Christa², Nur Hanna Mardhiyyah², Daru Seto Bagus Anugrah³, Tatik Widiaroh⁴, Parsaoran Siahaan¹

¹ Department of Chemistry, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia

² Chemistry Program, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia

³ Department of Biotechnology, Faculty of Biotechnology, Atma Jaya Catholic University of Indonesia, Tangerang, Indonesia

⁴ Department of Statistics, Faculty of Science and Mathematics, Diponegoro University, Semarang, Indonesia

Corresponding author: Dwi Hudyanti (dwi.hudyanti@live.undip.ac.id)

Received 30 January 2022 ♦ Accepted 13 April 2022 ♦ Published 10 May 2022

Citation: Hudyanti D, Christa SM, Mardhiyyah NH, Anugrah DSB, Widiaroh T, Siahaan P (2022) Dynamics insights into aggregation of phospholipid species with cholesterol and vitamin C. *Pharmacia* 69(2): 385–391. <https://doi.org/10.3897/pharmacia.69.e81435>

Abstract

This paper provides dynamic insight into the aggregation profile of systems containing six different phospholipid species, cholesterol, and vitamin C thru Coarse-Grain Molecular Dynamics (CGMD) simulations. The simulation used 42 systems, and each system was composed of 220 molecules of each phospholipid species, a varied number of cholesterol molecules (0, 11, 22, 33, 66, 88), and 10 vitamin C molecules. The phospholipid species were DLPE, DOPE, DLiPE, DOPS, DLiPS, and DLiPC. We found curved bilayer, toroidal bilayer, concave micelle, disc-like bilayer, planar bilayer, and liposome structures in the systems during the 40 ns simulation. The systems with a ratio cholesterol:phospholipid between 15% and 40% formed liposomes regardless of the phospholipid species. Cholesterol is positioned in the liposome bilayer while vitamin C is encapsulated in the aqueous core of liposomes for all cholesterol compositions. The cholesterol influences the liposome formation of various phospholipid species and the encapsulation of vitamin C in the liposome structure.

Keywords

Coarse-Grain Molecular Dynamics, liposome, membrane, self-assembly, vesicle

Introduction

Over the past few years, the use of vitamin C as an active agent has increased rapidly. People consider that vitamin C has antioxidant and other beneficial properties such as whitening effect. In addition, vitamin C boosts the body's immune system by speeding up the production of T cells and B cells that play a role in killing bacteria and viruses, also aiding other cell types in the immune system (Alkandahri et al. 2018). Despite the above properties,

vitamin C is unstable to light, temperature, and changes in pH (Sheraz et al. 2015). Due to these shortages, we need an effective delivery method for vitamin C.

Phospholipid encapsulation is the right choice because they are more affordable and easy to obtain than other delivery methods. Phospholipids have the self-assembly ability to form structures such as liposomes. Research showed that liposomes encapsulate vitamin C and that cholesterol addition can overcome leakage of liposomes (Hudyanti et al. 2018, 2019a; Liu et al. 2020). However, molecular

dynamics information on structural characteristics and the aggregation profile of phospholipid self-assembled structure with cholesterol and vitamin C are not established yet.

Coarse-Grained Molecular Dynamics (CGMD) simulation suits an exemplary method (Joshi and Deshmukh 2020; Liwo et al. 2021). It provides information about the molecular dynamics of the systems that we may not obtain experimentally without using a high-specification computer. The CGMD simplifies one to four heavy atoms into one bead while still carrying the properties and dynamics of those molecules. It also allows modification of the simulated environment and presents similar data as standard computer software. Previously, molecular dynamics simulation with coarse-grained modeling has determined the aggregation profile of self-assembled phospholipid structure and proved that the liposomes are metastable structures and water molecules are within the liposome core (Hudiyanti et al. 2014). However, the molecular dynamics of phospholipids with cholesterol and vitamin C in their self-assembled structures have not been fully established. Therefore, this study provides a new understanding of the liposomes' structural characteristics and aggregation profiles that contain phospholipids, cholesterol, and vitamin C.

Materials and methods

The research consisted of three main stages, namely (i) the system preparation consisting of phospholipid molecules, cholesterol, and vitamin C, (ii) the molecular dynamics simulations, and (iii) the analysis of phospholipid aggregation profile data. Molecular models were derived from Protein Data Bank, and PubChem (Wang et al. 2009), then prepared with the Open Babel package (O'Boyle et al. 2011) and modified with Molefactory on Visual Molecular Dynamics (VMD) package (Humphrey et al. 1996). Fig. 1 presents all the phospholipids, cholesterol, and vitamin C structures used in the simulation.

The coarse-grained structure of phospholipids follows Hudiyanti (Hudiyanti et al. 2014), cholesterol coarse-grained structure follows the "angle-corrected" model (CT3-Me2b) of the Daily (Daily et al. 2014). The coarse-grained structure of vitamin C modifies the model of HSP of Periole & Marrink (Periole and Marrink 2013). Ten to 14 beads represented each phospholipid molecule, ten beads modeled cholesterol molecules, and four beads modeled vitamin C molecules. Fig. 2 presents the coarse-grained structure of all molecules used in this simulation. The systems composition contained 220 phospholi-

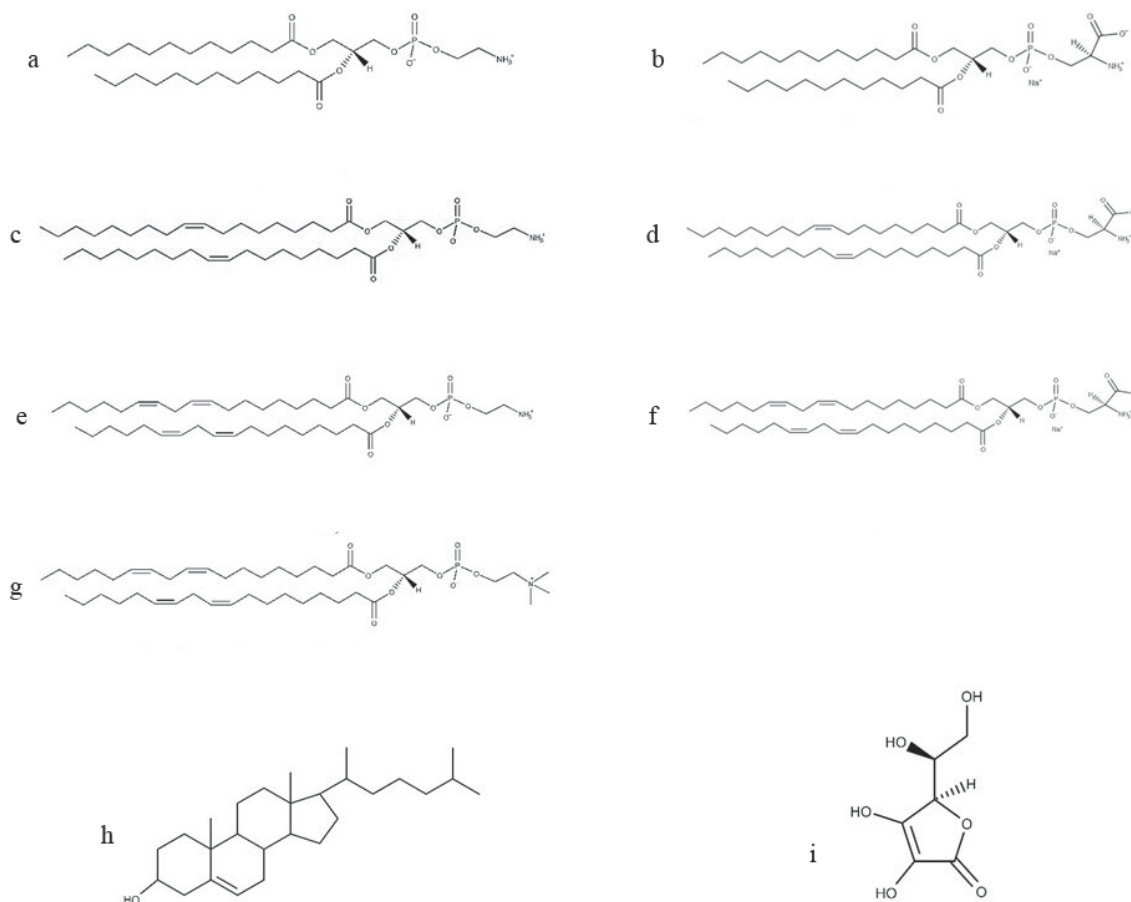


Figure 1. Molecular structure of Phospholipids (a–f), cholesterol (h), and vitamin C (i). **a** 1,2-Dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE) **b** 1,2-Dilauroyl-*sn*-glycero-3-phosphoserine (DLPS) **c** 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) **d** 1,2-Dioleoyl-*sn*-glycero-3-phosphoserine (DOPS) **e** 1,2-Dilinoleoyl-*sn*-glycero-3-phosphoethanolamine (DLiPE) **f** 1,2-Dilinoleoyl-*sn*-glycero-3-phosphoserine (DLiPS) **h** Cholesterol **i** Vitamin C.

pid molecules (Table 1), cholesterol (0; 11; 22; 33; 66; 88 molecules), and ten vitamin C molecules. Molecules were randomly placed in an 11 nm cube-shaped box using the Packmol package (Martínez et al. 2009).

Table 1. Phospholipids species used in this study.

Phospholipid species	Number C atoms: Number of Double Bonds of Phospholipid acyl groups		
	12:0 (DL)	18:1 (DO)	18:2 (DLi)
Ethanolamine Head Group (PE)	DLPE	DOPE	DLiPE
Serine Head Group (PS)	DLPS	DOPS	DLiPS
Choline Head Group (PC)	–	–	DLiPC

Coarse-grained-based residues are applied and identified on the system with AutoPSF based on MARTINI Force Field (Periole and Marrink 2013) using the VMD package. VMD package is used to convert all-atom structures into coarse-grained structures. We applied a solvation process to the system to provide an

aqueous environment. Meanwhile, ionization is designed to neutralize the system by adding Na⁺ and Cl⁻ ions. Based on the MARTINI Force Field, each bead interacts with the potential pair of Lennard-Jones (LJ) and van der Waals forces. As for modeling electrostatic interactions between zwitterion head groups on the system based on Coulomb's (Marrink et al. 2007). The total energy system was initially minimized and simulated in an aqueous milieu with a density of 0.00609 atoms/A³ using the Scalable Molecular Dynamics (NAMD) package to determine the self-assembled phospholipid structure. We performed the simulation process in the NPT ensemble (conditioning pressure state constant around 1.01325 bar). The minimization was applied to reach the system's lowest energy state by minimizing interaction between atoms, adjusting the structure to the force field, providing homogenous solvent distribution, and reducing steric collisions in the system (Hudiyanti et al. 2014). This lowest energy indicated the system's most stable conformation before aggregation proceeded. The achievement of energy convergence during minimization in 0.6 ns shows the lowest

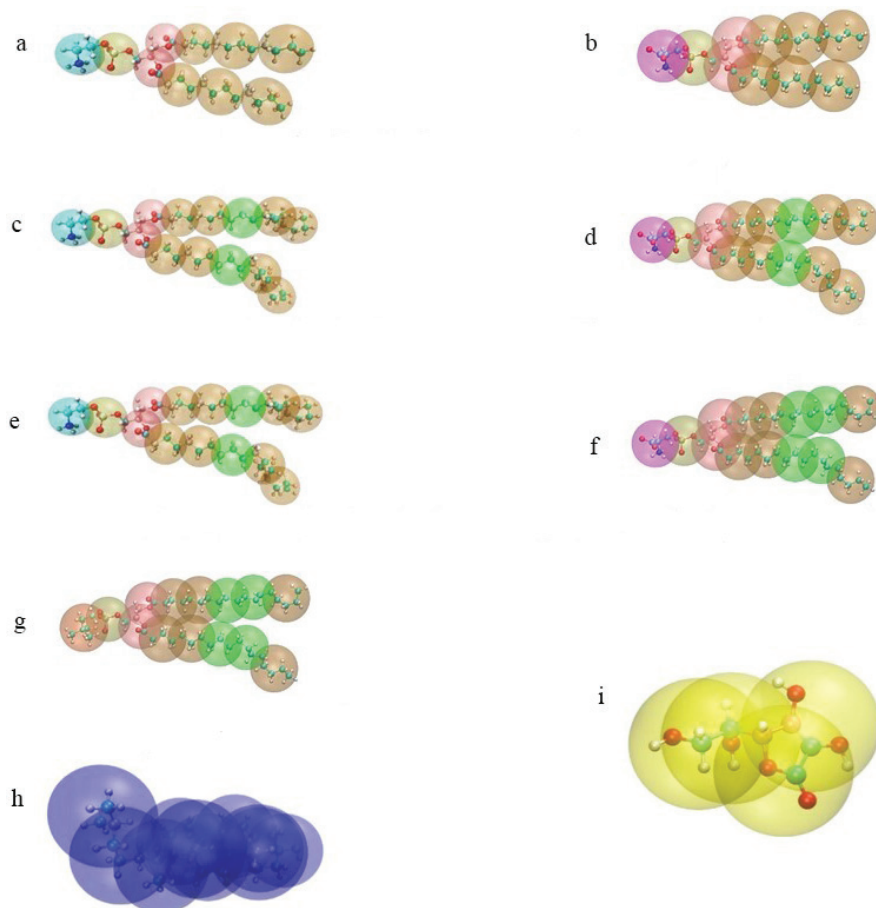


Figure 2. The coarse-grained structure of all molecules in the simulation. The phospholipid in fig (a–g): light blue bead for ethanolamine head group, purple for serine head group, orange for choline head group, light brown for phosphate, pink for glycerol backbone, dark brown for hydrocarbon tail group, and green for the double bond on hydrocarbon tail group; dark blue beads for cholesterol in fig (h); and yellow beads for vitamin c in fig (i). **a** 1,2-Dilauroyl-*sn*-glycero-3-phosphoethanolamine (DLPE) **b** 1,2-Dilauroyl-*sn*-glycero-3-phosphoserine (DLPS) **c** 1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) **d** 1,2-Dioleoyl-*sn*-glycero-3-phosphoserine (DOPS) **e** 1,2-Dilinoleoyl-*sn*-glycero-3-phosphoethanolamine (DLiPE) **f** 1,2-Dilinoleoyl-*sn*-glycero-3-phosphoserine (DLiPS) **h** Cholesterol **i** Vitamin C.

total energy system to perform dynamics simulations. The dynamics simulation duration is 40 ns. We used periodic boundary conditions (PBC) for the simulations and 40 fs time step integration. The VMD package visualizes and analyzes both the preparation and simulation outputs. The simulation gives the aggregation profile of the self-assembled structures. We analyzed important parameters: Root Mean Square Deviation (RMSD), aggregate structure, the total energy of the system, liposomes lifetime, area per lipid, membrane thickness, and liposomes size.

Results and discussion

The molecular dynamics simulation provides an overview of the self-assembled process and properties of phospholipids with cholesterol and vitamin C. Table 2 presents aggregate structures and total energy related to the systems. Parameters related to liposome structures during 40 ns of simulation, namely liposome lifetime, area per lipid, membrane thickness, and liposome size, were presented in Table 3. The phospholipid species, the cholesterol concentration in the system, and the addition of vitamin C affect the aggregate structures and parameters. Particular attention was directed to the systems that formed liposomes during simulations. Based on previous research conducted by Hudiyanti (Hudiyanti et al. 2014), the structure of liposome as a self-assembled phospholipid structure can be obtained in about 40 ns simulation time for as many as 256 phospholipid molecules. Therefore, in this research, the simulation time of 40 ns is assumed to be sufficient to simulate 220 phospholipid molecules until liposomes are formed.

RMSD analysis

RMSD value describes the average distance between each atom in the system at a specific time. A molecule with an RMSD value that does not change much over time indicates a stable molecular conformation. The structural stability of the systems during simulation is suggested by the converging curve of RMSD, for example, of system 220DLiPE-88Chol-10VitC, as shown in Fig. 3. Convergency of RMSD value during simulation becomes the basis for further parameter analysis. All data presented are collected and calculated after conforming to the convergency of the RMSD value between 4.21–4.83 Å during 40 ns simulation time.

Aggregate structures

We initiated the simulation with a random molecular position. After some time, the scattered molecules will interact and form various self-assembled structures such as toroidal bilayer, disc-like bilayer, liposomes, and planar bilayer, as presented in Fig. 4. The formation of liposomes can be as early as 3.48 ns for DOPS and as late as 36.48 ns for DLPS after the simulation commence, see Table 3. In Fig. 4 a system formed liposomes, and vitamin C with its hydrophilic property was encapsulated in the aqueous compartment or the liposomes core together with water

Table 2. Aggregate structures and total energy of system during 40 ns of simulation.

Phospholipid Species	System Composition [Phospho-Chol-VitC]	Aggregate Structure	Total energy (kcal/mol)
DLPE	220-00-00	Curved bilayer	-63,888
	220-00-10	Toroidal bilayer	-63,749
	220-11-10	Toroidal bilayer	-63,224
	220-22-10	Curved bilayer	-62,555
	220-33-10	Liposome	-62,138
	220-66-10	Liposome	-61,142
DLPS	220-88-10	Liposome	-58,692
	220-00-00	Concave Micelle	-64,614
	220-00-10	Toroidal bilayer	-64,624
	220-11-10	Disc-like bilayer	-64,157
	220-22-10	Disc-like bilayer	-63,381
	220-33-10	Liposome	-62,939
DOPE	220-66-10	Liposome	-61,339
	220-88-10	Liposome	-59,412
	220-00-00	Disc-like micelle	-60,832
	220-00-10	Planar bilayer	-60,566
	220-11-10	Curved bilayer	-59,839
	220-22-10	Liposome	-59,311
DOPS	220-33-10	Curved bilayer	-58,862
	220-66-10	Liposome	-56,922
	220-88-10	Liposome	-56,014
	220-00-00	Disc-like Micelles	-61,445
	220-00-10	Disc-like bilayer	-61,219
	220-11-10	Disc-like bilayer	-60,506
DLiPE	220-22-10	Disc-like bilayer	-60,184
	220-33-10	Disc-like bilayer	-59,638
	220-66-10	Liposome	-57,997
	220-88-10	Liposome	-56,534
	220-00-00	Disc-like bilayer	-60,826
	220-00-10	Disc-like bilayer	-60,604
DLiPS	220-11-10	Disc-like bilayer	-60,075
	220-22-10	Liposome	-59,596
	220-33-10	Liposome	-58,985
	220-66-10	Liposome	-57,440
	220-88-10	Liposome	-56,200
	220-00-00	Planar Bilayer	-62,282
DLiPC	220-00-10	Disc-like bilayer	-61,954
	220-11-10	Disc-like bilayer	-61,454
	220-22-10	Disc-like bilayer	-60,902
	220-33-10	Disc-like bilayer	-60,323
	220-66-10	Liposome	-58,627
	220-88-10	Liposome	-57,111
DLiPC	220-00-00	Liposome	-60,229
	220-00-10	Disc-like bilayer	-60,117
	220-11-10	Disc-like bilayer	-59,262
	220-22-10	Liposome	-58,686
	220-33-10	Liposome	-58,421
	220-66-10	Liposome	-56,569
	220-88-10	Liposome	-55,778

molecules. Besides that, unencapsulated vitamin C molecules are also found outside the liposomes and reside among water molecules of the system.

Further from Table 2, we saw that the aggregate structures and the total energy did not significantly change when introducing vitamin C to the systems. These phenomena suggested that the addition of vitamin C did not influence the formation of liposomes in all scenarios. Meanwhile, cholesterol with the hydrophobic

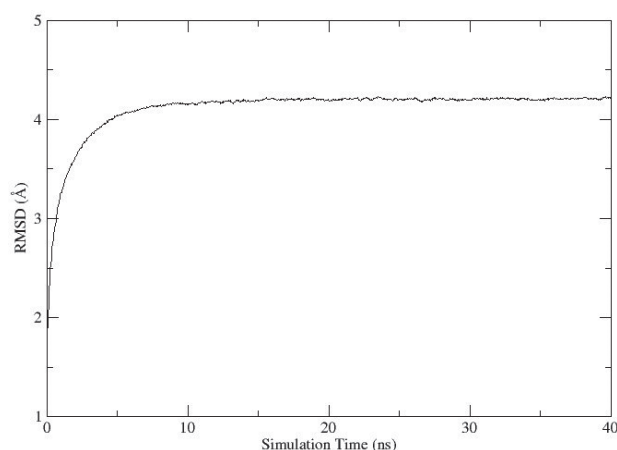


Figure 3. The graph of RMSD of system 220dlpe-88chol-10vitc during 40 ns of simulation.

property was encapsulated in the liposome bilayer membrane. Other systems formed self-assembled structures other than liposomes (Fig. 4b–e). The system compositions influence the formation of the liposomes. A certain amount of cholesterol is required to form liposomes that will encapsulate the vitamin C. Small amount of cholesterol, i.e., 0, 11, 22 molecules, for most systems were inadequate to induce the formation of the liposomes. Contrarily systems with high amounts of cholesterol, i.e., 33, 66, and 88 molecules, are more capable of forming liposomes and at the same time encapsulating vitamin C. Besides increasing the possibility of forming liposomes, an increase in cholesterol also impacts the stability of liposomes, as indicated by the increasing lifetime of liposomes, as listed in Table 3. Cholesterol inhibits the deformation of the membrane, causing the liposomes to stay intact until the end of the simulation.

Total energy system

Structural changes that occur during the aggregation process of forming a self-assembled structure were accompanied by a decrease in the system's total energy, as seen in Fig. 5. The total energy decreases until it reaches minimum energy when the simulation ends and forms a converging curve indicating the stability of the system's final structure. The total energy of liposome formation was the highest amongst other self-assembled structures that is due to the liposomes bilayer's high curvature. Liposomes formation occurred at total energy from -62,939 to -55,778 kcal/mol. In general, the bilayer structures had lower total energy compared to liposomes. The higher the amount of cholesterol, the higher is the total energy of the system. Amongst all phospholipid species with different head groups, the total energy of the system composed of PC was higher than PE and PS, as seen from the system total energy data in Table 2. The system's total energy from the lowest was PS<PE<PC. The system composed of phospholipids with 18 carbon atoms in their acyl chains has higher total energy than the 12 carbon atoms. Meanwhile, the system composed of phospholipids with one double bond has higher total energy than two double bonds. The order of the total energy based on the nature of the acyl chains in each phospholipid species from the lowest was DLPS(12:0)<DLPE(12:0)<DLiPS(18:2)<DOPS(18:1)<DLiPE(18:2)<DOPE(18:1)<DLiPC(18:2).

Liposomes size, membrane thickness, and area per lipid

Table 3 showed that cholesterol affected the liposomes size, membranes thickness, and area per lipid of the liposome membrane of each system. Increasing the amount of cholesterol in the system increases the liposome's size and the

Table 3. Liposome Profiles during 40 ns of Simulation.

Phospholipid Species	Liposomes Composition [Phospho-Chol-VitC]	Total energy (kcal/mol)	Occurrence Time ($t_{\text{initial}}-t_{\text{final}}$) ns	Lifetime (ns)	Area per lipid (nm^2/lipid)	Membrane thickness (nm)	Liposome Size (nm)
DLPE	220-33-10	-62,138	31.76 – 40.00	8.27	1.20	4.42	8.00
	220-66-10	-61,142	26.80 – 40.00	13.20	1.07	4.36	8.30
	220-88-10	-58,692	18.08 – 40.00	21.92	0.98	4.78	8.40
DLPS	220-33-10	-62,939	36.48 – 40.00	3.52	1.15	4.72	8.50
	220-66-10	-61,339	34.84 – 40.00	5.16	1.03	4.96	9.00
	220-88-10	-59,412	13.76 – 40.00	26.24	0.95	4.85	8.50
DOPE	220-22-10	-59,311	30.00 – 40.00	10.00	1.23	4.97	8.50
	220-66-10	-56,922	28.80 – 40.00	11.20	1.05	4.99	9.00
	220-88-10	-56,014	27.08 – 40.00	12.92	0.98	5.20	9.20
DOPS	220-66-10	-57,997	6.36 – 40.00	33.64	1.01	5.29	9.00
	220-88-10	-56,534	3.48 – 40.00	36.52	0.94	5.43	9.50
DLiPE	220-22-10	-59,596	29.44 – 40.00	10.56	1.23	4.88	8.72
	220-33-10	-58,985	28.68 – 40.00	11.32	1.18	5.12	8.72
	220-66-10	-57,440	29.68 – 40.00	10.32	1.05	5.07	8.90
DLiPS	220-88-10	-56,200	21.80 – 40.00	18.20	0.98	5.06	9.00
	220-66-10	-58,627	23.36 – 40.00	16.64	1.02	5.07	9.00
	220-88-10	-57,111	20.32 – 40.00	19.68	0.94	5.27	9.50
DLiPC	220-22-10	-58,686	16.44 – 40.00	23.56	1.17	5.08	9.50
	220-33-10	-58,421	29.60 – 40.00	10.40	1.18	4.94	9.00
	220-66-10	-56,569	29.60 – 40.00	10.40	1.04	5.22	9.00
	220-88-10	-55,778	20.08 – 40.00	19.92	0.97	5.37	8.90

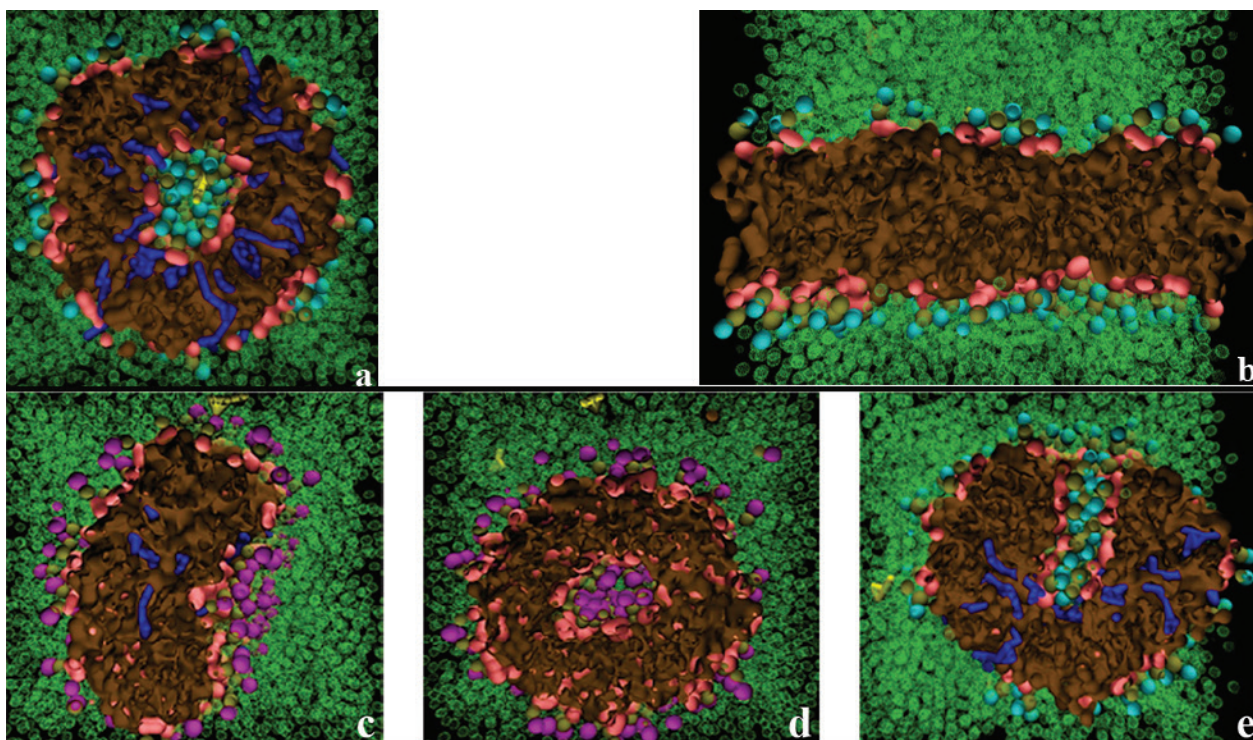


Figure 4. Various shapes of system self-assembled structures during 40 ns simulation time. Part of phospholipids was presented by: light blue beads for ethanolamine head groups, purple beads for serine head groups, light brown beads for phosphate, pink beads for glycerol backbone, and dark brown beads for hydrocarbon tail group. cholesterol was presented by dark blue beads, whereas yellow beads presented vitamin c and water molecules were lime beads. **a** Liposome of system 220LiPE-88Chol-10VitC at $t = 21.88$ ns (lifetime of liposome = 21.8 - 40 ns). Cholesterol was encapsulated in liposome bilayer (hydrophobic part of liposomes), meanwhile vitamin C was encapsulated in the core of liposome (hydrophilic part of liposomes) along with water molecules **b** Planar bilayer of system 220DOPE-0Chol-10VitC at $t = 40$ ns (the end of the simulation). **c** Disc-like bilayer of system 220DLiPS-33Chol-10VitC at $t = 40$ ns. Unlike liposome, there were no core formed and no water encapsulated in self-assembled structure. **d** Toroidal bilayer of system 220DLPS-0Chol-10VitC at $t = 40$ ns. **e** A curved bilayer of system 220DOPE-33Chol-10VitC at $t = 40$ ns.

thickness of the membrane. On the contrary, increasing the amount of cholesterol in the system decreased the area per lipid of the liposome membrane. The phospholipid species that compose the system also influence the liposome size and membrane thickness. Liposome membrane thickness is calculated by measuring the distance between two phosphate head group peaks per phospholipid bilayer (i.e., the peak on the first layer of the phosphate head group and the peak on the second layer of the phosphate head group) and locating in the middle point between them (Guixà-González et al. 2014). As shown in Table 3, the membrane thickness of all simulation systems capable of forming liposomes ranges between 4.36 – 5.43 nm, indicating the maximum membrane thickness that a phospholipid bilayer can achieve when forming liposomes during 40 ns of simulation. Meanwhile, the liposome size obtained in this study ranged from 8.00 – 9.50 nm. As Hudiyanti (Hudiyanti et al. 2014) has explained, the liposome is not a stable self-assembled structure. Its curved bilayer can deform the system into a more stable planar structure as the simulation time increases. Therefore, the liposome size obtained in this study is the smallest size that can be obtained when liposomes are formed during the 40 ns time of the simulation. The data indicated that, in general, the liposomes size and the thickness of the membrane increased with increasing

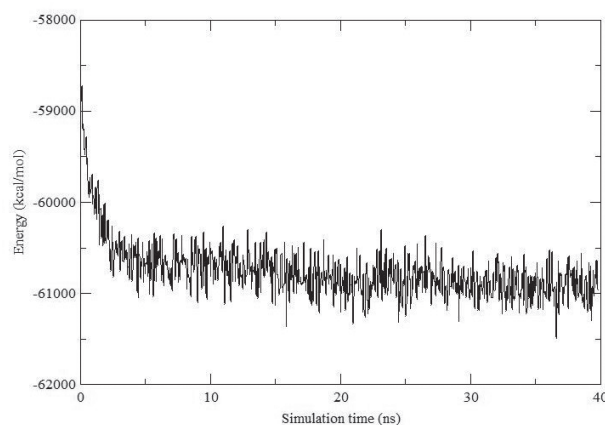


Figure 5. The total energy of the system contains 220DLiPS-22Chol-10VitC.

head groups size (Hudiyanti et al. 2019b) and the number of carbon atoms in the acyl chains. The number of double bonds in each hydrocarbon tail of the phospholipid in the system did not significantly influence the liposomes size, the membrane's thickness, and the area per lipid of the liposome membrane. Based on the phospholipid head groups from the lowest, the membrane thickness and liposome size were PE < PS < PC, while based on the number of the carbon

atom and the number of a double bonds in the hydrocarbon tails, the order was 12:0 < 18:2 < 18:1. The thickness of liposome membranes and the size of liposomes are due to the cholesterol condensation effect. The condensation effect arises from the rigid sterol ring of the cholesterol structure that suppresses the density of the phospholipid hydrocarbon tail; the greater the amount of cholesterol in the system, the greater the condensation effect. The phospholipid acyl chains in the liposome bilayer become denser and more regular and increase the thickness of the membrane and the liposome size (Alwarawrah et al. 2010).

Conclusion

The simulations portray the aggregation process of the system with 6 different phospholipid species, cholesterol, and vitamin C during the 40 ns simulation. At least 6 other aggregate structures formed: curved bilayer, toroidal

bilayer, concave micelle, disc-like bilayer, planar bilayer, and liposome. The formation of the aggregated structure is determined by the ratio of cholesterol:phospholipid. The ratio between 15% and 40% will form liposomes regardless of the phospholipid species. Cholesterol is located in the liposome bilayer, while vitamin C is located in the liposomes' core for all cholesterol composition. This study better understands the cholesterol effect on the liposome formation of various phospholipid species and the encapsulation of vitamin C in liposome structure. The simulation provides the basis for cultivating phospholipid-based drug delivery systems.

Acknowledgements

The Minister of Research and Technology/BRIN Republic Indonesia supported this research through PDUPT Research Scheme 2021. Grant No. 187-27/UN7.6.1/PP/2021.

References

- Alkandahri MY, Subarnas A, Berbudi A (2018) Review: aktivitas immunomodulator tanaman sambiloto (*Andrographis paniculata* Nees). *Farmaka* 16: 16–21. <https://doi.org/10.24198/JF.V16I3.14237>
- Alwarawrah M, Dai J, Huang J (2010) A Molecular View of the Cholesterol Condensing Effect in DOPC Lipid Bilayers. *Journal of Physical Chemistry B* 114: 7516–7523. <https://doi.org/10.1021/JP101415G>
- Daily MD, Olsen BN, Schlesinger PH, Ory DS, Baker NA (2014) Improved coarse-grained modeling of cholesterol-containing lipid bilayers. *Journal of Chemical Theory and Computation* 10: 2137–2150. <https://doi.org/10.1021/ct401028g>
- Guixà-González R, Rodríguez-Espigares I, Ramírez-Anguita JM, Carrió-Gaspar P, Martínez-Seara H, Giorgino T, Selent J (2014) MEMB-PLUGIN: studying membrane complexity in VMD. *Bioinformatics*, 30(10): 1478–1480. <https://doi.org/10.1093/bioinformatics/btu037>
- Hudiyanti D, Fawrin H, Siahaan P (2018) Simultant encapsulation of vitamin C and beta-carotene in sesame (*Sesamum indicum* L.) liposomes. *IOP Conference Series: Materials Science and Engineering* 349 pp. <https://doi.org/10.1088/1757-899X/349/1/012014>
- Hudiyanti D, Aminah S, Hikmahwati Y, Siahaan P (2019a) Cholesterol implications on coconut liposomes encapsulation of beta-carotene and vitamin C. In: *IOP Conference Series: Materials Science and Engineering*. IOP Publishing 509: e012037. <https://doi.org/10.1088/1757-899X/509/1/012037>
- Hudiyanti D, Hamidi NI, Anugrah DSB, Salimah SNM, Siahaan P (2019b) Encapsulation of Vitamin C in Sesame Liposomes: Computational and Experimental Studies. *Open Chemistry* 17: 537–543. <https://doi.org/10.1515/chem-2019-0061>
- Hudiyanti D, Radifar M, Raharjo TJ, Narsito N, Noegrohati S (2014) A Coarse-Grained Molecular Dynamics Simulation Using NAMD Package to Reveal Aggregation Profile of Phospholipids Self-Assembly in Water. *Journal of Chemistry* 2014: 1–6. <https://doi.org/10.1155/2014/273084>
- Humphrey W, Dalke A, Schulten K (1996) VMD: Visual molecular dynamics. *Journal of Molecular Graphics* 14: 33–38. [https://doi.org/10.1016/0263-7855\(96\)00018-5](https://doi.org/10.1016/0263-7855(96)00018-5)
- Joshi SY, Deshmukh SA (2020) A review of advancements in coarse-grained molecular dynamics simulations. *Molecular Simulation* 47(10–11): 786–803. <https://doi.org/10.1080/08927022.2020.1828583>
- Liu X, Wang P, Zou YX, Luo ZG, Tamer TM (2020) Co-encapsulation of Vitamin C and β -Carotene in liposomes: Storage stability, antioxidant activity, and in vitro gastrointestinal digestion. *Food Research International* 136: e109587. <https://doi.org/10.1016/J.FOODRES.2020.109587>
- Liwo A, Czaplowski C, Sieradzan AK, Lipska AG, Samsonov SA, Murarka RK (2021) Theory and Practice of Coarse-Grained Molecular Dynamics of Biologically Important Systems. *Biomolecules* 11: 1347–1347. <https://doi.org/10.3390/biom11091347>
- Marrink SJ, Risselada HJ, Yefimov S, Tieleman DP, de Vries AH (2007) The MARTINI Force Field: Coarse Grained Model for Biomolecular Simulations. *The Journal of Physical Chemistry B* 111: 7812–7824. <https://doi.org/10.1021/jp071097f>
- Martínez L, Andrade R, Birgin EG, Martínez JM (2009) PACKMOL: A package for building initial configurations for molecular dynamics simulations. *Journal of Computational Chemistry* 30: 2157–2164. <https://doi.org/10.1002/jcc.21224>
- O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011) Open Babel: An open chemical toolbox. *Journal of Cheminformatics* 3: e33. <https://doi.org/10.1186/1758-2946-3-33>
- Periole X, Marrink S-J (2013) The Martini coarse-grained force field. *Methods in molecular biology* (Clifton, N.J.) 924: 533–565. https://doi.org/10.1007/978-1-62703-017-5_20
- Sheraz MA, Khan M, Ahmed S, Kazi H, Ahmad I (2015) Stability and Stabilization of Ascorbic Acid. *H&PC Today - Household and Personal Care Today* 10: 22–25. [January 29, 2022]
- Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Bryant SH (2009) PubChem: a public information system for analyzing bioactivities of small molecules. *Nucleic acids research* 37(Suppl 2): W623–W633. <https://doi.org/10.1093/nar/gkp456>