

# Encapsulation of Vitamin C in Sesame Liposomes: Computational and Experimental Studies

*by Parsaoran Siahaan*

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**Submission date:** 13-Jan-2022 02:53PM (UTC+0700)

**Submission ID:** 1741021615

**File name:** Artikel-06.pdf (454.84K)

**Word count:** 3541

**Character count:** 18652

## Research Article

Dwi Hudyanti\*, Noor Ichsan Hamidi, Daru Seto Bagus Anugrah, Siti Nur Milatus Salimah, Parsaoran Siahaan

# Encapsulation of Vitamin C in Sesame Liposomes: Computational and Experimental Studies

<https://doi.org/10.1515/chem-2019-0061>

received September 8, 2018; accepted February 5, 2019.

**Abstract:** An experimental and computational study was carried out for encapsulation of vitamin C in sesame, *Sesamum indicum* L., liposomes. Based on computational studies, the packing parameter (P) of sesame phospholipids was found to be  $0.64 \pm 0.09$ . This indicates that the molecular shape of sesame phospholipids is in the form of truncated cone and, in aqueous solution, it self-assembles to form liposomes. In the liposomes, no chemical interaction was observed between phospholipid molecules and vitamin C. However, medium-strength hydrogen bonds ( $E$ ) from  $-87.6$  kJ/mol to  $-82.02$  kJ/mol with bond lengths ranging from  $1.746$  Å to  $1.827$  Å were formed between vitamin C and phospholipid molecules. Because of this weak interaction, vitamin C gets released easily from the inner regions of liposome. Empirical experiments were performed to confirm the computation outcomes, where sesame liposomes were found to encapsulate almost 80% of vitamin C in their interior cavities. During the 8 days storage, release of vitamin C occurred gradually from the liposome system, which signifies weak interactions in the liposome membranes amongst phospholipid molecules and vitamin C.

**Keywords:** drug delivery; hydrogen bond; phospholipids; packing parameter; intermolecular interactions.

## 1 Introduction

Hudyanti et al. are currently investigating natural liposomes as drug delivery systems [1–3]. Liposomes are made up of phospholipid molecules [4], which are amphiphilic in nature and, in aqueous solution, these instantly form bilayer membranes. Self-assembly of the membrane into liposomes occurs, which are then employed in drug delivery systems [5]. Hudyanti et al. employed liposomes from sesame phospholipid for encapsulating vitamin C [6]. Vitamin C is a vital nutrient used in many human functions. Unfortunately, vitamin C cannot be synthesised by human, and needs to be taken as supplement from other sources. Also, human systems are not designed to store vitamin C. When the metabolism ends, it is immediately excreted. Vitamin C also gets oxidised easily [7,8]. Previous studies have reported 88% encapsulation of vitamin C in sesame liposomes [2]. Thus, employing liposomes for encapsulation will give ideal solution for vitamin C.

Liposomes are made up of phospholipid molecules. The success of sesame liposomes as a carrier for vitamin C is based on the interaction between vitamin C and phospholipid molecules. The energy interaction profile pertaining to phospholipids self-assembly in the production of liposomes was revealed with the study of dynamic interaction amongst different phospholipid species. Assembly of liposomes occurs at energy levels of  $-25.900$  to  $26.900$  kcal/mol based on the acyl chains and the head group types [9].

This research study is aimed at evaluating self-assembled sesame, *Sesamum indicum* L., phospholipid species as well as the interaction occurring between vitamin C and phospholipid molecules. Studies were performed based on computation and wet experiments. The parameter packing concept of molecules [10] was applied to predict self-assembled shape, which revealed that aggregation of sesame phospholipids occurs to assemble into liposomes. Based on the interaction energy calculation, a weak interaction between sesame

\*Corresponding author: Dwi Hudyanti, Chemistry Department,

Diponegoro University, Semarang, Indonesia,

E-mail: [dwi.hudyanti@live.undip.ac.id](mailto:dwi.hudyanti@live.undip.ac.id)

Noor Ichsan Hamidi, Daru Seto Bagus Anugrah, Siti Nur Milatus

Salimah: Undergraduate Program, Chemistry Department,

Diponegoro University, Semarang, Indonesia

Parsaoran Siahaan: Chemistry Department, Diponegoro University,

Semarang, Indonesia

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phospholipids and vitamin C was found. These were an intermolecular interaction and not chemical interaction. The encapsulation data showed that vitamin C was encapsulated by sesame liposomes with a high efficiency. Moreover, during storage, some vitamin C is released by the liposomes into the liposomes medium. Based on this data, no bond formation was found to exist between phospholipid molecules and vitamin C in liposomes. Vitamin C gets released from liposomes due to weak interaction between sesame phospholipids and vitamin C. For encapsulation of vitamin C, sesame liposomes were found to be optimum.

## 2 Research Methods

In this research work, in-house isolation of sesame phospholipids was done with the head groups consisting of serine, choline and ethanolamine; the acyl chain were C12:0, C16:0, C18:0, C18:1, and C18:2 [2,3].

### 2.1 Prediction of phospholipids self-assembled

As mentioned above, combining of the head and tail groups of sesame phospholipids was done to forecast self-assembly by the Marvin Sketch program. Through the packing parameter concept, prediction study of self-assembled shape was done [11]. Packing parameters (Figure 1) would describe amphiphilic molecules' aggregate structure in aqueous solution in terms of molecular geometry, i.e. the head group's cross-sectional area ( $a$ ), optimum chain length ( $I_{opt}$ ) and hydrocarbon chain volume ( $V$ ). Packing parameter ( $P$ ) defines the relation between these geometry parameters, given as:

$$P = \frac{V}{(a \cdot I_{opt})} \dots\dots (1)$$

### 2.2 Intermolecular interaction between vitamin C and phospholipids

The computation method was employed to analyse the interactions between sesame phospholipids and vitamin C [12]. Calculation of interaction energy was done for vitamin C and phospholipid molecule C4:0 (vitC•••phospho4) and phospholipid molecule C20:0 (vitC•••phospho20). An energy difference could be seen between the molecular

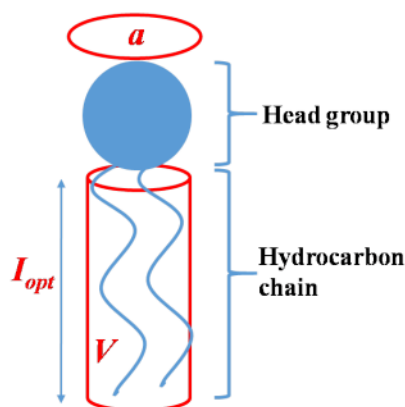


Figure 1: Packing parameters of phospholipid molecules.

association energy for each molecule, phospholipids and vitamin C. This formed the energy pertaining to the most stable interaction amongst stable structures. The interaction energy ( $E_i$ ) comprises the molecular energy of vitamin C ( $E_{vitC}$ ), the energy of association molecules ( $E_{vitC \cdot \cdot \cdot phospho}$ ) and the molecular energy of phospholipid ( $E_{phospho}$ ).

$$E_i = E_{vitC \cdot \cdot \cdot phospho} - (E_{vitC} + E_{phospho}) \dots\dots (2)$$

As presented in equation 2, phospholipid's ( $E_{phospho}$ ) molecular energy was regarded the molecular energy of phospholipid C20:0 ( $E_{phospho20}$ ) or phospholipid C4:0 ( $E_{phospho4}$ ).

### 2.3 Preparation of sesame liposomes

The method of Hudyanti *et al.* was employed to isolate sesame phospholipid from sesame seeds [13]. The liposomes preparation followed the method of Hudyanti *et al.* [2], which included thin layer formation, ultrasonication and hydration in PBS buffer solution. Into chloroform, phospholipid was dissolved in the thin layer formation stage. The solution was kept inside a reaction tube with nitrogen passing above it, which helped in the formation of a thin layer. In the hydration stage, addition of vitamin C to the PBS buffer solution in the tube was done ( $C_{initial}$ ), which had the thin layer. Post this, the freeze thawing sequence was performed, involving cooling (at  $-5^\circ\text{C}$ ), heating (at  $50^\circ\text{C}$ ) and employing a vortex mixer for stirring. In each sequence, these steps were performed for 5 minutes. Repetition of freeze thawing was done until dissolution of the thin layer completely. During the



a. Molecular shape, Truncated cone    b. Self-assembled shape, liposome

Figure 2: Self-assembled prediction of phospholipid molecules.

sonication stage, the solution was placed in an ultrasonic bath for almost 1.5 hours.

The encapsulation efficiency ( $EE$ ) was employed to determine the ability of liposomes to encapsulate vitamin C. In the liposome medium, the free vitamin C concentration was measured to determine  $EE$  pertaining to sesame liposomes. Centrifugation of the liposomes solution was done at 6,000 rpm for 30 minutes to segregate the liposomes from the medium *viz.* supernatants. A UV-Visible spectrophotometer at 265 nm wavelength was employed to analyse supernatant for determining un-encapsulated vitamin C concentration ( $C_{free}$ ). Then,  $EE$  was calculated by the following equation 3:

$$\%EE = \left( \frac{C_{initial} - C_{free}}{C_{initial}} \right) \times 100 \dots\dots\dots (3)$$

The solution was stored for 8 days in low temperatures ( $5^{\circ}\text{C}$ ) to determine if there is leakage of sesame liposomes. Centrifugation was carried out for the dispersion, and then analysis of the supernatant is performed for each day. The increment in vitamin C concentration signified the leakage in the liposomes medium.

**Ethical approval:** The conducted research is not related to either human or animal use.

### 3 Result and Discussion

This research was aimed at studying encapsulation of vitamin C in sesame liposomes. Computational and empirical experiments were performed for the studies. The computation allowed calculating possibilities that may occur during encapsulation when mixing sesame phospholipids and vitamin C, while empirical data was provided via the experiment.

#### 3.1 Prediction of sesame phospholipid self-assembled

The packing parameters ( $P$ ) employing the Marvin Sketch program were computed for predictions of self-assembled sesame phospholipid. A description regarding the molecule shape of phospholipid and self-assembled bilayer is provided by packing parameters [10]. Volume of the hydrocarbon chain ( $V$ ), cross-sectional area of the head group ( $a$ ) as well as the optimum chain length ( $I_{opt}$ ) pertaining to every head and tail groups for each of sesame phospholipid molecular types were calculated for packing parameters. For each combination of tail and head groups, these parameters were integrated in equation 1.

Based on the results (Table 1), the highest value of 0.85 was achieved for packing parameters pertaining to (C18:1/C18:1) the phosphatidyletanolamine molecule, while the lowest value of 0.50 was achieved on (C16:0/C16:0) the phosphatidylcholine molecule. On average, packing parameter ( $P$ ) of  $0.64 \pm 0.09$  was associated with sesame phospholipids, which was in the range  $\frac{1}{2} < P < 1$ . Theoretically, this value indicates truncated cone shape of sesame phospholipids [14]. Therefore, when sesame phospholipids were kept in solution, they self-assembled into liposomes (Figure 2).

#### 3.2 Intermolecular interaction between vitamin C and phospholipids

NWChem programs were employed to analyse the interactions between phospholipids and vitamin C. The program allows optimising the geometry position as well as calculating the interaction energy [15]. Calculation of interactions was done between vitamin C (vitC) along with two different types of phospholipids: C20:0/C20:0 phosphatidylcholine (phospo20) and C4:0/C4:0 phosphatidylcholine (phospo4). Different lengths of the

**Table 1:** Packing parameter ( $P$ ) determination of sesame phospholipids.

1 <sup>st</sup> tail group	2 <sup>nd</sup> tail group	Phospholipid molecular type	$l_{r1}$ (Å)	$l_{r2}$ (Å)	$V_{r1}$ (Å <sup>3</sup> )	$V_{r2}$ (Å <sup>3</sup> )	$V$ (Å <sup>3</sup> )	$lopt$ (Å)	$a$ (Å <sup>2</sup> )	$P$	
1 palmitic acid (C16:0)	oleic acid	phosphatidyl-ethanolamine	23.21	19.84	292.93	320.17	613.10	23.21	37.88	0.70	
		linoleic acid	23.21	21.11	292.93	311.63	604.56	23.21	37.88	0.69	
		palmitic acid	23.21	23.21	292.93	292.93	585.86	23.21	37.88	0.67	
	oleic acid	phosphatidyl-choline	phosphatidyl-ethanolamine	23.21	19.84	292.93	320.17	613.10	23.21	49.70	0.53
			linoleic acid	23.21	21.11	292.93	311.63	604.56	23.21	49.70	0.52
			palmitic acid	23.21	23.21	292.93	292.93	585.86	23.21	49.70	0.50
	oleic acid	phosphatidyl-serine	phosphatidyl-ethanolamine	23.21	19.84	292.93	320.17	613.10	23.21	44.85	0.59
			linoleic acid	23.21	21.11	292.93	311.63	604.56	23.21	44.85	0.58
			palmitic acid	23.21	23.21	292.93	292.93	585.86	23.21	44.85	0.56
2 oleic acid (C18:1)	palmitic acid	phosphatidyl-ethanolamine	19.84	23.21	320.17	292.93	613.10	23.21	37.88	0.70	
		linoleic acid	19.84	21.11	320.17	311.63	631.80	21.11	37.88	0.79	
		oleic acid	19.84	19.84	320.17	320.17	640.34	19.84	37.88	0.85	
	palmitic acid	phosphatidyl-choline	phosphatidyl-ethanolamine	19.84	23.21	320.17	292.93	613.10	23.21	49.70	0.53
			linoleic acid	19.84	21.11	320.17	311.63	631.80	21.11	49.70	0.60
			oleic acid	19.84	19.84	320.17	320.17	640.34	19.84	49.70	0.65
	palmitic acid	phosphatidyl-serine	phosphatidyl-ethanolamine	19.84	23.21	320.17	292.93	613.10	23.21	44.85	0.59
			linoleic acid	19.84	21.11	320.17	311.63	631.80	21.11	44.85	0.67
			oleic acid	19.84	19.84	320.17	320.17	640.34	19.84	44.85	0.72
3 linoleic acid (C18:2)	palmitic acid	phosphatidyl-ethanolamine	21.11	23.21	311.63	292.93	604.56	23.21	37.88	0.69	
		oleic acid	21.11	19.84	311.63	320.17	631.80	21.11	37.88	0.79	
		linoleic acid	21.11	21.11	311.63	311.63	623.26	21.11	37.88	0.78	
	palmitic acid	phosphatidyl-choline	phosphatidyl-ethanolamine	21.11	23.21	311.63	292.93	604.56	23.21	49.70	0.52
			oleic acid	21.11	19.84	311.63	320.17	631.80	21.11	49.70	0.60
			linoleic acid	21.11	21.11	311.63	311.63	623.26	21.11	49.70	0.59
	palmitic acid	phosphatidyl-serine	phosphatidyl-ethanolamine	21.11	23.21	311.63	292.93	604.56	23.21	44.85	0.58
			oleic acid	21.11	19.84	311.63	320.17	631.80	21.11	44.85	0.67
			linoleic acid	21.11	21.11	311.63	311.63	623.26	21.11	44.85	0.66

tail groups were considered to determine its effect on interaction.

The formation of hydrogen bonds between vitamin C and the head groups shows two interactions between phospholipid molecules and vitamin C as demonstrated in figure 3a (vitC•••phospho4) and figure 3b (vitC•••phosp20). For vitC•••phospho4 O6O•••H24 and H73•••O23, the interactions occur with  $E_f = -87.6$  kJ/mol. In vitC•••phospho20 O156•••H24 and H169•••O23, the interactions occur with  $E_f = -82.02$  kJ/mol. In both vitC•••phospho4 and

vitC•••phospho20, the lengths of hydrogen bonds (R) were changed. In vitC•••phospho4, 1.746 Å ( $R_{O60\cdots H24}$ ) and 1.819 Å ( $R_{H73\cdots O23}$ ) were the bond lengths; whereas 1.753 Å ( $R_{O156\cdots H24}$ ) and 1.827 Å ( $R_{H169\cdots O23}$ ) were the bond lengths in vitC•••phospho20.

Based on energy ( $E_f$ ) and bond length data, vitamin C was seen to interact stronger with short acyl chain (C4:0) than long acyl chain (C20:0) phospholipids. Based on the data, both interactions were found to occur with medium strength of hydrogen bond [16]. Also, information was provided confirming that in the self-assembled process,

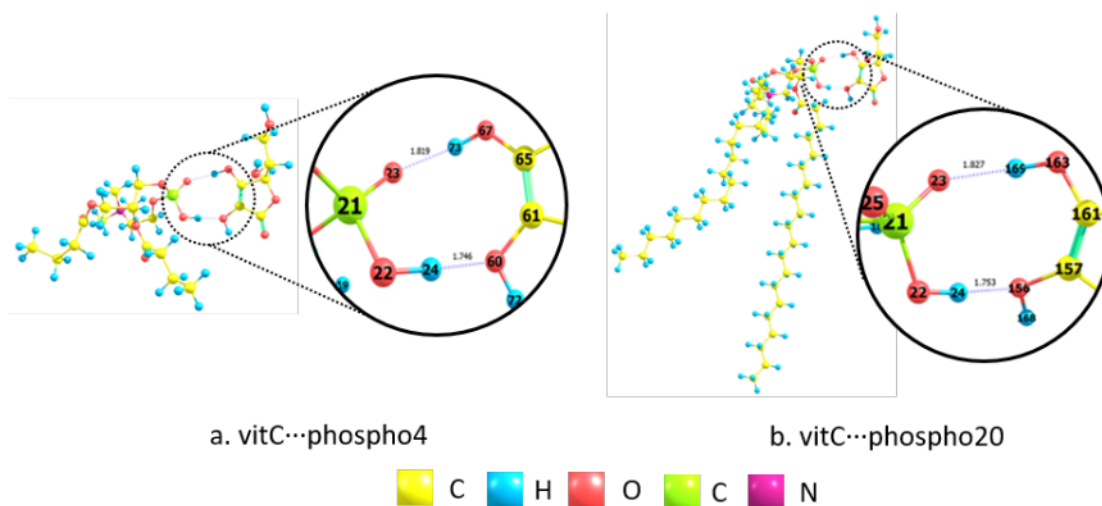


Figure 3: Intermolecular interaction between vitamin C and phospholipid molecules.

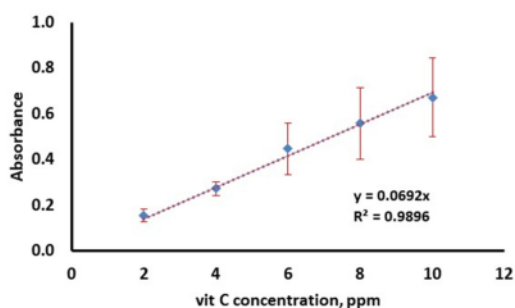


Figure 4: Standard curve for vitamin C.

Table 2: Encapsulation parameter in sesame liposomes.

Parameter	Value
$C_{\text{initial}}$	94.67 ppm
$C_{\text{free}}$	18.93 ppm
$EE$	80%

no chemical interaction occurred between vitamin C and phospholipid molecules. These conditions favour easy release of vitamin C from liposomes cavity.

Based on the following discussion, the empirical experiments were seen to support the computation results or vice versa. The computation data were confirmed based on the leakage data and encapsulation efficiency. The

encapsulation ability of sesame liposomes is determined by encapsulation efficiency to entrap vitamin C, while the ease of vitamin C release from liposomes cavity is confirmed with the leakage data on storing for 8 days.

### 3.3 Encapsulation efficiency (EE) of vitamin C

Vitamin C concentration was analysed by constructing a standard vitamin C curve (Figure 4).

Encapsulation parameters associated with sesame liposomes are presented in Table 2. The data were found to be in line with the computation results that showed sesame phospholipids formed liposomes when immersed in solution. 80% of vitamin C was found to be encapsulated by the liposome of the total added vitamin C. Based on the  $EE$  value and the computation results, sesame liposomes have demonstrated valuable potency as encapsulation materials, particularly for hydrophilic substances like vitamin C versus  $EE$  values for soybean and coconut [6].

### 3.4 Leakage of sesame liposomes

On spontaneous exiting of encapsulated vitamin C via the liposome membrane because of membrane permeability, a leakage occurs [3]. With the leakage, in the liposome medium ( $C_{\text{free}}$ ), the concentration of vitamin C was seen to escalate as observed in 8 days storage period. Figure 4 displays leakage occurring for vitamin C to the liposome

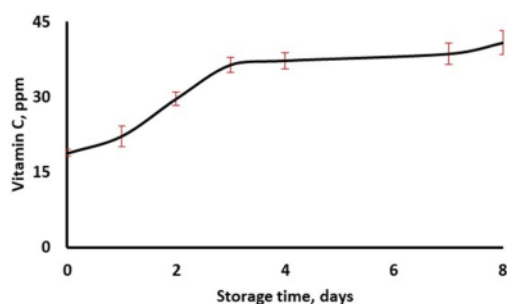


Figure 5: Leakage of vitamin C to the liposome medium in 8 days.

medium during 8 days. A concentration gradient is formed in the outer region should vitamin C get encapsulated inside liposomes. Moreover, the bilayer membrane formed by sesame phospholipids appears to be tenuous, since oleic and linoleic fatty acids dominate the sesame phospholipid acyl groups [2,13]. These long fatty acid chains include an unsaturated bond that aids in the formation of a mobile bilayer [17]. To adjust the concentration differences, the spontaneous stream pertaining to vitamin C is driven by both conditions from the inner portion of liposomes to the medium [18,19]. This process induces increasing of vitamin C concentration in the medium as presented in figure 5. The vitamin C leakage to the liposome medium was in line with the computation results. Based on the computational results, only intermolecular interactions were found to exist between sesame phospholipids and vitamin C in the form of hydrogen bonds. Based on the concentration gradient, intermolecular interaction and a mobile bilayer, it was concluded that the release of vitamin C from the liposomes occurred with ease. Also, there was a possibility that the cavities formed in the liposome membrane because of the double bond could have a role in facilitating the release of vitamin C from the liposome system.

## 4 Conclusion

Based on the computation study, sesame phospholipids were found to be in a truncated cone shape, which in an aqueous solution would form liposomes. The molecules in the liposome system experienced interactions due to cooperative hydrogen bonds that had energy ( $E$ ) ranging from  $-87.6$  kJ/mol to  $-82.02$  kJ/mol, and bond length ranging from  $1.746$  Å– $1.827$  Å. Based on the experimental studies, sesame liposomes were found to encapsulate

vitamin C with 80% encapsulation efficiency ( $EE$ ). During 8 days storage, an easy release of vitamin C from sesame liposomes occurred, as confirmed with the increase in vitamin C concentration in the liposome medium. Based on experimental and computational studies, the presence of intermolecular interaction between vitamin C as well as phospholipid molecules in liposomes was confirmed, signifying that vitamin C could be easily released from liposomes.

**Acknowledgement:** This research was supported (in part) by the Diponegoro University PNPB Fundamental Research scheme 2014.

**Conflict of interest:** Authors declare no conflict of interest.

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