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Enhancing vitamin C stability through liposomal encapsulation with optimised pressure and cycle conditions

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Abstract: Encapsulation technology offers an effective strategy to enhance the bioavailability and stability of vitamin C by addressing its sensitivity to environmental factors. This study investigates the impact of formulation parameters, particularly lecithin concentration and high-pressure processing conditions, on the physicochemical properties, gastrointestinal stability, cytotoxicity, and shelf life of liposomal vitamin C formulations. Among the tested samples, Sample 1, prepared with 20% soybean lecithin and 20% ascorbic acid and processed at 400 bar with a single cycle, demonstrated superior performance. It exhibited a high zeta potential (–23.17 mV), uniform size distribution (317.5 ± 8.863 nm) and encapsulation efficiency of 77.6%, along with 85% vitamin C retention under simulated gastrointestinal conditions. Cellular uptake in Caco-2 cells reached 30%, and structural integrity was preserved for 240 days at 40 °C, indicating strong thermal stability. The results underscore that lecithin concentration had the most significant influence on encapsulation efficiency and liposome stability, compared to pressure intensity or the number of processing cycles. Furthermore, modulating the zeta potential through lipid composition and the energy applied to phospholipid solutions was found to be critical for improving bioavailability and ensuring long-term dispersion stability. In conclusion, the optimised liposomal formulation offers a promising vehicle for advanced vitamin C delivery with enhanced protection, bioaccessibility, and storage potential.

Keywords: hospholipid-based delivery systems; high-pressure processing; stability; size; encapsulation efficiency

The evolving lifestyle dynamics in modern society has amplified interest in healthy lifestyles, increasing demand for functional food products and dietary supplements. This trend is driven by growing awareness of the pivotal role of nutrition in health maintenance and collective aspiration for healthier living. The demand for dietary supplements with high bioavailability has become more pronounced, particularly during the Covid-19 pandemic.

Vitamins and minerals are the most commonly researched products among dietary supplements. The global market size for dietary supplements was valued at USD 167 billion in 2023 and is projected to reach USD 239.4 billion by 2028 (Marketsandmarkets 2024). While over 50% of American adults consume at least one dietary supplement, 43.9% of Turkish adults reported using them during the pandemic (Gurhan et al. 2022).

Interest in traditional vitamin C has shifted to its liposomal form due to the poor stability of conventional vitamin C. Liposomal vitamin C offers several notable biological advantages. For instance, it demonstrates significantly enhanced bioavailability compared to traditional forms of vitamin C, resulting in higher plasma concentrations ($P < 0.05$).

Vitamin C also serves as a potent antioxidant, mitigating cellular oxidative stress and protecting against

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cellular damage (Bedhiafi et al. 2023). Additionally, it can be used as a skin rejuvenating agent due to its antioxidant activity (Vinardell and Mitjans 2015). Moreover, at lower concentrations, liposomal vitamin C can exert pro-oxidant effects on cancer cells, promoting apoptosis (Pal and Jana 2022). Finally, the liposomal formulation prolongs the elevated levels of vitamin C in the bloodstream, thereby enhancing its therapeutic efficacy.

Several factors influence the chemical stability of traditional vitamin C, including pH, temperature, enzymatic oxidation, light, atmospheric oxygen, and metal ions (Bedhiafi et al. 2023). Liposomes are effective delivery systems for vitamins and minerals, offering higher absorption rates compared to traditional forms. They also present advantages such as supporting sustainable technologies and optimising the utilisation of active ingredients.

Liposomes are spherical vesicles composed of one or multiple phospholipid bilayers, capable of encapsulating active molecules of varying polarities, including hydrophilic, lipophilic, or amphiphilic compounds (Mehta et al. 2023).

These vesicles function as efficient carriers, encapsulating bioactive molecules within the hydrophilic core, the lipophilic bilayer, or intermediate layers, depending on the molecular properties of the encapsulated substance (Nsairat et al. 2022). Liposomes, with their lipid bilayers generally composed of phospholipids, can be used to enhance the stability and reduce the degradation of vitamin C (Łukawski et al. 2020).

In addition to the biological benefits of liposomal vitamins, which are known for their high absorption rates through human cell membranes, a common issue with liposomal products is their limited long-term stability, often resulting in phase separation.

In our study, we used liposomal vitamin C at a commonly consumed concentration of 20% as a model for liposomal products. We tested methods to evaluate its physicochemical stability, absorption, high-dose cytotoxicity, and bioavailability.

The objective of this study is to enhance the stability, bioavailability, and encapsulation efficiency of vitamin C through liposomal formulations by protecting it against environmental factors such as pH, enzymes, light, and temperature. Additionally, the study aims to evaluate the physicochemical properties, gastrointestinal stability, cytotoxicity, and estimated shelf life of the formulations in order to assess their potential for long-term applications in liposomal vitamin C.

MATERIAL AND METHODS

Material. Caco-2 colorectal adenocarcinoma cells were obtained from the Proteomics and Mass Spectrometry Laboratory of Yeditepe University. The MTS reagent was the BlueGene MTS Assay Kit (China). Transwell plates with permeable inserts of 12 mm diameter and 0.4 μm pore size were purchased from Corning (USA). DMEM/F12 medium was supplied by Sigma-Aldrich (Germany).

Preparation of liposomal vitamin C. The experimental design for the formation of liposomal structures was developed using lecithin and vitamin C as key components. The concentration of vitamin C was set at 20%, corresponding to 1 000 mg·(5 mL)$^{-1}$, as this is the most commonly used concentration and it presents most challenges in terms of stabilisation. Lecithin concentrations were selected at 10% and 20%, based on the necessary ratios for effective encapsulation of vitamin C. The pressure values were determined by considering the maximum, minimum, and average pressures achievable with the high-pressure homogeniser. Utilising these parameters, samples were prepared under 24 distinct experimental conditions. The ingredients were first mixed, followed by the application of high pressure and multiple homogenisation cycles (Table 1). Each experiment was conducted in triplicate. The results were evaluated by monitoring vitamin C content, liposome size, and encapsulation efficiency.

Determination of vitamin C amount. Each sample was injected into an HPLC column (Shimadzu, Japan) and eluted isocratically using mobile phase A (25 mM KH_2PO_4) and mobile phase B (methanol) at a ratio of mobile phases of 75 : 25 (A : B; v : v). The flow rate was set at 2 mL·min^{-1}. A reverse phase C18 column (BDS Hypersil, 5 μm, 4.6 mm × 250 mm, Thermo Scientific, USA) maintained at 40 °C was used to determine the amount of vitamin C. Detection was performed at an absorbance wavelength of 254 nm.

Encapsulation efficiency. In this study, 1 mL of the sample was mixed with distilled water to achieve a final total volume of 10 mL. The 10 mL sample was then transferred into a centrifugal filtration tube and centrifuged (Sigma 3K30, Germany) at 8 000 × g for 15 min. After centrifugation, the volume of the filtrate (V_f) was measured. The vitamin C concentrations (ρ_f) of samples were analysed by HPLC. The encapsulation efficiency was calculated by the following formula:

$$\text{Encapsulation efficiency}(\%) = \left(1 - \frac{\rho_f \times V_f}{\rho_C \times V_T}\right) \times 100 \quad (1)$$

where: ρ_C – the concentration of vitamin C; V_T – the volume of total solution.

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Table 1. The experimental design sets for liposome preparation

Samples	Lecithin (%)	Vitamin C (%)	Pressure (bar)	Cycles
1	20	20	400	1
2	20	20	400	2
3	20	20	400	3
4	20	20	400	4
5	20	20	700	1
6	20	20	700	2
7	20	20	700	3
8	20	20	700	4
9	20	20	1 000	1
10	20	20	1 000	2
11	20	20	1 000	3
12	20	20	1 000	4
13	10	20	400	1
14	10	20	400	2
15	10	20	400	3
16	10	20	400	4
17	10	20	700	1
18	10	20	700	2
19	10	20	700	3
20	10	20	700	4
21	10	20	1 000	1
22	10	20	1 000	2
23	10	20	1 000	3
24	10	20	1 000	4

Liposome size and physical properties. The average size and the polydispersity index (PDI) of each liposomal sample were measured by using a Malvern Zetasizer Nano ZS instrument (Malvern Instruments, Worcestershire, UK). Samples were diluted with distilled water (1 : 10) before measurements.

***In vitro* gastrointestinal digestion simulation.** Simulated *in vitro* gastrointestinal digestion was applied to liposomal samples according to the method described by Minekus et al. (2014). Both gastric and intestinal phases were conducted sequentially. For the gastric phase, 10 mL of samples was mixed with simulated gastric fluids and $CaCl_2$. The pH was adjusted to 3.0, and 1.6 mL of pepsin (25 000 U) was added to the mixture. The final volume was completed to 20 mL with ddH_2O, and the samples were incubated at 37 °C for 2 h with shaking at 150 rpm. Afterwards, Pefabloc (5 mM) was added to stop the reaction. For the intestinal phase, gastric chyme was transferred to simulated intestinal fluids. Then, 40 µL $CaCl_2$ was added, and the pH was adjusted to 7.0 with 1 M NaOH. Pancreatin (100 $U \cdot mL^{-1}$) and bile salts (10 mM) were added to the mixture. Intestinal digestion was performed at 37 °C and 150 rpm, and after 2 h, 8 µL Pefabloc (5 mM) was added to stop the enzymatic reaction.

The digested samples were ultrafiltered using 50 kDa molecular weight cut-off ultrafiltration tubes at 9 500 × g for 30 min. The retentates and permeates were subsequently analysed to calculate encapsulation efficiency. The retentates contained the liposomal vesicles, while the permeates contained the free vitamin C molecules.

Cytotoxicity and absorption of the liposomes in Caco-2 cells MTS assay. The effect of liposomal vitamin C on cell viability was assessed by the MTS cell viability assay (Al Sabbagh et al. 2015). During the logarithmic growth phase, Caco-2 cells were seeded in 96-well plates (20-fold dilution) at a density of approximately 106 $cells \cdot well^{-1}$, and 600 µL of liposome solution was added to each well.

After 24 h of incubation at 37 °C with a 5% CO_2 atmosphere, the supernatant was discarded and replaced with 600 µL of fresh liposome solution. Following an additional 24-hour period of incubation, 10 µL of MTS reagent was added to each well and incubated for approximately 2 h. Absorbance was measured at 490 nm. Caco-2 cells were also cultured in transwell inserts and monitored to ensure complete confluency across the membrane surface.

To determine the bioavailability of liposomal vitamin C, the Caco-2 cellular model was used to assess permeability and uptake. Liposome solutions were filtered and sterilised using a 0.45 µm membrane. When the transepithelial electrical resistance (TEER) of the Caco-2 monolayer exceeded 300 $\Omega \cdot cm^{-2}$ and full surface coverage was confirmed, transport experiments were initiated.

The Caco-2 cell layer was washed three times with DMEM/F12 solution (pH = 7.4), and the cells were incubated at 37 °C. For transport from the apical to the basolateral side, 600 µL of the sample solution was added to the apical side (donor compartment), and 1.2 mL of DMEM/F12 solution was added to the basolateral side (receiving compartment). For transport from the basolateral to the apical side, 1.2 mL of the sample solution was added to the basolateral side, and 0.6 mL of HBSS solution was added to the apical side. Samples (up to 300 µL) were collected at 5, 10, 30, and 60 min, with an equivalent volume of 300 µL of DMEM/F12 solution added to maintain the volume and osmotic balance (Hubatsch et al. 2007). Vitamin C concentrations in apical and basal compartments were quantified using HPLC. In this experiment, the bioavailability is expressed as the

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relative difference in active ingredient content in the basal layer of the Caco-2 model.

Storage stability. Stability is a crucial factor in the formulation and design of liposomal products. The physical instability of liposomal formulations is often attributed to an increase in particle size, which results from self-aggregation of liposomes due to processing techniques and/or extended storage periods. Sample 1 (50 mL), which needs to be stored at room temperature and in a cool place, was exposed to 4, 25, and 40 °C at 75% relative humidity to perform an accelerated storage stability test and monitor sedimentation and phase formation in the climate chamber over a period of 270 days (FDA 2003). At the end of the storage period, accelerated shelf-life analysis was conducted to calculate the average shelf life of the samples.

At the conclusion of the stability testing experiment, the accelerated shelf life (ASL) of the product was calculated using the obtained data. The fundamental principle of ASL is to accelerate the ageing rate of the product, with temperature being the most commonly recognised accelerating factor (Phimolsiripol and Suppakul 2016). ASL serves as an effective method for estimating the shelf life of food and pharmaceutical products and it was applied in this study to determine the average shelf life of Sample 1 according to the relevant equation:

$$ASL = real\ shelf\ time / AAF \quad (2)$$

where: AAF – the accelerated ageing factor, calculated as:

$$AAF = Q_{10}\left[\left(T_{AA} - T_{RT}\right)/10\right] \quad (3)$$

where: T_{AA} – the accelerated ageing temperature (°C); T_{RT} – the ambient temperature (°C). Typically, a Q_{10} value of 2 is used for food or pharmaceutical products; however, Al-Haushey and Moussa reported a value of 1.218 666, which was used here (Moussa and Al-Haushey 2015).

Principal component analysis (PCA) and partial least squares (PLS) were performed by Python 3.5 (Mendez et al. 2020).

RESULTS

Impact of formulation on liposomal vitamin C encapsulation efficiency. The experimental design sets outlined in Table 1 were implemented individually, with the results displayed in Figure 1. This figure demonstrates how different pressure levels and cycle numbers impact encapsulation efficiency. Encapsulation efficiency refers to the amount of a compound that is encapsulated by liposomes relative to the total amount used (Moghimipour et al. 2018). The encapsulation efficiency

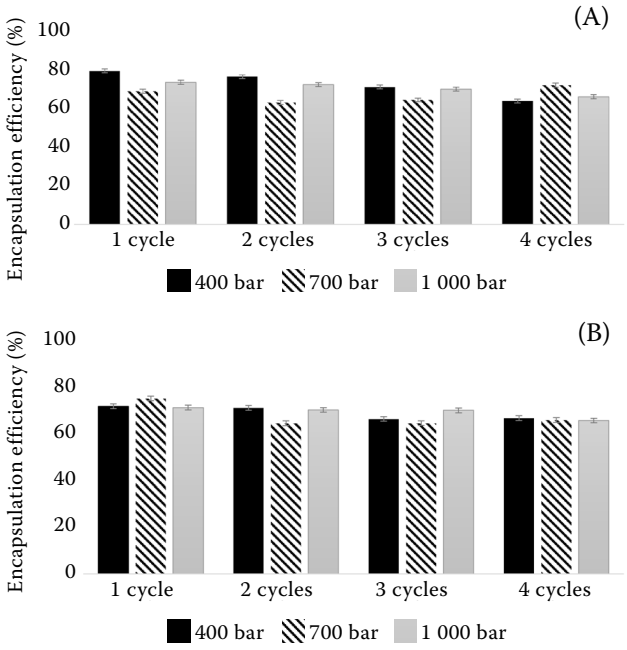

Figure 1. Encapsulation efficiency of liposomal vitamin C samples (A) 1–12, (B) 13–24

of samples 1–24 generally decreased with increasing pressure values (Figure 1). Notably, the encapsulation efficiency of samples 1–12 decreases more gradually and consistently at pressures of 400 bar and 1 000 bar. The highest encapsulation efficiency achieved was 77.6% at 400 bar with a formulation of 20% lecithin and 20% vitamin C (Sample 1). This was followed by 77.4% at 400 bar with a formulation of 10% lecithin and 20% vitamin C (Sample 14) and 76.8% at 700 bar with a formulation 10% lecithin and 20% vitamin C (Sample 17). The number of homogenisation cycles did not significantly impact the encapsulation efficiency for samples 13–24 at 1 000 bar with a formulation containing 10% lecithin and 20% vitamin C.

Although the encapsulation efficiency values presented in Figure 1 are relatively close, the three best-performing samples (Sample 1, 14, and 17) were selected for further analysis. Zeta potential and particle size measurements were conducted for these samples and compared with the control group (unloaded liposome) (Table 2).

The size and physical properties of liposomes. The control group exhibited the smallest liposome size and the highest zeta potential (Table 2). In contrast, the liposome-loaded samples (1, 14, and 17) displayed larger particle sizes and lower zeta potential values. Among these, Sample 1 demonstrated a more uniform size distribution, higher encapsulation efficiency, and a relatively high zeta potential value compared to the other formulations.

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Table 2. The physical properties of samples

Samples	Zeta potential (mV)	Size (nm)
Control (unloaded liposome)	–26.01 ± 0.574	288.6 ± 5.632
1	–23.17 ± 0.534	317.5 ± 8.863
14	–12.35 ± 0.458	757.9 ± 13.069
17	–18.37 ± 0.974	587.5 ± 7.769

***In vitro* gastrointestinal digestion simulation.** To evaluate the stability of Sample 1 under physiological conditions, an *in vitro* gastrointestinal digestion simulation was conducted. Vitamin C release from liposomes was tracked during both gastric and intestinal phases to assess integrity retention and controlled release characteristics (Figure 2).

Approximately 15% of the encapsulated vitamin C was released by the end of the digestion process (Figure 2). Initially, Sample 1 showed an encapsulation efficiency of 77.6%. Following an exposure to the gastric phase, 95.9% of vitamin C remained encapsulated, indicating minimal degradation. After 2 h in the intestinal phase, encapsulation efficiency decreased to 84.7%, suggesting that the formulation preserved liposomal integrity under gastrointestinal conditions.

Cytotoxicity and absorption in Caco-2 cells. The cytotoxicity of Sample 1 was evaluated by exposing Caco-2 cells to the liposomal formulation for 60 min, followed by an MTS assay (Figure 3A). The goal was to assess potential adverse effects of liposomal vitamin C on intestinal epithelial cells during digestion. Caco-2 monolayer integrity was verified through visual observation under transwell conditions (Figure 3B).

According to HPLC results, liposomal vitamin C exhibited 30% higher absorption in the basal compartment compared to its traditional (non-liposomal) form. Following the collection of all physical and biological responses from the analyses, Sample 1 demonstrated significant properties, making it a strong candidate for a liposomal product.

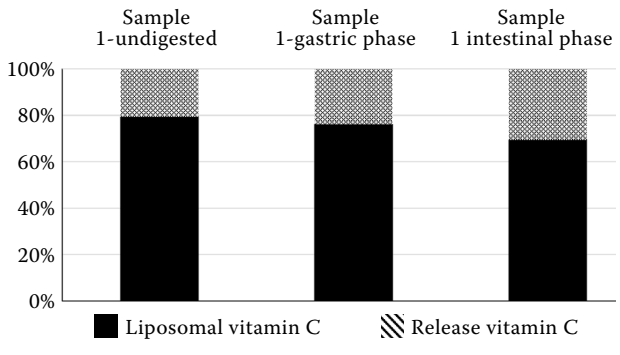

Figure 2. Encapsulation efficiency of Sample 1 during digestion

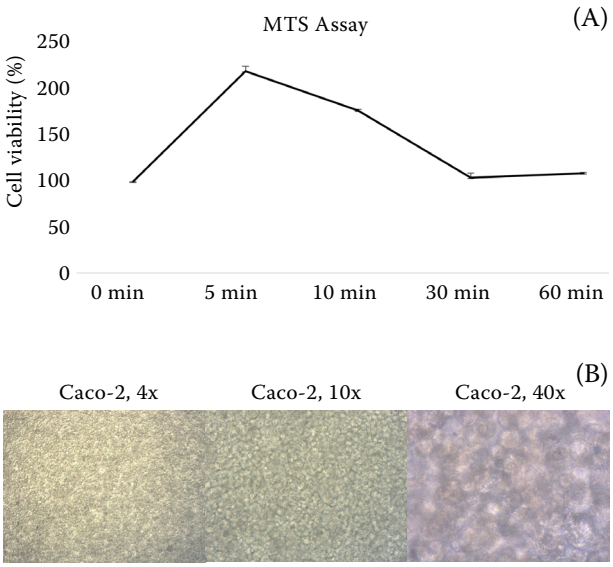

Figure 3. (A) Cell viability of the Caco-2 cell line after exposure to ascorbic acid with the MTS assay (B) Caco-2 cells were observed under an inverted phase-contrast microscope (ZEISS Primovert - Axiocam105, magnifications 4×, 10×, and 40×)

Storage stability of liposomal vitamin C. Sample 1 was monitored over a 270-day period to observe any potential phase separation or sedimentation formation. The physical stability of Sample 1 was evaluated by recording phase formation and sedimentation for 240 days (Figure 4).

The data obtained from this analysis showed the stability of liposomal vitamin C exposed to 40 °C and 75% relative humidity, with no sedimentation or phase change detected during the first 240 days of storage at three different temperatures. However, sediments were observed on the 270th day at 40 °C. Figure 4A presents the particle size variations of Sample 1 throughout storage: 317.5 (Day 0), 337.9 (Day 180), 345.2 (Day 210), and 366 (Day 240). Figure 4B provides a visual representation of Sample 1 appearance over time, while Figure 4C illustrates its liposomal morphology. The image in Figure 4C reveals a well-defined, spherical liposome with a diameter of 335.3 nm, consistent with expected size ranges for liposomal carriers. The darker interior indicates the potential encapsulation of vitamin C. Throughout the storage duration at 25 °C, the formulation maintained an average particle size within the 300–400 nm range, with only minimum size fluctuation toward the end of the study. The results

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confirm that Sample 1 maintained its structural integrity and high physical stability under various storage conditions.

Using the Equation (2), the estimated real shelf life of the product was calculated to be 585 days.

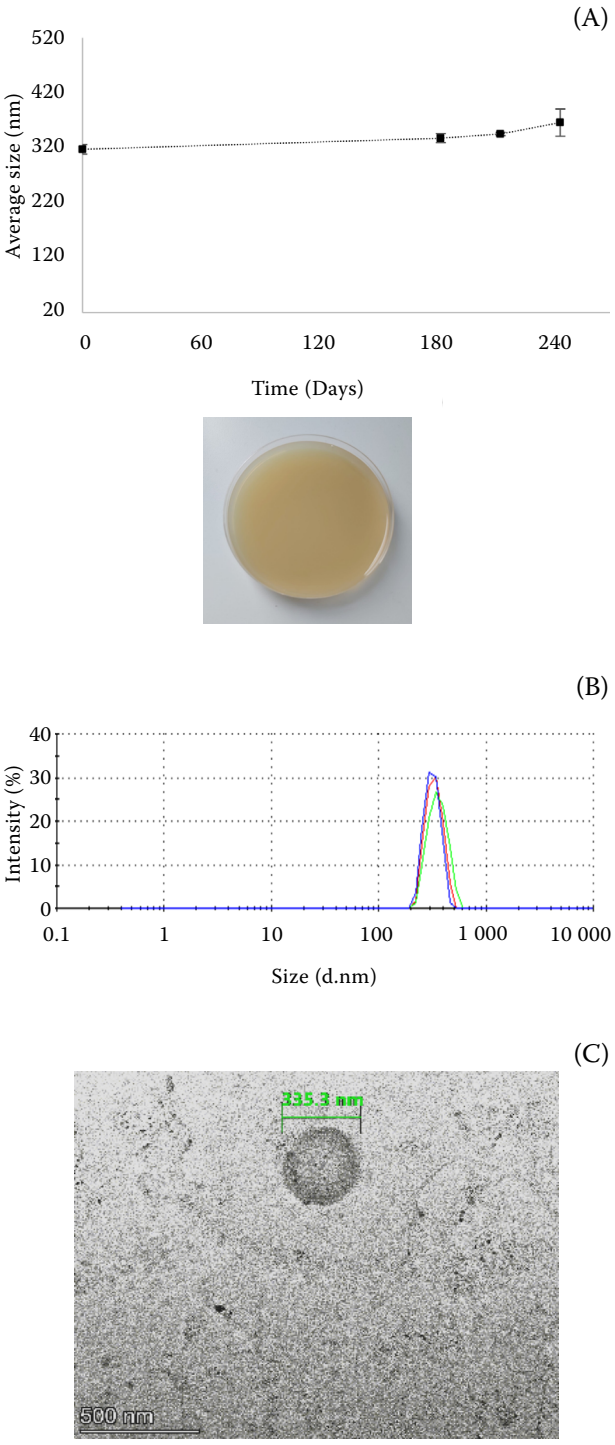

Figure 4. Effect of storage conditions of liposomal vitamin C over 6 months in the dark at 25 °C on (A) particles' average size; (B) intensity and size of Sample 1; (C) TEM imaging.

DISCUSSION

The aim of this study was to evaluate the effects of formulation and pressure parameters in the preparation of high-concentration liposomal vitamin C and to determine the impact of high pressure and multiple cycles on the liposome stability. The experimental results demonstrated that the increase in applied pressure and in the number of cycles did not significantly affect the stability of the liposomal product. However, the findings highlight the crucial role of lecithin concentration in optimising encapsulation efficiency, liposome stability, bioavailability, and overall performance in the design of effective liposomal drug delivery systems.

In our study, the phospholipids selected for liposome production exhibited amphiphilic properties, enabling them to self-assemble into bilayer membranes in aqueous solutions. During liposome formation, the hydrophilic head groups of the phospholipids interacted with the aqueous phase, while the hydrophobic tails interacted with each other. This organisation resulted in a vesicle with a hydrophilic core surrounded by a hydrophobic bilayer. Structurally, this phospholipid layer resembles a cell membrane, which facilitates the transfer of bioactive molecules dissolved within the liposome into cells through a process known as fusion.

During fusion, the phospholipids on the outer layer of the liposome merge with the cellular membrane, integrating into its structure. In addition to the liposomal structure ability to fuse with the cell membrane, the number of active molecules it can carry, referred to as encapsulation efficiency, is a critical factor influencing the success of a liposomal formulation. Therefore, encapsulation efficiencies were compared across different experimental conditions.

The study results showed that Sample 1 exhibited the highest encapsulation efficiency among all the tested formulations. Differences in the zeta potential were attributed to several physicochemical factors, primarily influenced by the lipid composition, the properties of the encapsulated compound, and surface charge density. The zeta potential, which represents the electrical charge on the surface of liposomes, plays a crucial role in determining colloidal stability and cellular interactions. The surface charge of liposomes is a key parameter in maintaining the physical stability of liposomal vesicles in suspension and is typically assessed through the zeta potential measurements (Rasmussen et al. 2020). The high zeta potential indicates stronger surface charge repulsion, thereby enhancing the stability of the dispersion (Smith et al. 2017). In this study,

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variations in the zeta potential were observed among different liposomal formulations. Sample 1, formulated with 20% lecithin, exhibited a higher zeta potential (–23.17 mV), suggesting improved dispersion stability due to increased electrostatic repulsion. In contrast, Sample 14, which had a lower lecithin concentration (10%), displayed a lower zeta potential (–12.35 mV), indicating reduced charge density and higher aggregation tendency. Additionally, the encapsulation of vitamin C may have influenced the zeta potential, as interaction between negatively charged ascorbate ions and the phospholipid bilayer can alter the surface charge distribution. In a study investigating the effect of vitamin C encapsulation on the zeta potential, the impact of different ratios of milk phospholipid, cholesterol, and phytosterol powders, along with various sonication durations, was evaluated in the development of a nanoliposome formulation. The results demonstrated that extending the sonication duration and lowering the phospholipid/phytosterol ratio significantly reduced the particle size of the nanoliposomes. Additionally, replacing phytosterol with cholesterol had a positive effect on encapsulation efficiency and vitamin C stability. These findings confirm that vitamin C encapsulation also affects the zeta potential and surface charge characteristics (Amiri et al. 2019).

Overall, these findings highlight the critical role of lipid composition and formulation parameters in modulating the zeta potential, which is an essential factor for maintaining liposomal stability, enhancing bioavailability, and ensuring the effectiveness of targeted drug delivery efficiency.

The data indicate that lecithin concentration plays a more critical role in achieving high encapsulation efficiency than variations in pressure or the number of processing cycles. This may be attributed to the lecithin stabilising effect on the dispersion, which effectively absorbs the energy from the high pressure applied during liposome formation. Such stabilisation likely enhances the encapsulation process, improving overall efficiency. Wang et al. (2017) reported that increasing the concentration of soy lecithin in oil/water emulsions improves emulsion quality by reducing the droplet size, increasing the zeta potential, improving stability, and enhancing encapsulation efficiency.

Liposomes are generally defined as delivery vehicles that mimic natural cell membranes, effectively transporting molecules from the oral cavity into the body via direct mucosal absorption. Additionally, they act as protective shields, safeguarding the encapsulated molecules from adverse environmental conditions. To evaluate the resilience of encapsulation efficiency under varying physiological conditions, *in vitro* digestion simulations were applied to Sample 1. This involved monitoring changes in encapsulation efficiency in response to stress factors such as fluctuating pH levels and enzymatic activity during digestion. Vergara et al. (2020) found that encapsulated lactoferrin is protected against gastric and intestinal digestion, suggesting its potential use in oral products for delivering sensitive molecules to the digestive system.

In the present study, the encapsulation efficiency of Sample 1 showed that the total amount of vitamin C was reduced by 15% following the intestinal digestion. Factors such as liposome particle size, wall materials, and surface modifications likely contributed to this digestive impact on the liposomal structure. This finding supports the intended purpose of liposomal products in protecting active ingredients during digestion.

The Caco-2 cell line, derived from human colon adenocarcinoma cells, is commonly employed to effectively mimic the conditions of the small intestine. It is widely used to study intestinal absorption, permeability, and transport mechanisms of various samples (Frank et al. 2017). Liposomal vitamin C did not exhibit any cytotoxic effects on the cells during the digestion (Figure 3A); rather, it promoted cell growth and proliferation. Previous studies support this finding, indicating that ascorbic acid does not exhibit toxicity toward the Caco-2 cell line (Korkmaz et al. 2023).

Żmuda et al. (2024) reported an absorption rate of 22.28% for liposomal vitamin C in the Caco-2 model, whereas in the present study, the absorption rate was measured at 30%. Additionally, it was observed that the Caco-2 cell line exhibited greater suppression when exposed to the applied liposomal active ingredients compared to the healthy cell line. As a result, the antioxidant properties of the active ingredients appeared to selectively eliminate damaged cells.

Several studies have investigated the influence of physical factors on liposome stability, identifying temperature as one of the most critical parameters affecting the shelf life and structural integrity of liposomes (Lin et al. 2022; Xia et al. 2022). Most of these studies evaluated stability over a storage period of 20–90 days, reporting retention rates at the end of the designated storage durations (Romano et al. 2024). In contrast, the present study extended the observation period to 270 days, significantly surpassing the duration reported in previous research. In this study, liposomal properties such as particle size, zeta potential, and intensity remained stable, although the amount

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of vitamin C slightly decreased to 935 mg·mL^{-1} by the end of the storage period. Anjani et al. (2023) similarly employed an accelerated storage strategy to evaluate the long-term stability of liposomal formulations.

Commercially, the expected shelf life of liposomal vitamin C typically ranges from 1 to 2 years. Based on these findings, the target shelf life is nearly achieved.

CONCLUSION

The study demonstrates that the formulation of the sample, mostly lipid composition, lecithin concentration and the energy input applied to the phospholipid solutions are the most critical factors for producing small-sized, homogeneous, and highly bioavailable liposomal products. The study serves as a notable example, as the stabilisation process was monitored over a 270-day period. By optimising these parameters, a more stable and effective formulation of liposomal vitamin C can be developed.

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