

## Modified Polymeric Surface Liposomal Vitamin C as an Advanced and Effective Formulation in Topical Skin Applications

Elin Gitlesen<sup>1</sup>, Susanne McGinn<sup>2</sup>, Nadia Mohamed<sup>3</sup>, Ali Saadani<sup>4</sup>, and Rahma H. Hagagy<sup>5</sup>

<sup>1</sup>Elin Gitlesen, Senior Technical Consultant, Scientific Department, Beautics Laboratories, Borre, Norway

<sup>2</sup>Susanne McGinn, Technical Executive, Scientific Department, Beautics Laboratories, Borre, Norway

<sup>3</sup>Nadia Mohamed, Professor Assistant, Medical Biochemistry Department, National Research Centre, Cairo, Egypt

<sup>4</sup>Ali Saadani, Chief Officer, Scientific Department, Polygon Technologies, Cairo, Egypt

<sup>5</sup>Rahma Hassan, Research Executive Specialist, Scientific Department, Polygon Research Centre, Cairo, Egypt

**Abstract:** This study evaluates and compares the anti-oxidation protective effects of two liposomal vitamin C formulations LipoVITA<sup>®</sup> C (conventional liposomal vitamin C formulation) and LipoVITA<sup>®</sup> C PLUS (an advanced modified polymeric surface liposome) against non-liposomal vitamin C (NL- VC). The study employs cell viability assay using human skin fibroblasts exposed to UV radiation module. Vitamin C is a potent but unstable antioxidant; liposomal encapsulation stabilizes it and enhances its efficacy. Human skin fibroblasts were treated with varying concentrations of LipoVITA<sup>®</sup> C, LipoVITA<sup>®</sup> C PLUS, and NL- Vitamin C (100, 10, 1, 0.1 µg/ml) and subjected to no UV exposure, 3 minutes UV exposure, or 8 minutes UV exposure. Cell viability was assessed using the MTT assay, and statistical analysis using one-way ANOVA, post-hoc tests, and SPSS software revealed highly significant differences between groups ( $p < 0.001$ ). Results showed that LipoVITA<sup>®</sup> C PLUS maintained the highest viability across all concentrations and UV exposures. Both liposomal formulations demonstrated concentration-dependent protection and retained over 90% viability at 0.1 µg/ml after 8 minutes of UV exposure, in contrast to the sharp decline in viability with NL- Vitamin C. In conclusion, LipoVITA<sup>®</sup> C PLUS proved superior in protecting fibroblasts against UV-induced damage compared to LipoVITA<sup>®</sup> C and NL- Vitamin C. The advanced modified polymeric surface liposomes enhanced vitamin C stability and intracellular delivery, thereby reducing cellular oxidation more effectively.

**Keywords:** Liposomal vitamin C, Liposomes, Vitamin C, LipoVITA<sup>®</sup> C, LipoVITA<sup>®</sup> C PLUS, UV radiation, Human skin fibroblasts, Antioxidant, Cell viability assay, Oxidative stress, Photoprotection.

### INTRODUCTION

Vitamin C, also known as ascorbic acid (AA), is a vital nutrient crucial in various physiological processes, including immune function, collagen synthesis, and antioxidant defense. It is commonly found in fruits and vegetables and is widely used as a dietary supplement to meet the recommended daily intake. As a potent scavenger of free radicals, vitamin C aids in mitigating oxidative stress and reducing the risk of chronic diseases [Morelli, M. B. *et al.*, 2020]. However, the stability and bioavailability of vitamin C can be compromised due to its susceptibility to environmental conditions and rapid degradation [Caritá, A. C. *et al.*, 2020].

The vulnerability of vitamin C to oxidation and degradation, when exposed to unfavorable conditions, hampers its efficacy as an antioxidant. Consequently, the challenges associated with the stability and bioavailability of vitamin C have sparked interest in innovative delivery systems [Caritá, A. C. *et al.*, 2020]. Among these approaches, liposomal encapsulation has emerged as a promising strategy. Liposomes, lipid-based vesicles, can encapsulate hydrophilic substances like vitamin C, providing various advantages such as enhanced stability, controlled release, and protection against degradation [Milano, G. *et al.*, 2022].

Among the emerging delivery systems, liposomal formulations have shown promise for improving the protection and absorption of vitamin C. Liposomes are lipid-based vesicles that can encapsulate hydrophilic substances, such as vitamin C, within their aqueous core [Łukawski, M. *et al.*, 2020]. This encapsulation shields vitamin C from degradation and facilitates its efficient delivery to target tissues. Consequently, liposomal vitamin C formulations have gained popularity as potential alternatives to conventional vitamin C supplements [Lv, X. *et al.*, 2022].

Previous research has focused on evaluating the protective effect of liposomal vitamin C formulations from commercial products against NL- Vitamin C. These studies have investigated various factors, such as liposomal composition, size, encapsulation efficiency, and release kinetics, to optimize the formulation and enhance the protective effects of vitamin C.

For example, a study demonstrated that liposomal encapsulation significantly improved the stability and bioavailability of vitamin C. The researchers formulated liposomes using a combination of phospholipids and cholesterol and found that the encapsulated vitamin C remained stable for a longer duration compared to NL- Vitamin C. This

finding suggests that liposomal encapsulation can protect vitamin C from degradation, potentially enhancing its shelf life and bioavailability [Csorba, A. et al., 2023].

Another study compared the antioxidant activity of liposomal vitamin C with non-liposomal vitamin C. The researchers evaluated the ability of liposomal vitamin C to scavenge free radicals and protect against oxidative stress. The results showed that liposomal vitamin C exhibited higher antioxidant activity than non-liposomal vitamin C, indicating its potential for enhanced protection against oxidative damage [Gopi, S. et al., 2021].

Furthermore, the combination of liposomal vitamin C with other antioxidants, such as vitamin E, glutathione, or alpha-lipoic acid, has shown synergistic effects in protecting against oxidative stress [Babbush, K. M. et al., 2020]. These findings suggest that liposomal vitamin C formulations have the potential to provide enhanced protection against free radicals and oxidative stress [Amini, L. et al., 2021].

Despite the promising outcomes reported in previous studies, there is still a need for further research to fully understand the mechanisms underlying the protective effect of liposomal vitamin C formulations and to optimize their formulation and delivery strategies. **This study aims** to evaluate and compare the protective effects of two different commercially available liposomal vitamin C formulations, **LipoVITA® C** and **LipoVITA® C PLUS**, against NL- Vitamin C, with a particular focus on their ability to safeguard human skin fibroblasts against UV-induced cellular damage. **LipoVITA® C** is a conventional liposomal vitamin C formulation, while **LipoVITA® C PLUS** is an enhanced formulation that combines vitamin C into advanced modified polymeric surface liposome. By elucidating the comparative efficacy and performance of these liposomal formulations, valuable insights can be gained regarding their potential applications in the field of skincare. Also, by assessing the protective effect of these liposomal formulations against NL-Vitamin C, we aim to shed light on their potential benefits in preserving the stability and bioavailability of this essential nutrient.

## 1 MATERIALS AND METHODS

### 1.1 Drugs

**LipoVITA® C and LipoVITA® C PLUS:** These liposomal Vitamin C formulations were procured from Beautics Laboratories (Head office at Horton,

Norway and manufacturing facility at Cairo, Egypt) and were of the highest cosmeceutical grade. The choice of these formulations was based on their liposomal encapsulation of vitamin C, providing enhanced stability and controlled release characteristics, which aligns with the aim of this study.

### 1.2 Cell Line, Cell Culture Propagation, and Maintenance

**Human skin fibroblast:** HSF cells were obtained from NAWAH Scientific Research Center (Cairo, Egypt). Procedures were conducted in a sterile Class II A2 biosafety cabinet (Labconco). Cells were cultured in DMEM supplemented with 10% heat-inactivated FBS (Gibco, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin sulfate (Lonza, Belgium) at 37°C in 5% CO<sub>2</sub>. At 70-90% confluence, cells were sub-cultured with trypsin-EDTA (Lonza, Belgium) and harvested in the logarithmic growth phase. Tested materials were dissolved in distilled water, stored at -20 °C, and diluted in culture medium immediately before each experiment.

### 1.3 Formulations Tested

**LipoVITA® C, LipoVITA® C PLUS, and NL-Vitamin C:** Three distinct vitamin C formulations were assessed in this study. LipoVITA® C and LipoVITA® C PLUS, both liposomal formulations, were compared with **Non-Liposomal Vitamin C (NL-VC)**. **LipoVITA® C** is a conventional liposomal formulation designed to encapsulate Vitamin C, enhancing its stability and providing moderate skin permeation. The liposomal vesicles enable Vitamin C to penetrate the skin barrier more effectively than NL- Vitamin C, offering controlled delivery and sustained release of the active ingredient, thus supporting its antioxidant properties. **LipoVITA® C PLUS** represents an advanced modified polymeric surface liposome, developed by *Beautics Laboratories*, significantly enhances skin permeation and retention. The technology incorporated to allow for increased stability and controlled permeation, making it highly effective in combating oxidative stress, minimizing aging signs, and evening skin tone. The surface of LipoVITA® C PLUS is developed with a specialized polymer matrix that modulates the stratum corneum's physicochemical characteristics, enabling better travel and permeation through skin layers while achieving extended retention.

## 1.4 Evaluation of Cytotoxicity by MTT Assay & IC50

### 1.4.1 MTT Assay

Cytotoxicity against HSF cells was evaluated using the MTT assay as per Mosmann (1983) [Mosmann, T, 1983; Mohamed, M. B. I. *et al.*, 2022]. Cells were seeded in 96-well plates at a density of  $1 \times 10^4$  cells/well in 100  $\mu$ l of growth medium and incubated overnight at 37°C with 2% CO<sub>2</sub>. Cells were treated with serial dilutions of each drug (100, 10, 1, 0.1  $\mu$ g/ml) for 48 hours, while untreated cells served as controls. After incubation, cells were washed with PBS, and 100  $\mu$ l of MTT solution (0.5 mg/ml) was added to each well and incubated for 4 hours. Formazan crystals were dissolved in 100  $\mu$ l of DMSO, and optical density (OD) was measured at 492 nm (reference at 630 nm) using a microplate reader. Cell viability was calculated as  $(OD_{\text{test}}/OD_{\text{control}}) \times 100$ , and IC50 values were derived from a four-parameter logistic curve using Sigma Plot software (version 11).

% Viability =  $\frac{\text{absorbance of drug}}{\text{absorbance of control}} \times 100$

### 1.4.2 IC50 Determination for Photoprotection

The IC50 values for LipoVITA<sup>®</sup> C, LipoVITA<sup>®</sup> C PLUS, and NL- Vitamin C were calculated to evaluate the dose at which each formulation reduced cell viability by 50% under UV exposure. This dose-response curve allowed for the comparative analysis of each formulation's photoprotective properties. The Sigma Plot software was used to fit a four-parameter logistic model to the data, plotting % cell viability against log-transformed concentrations to derive IC50 values for both 3-minute and 8-minute UV exposures.

### 1.5 UV Exposure

Cells were exposed to UV radiation for varying durations to simulate different levels of UV-induced stress on human skin fibroblasts. The exposure times included short-term (3 minutes) and prolonged (8 minutes) durations, with a control group that received no UV exposure (0 minutes). This design allowed for the assessment of the formulations' protective effects under distinct conditions, mimicking relevant environmental stressors.

Following UV exposure, samples from each group were taken for absorbance measurement using a spectrophotometer at a specified wavelength. Each measurement was repeated three times to ensure

accuracy. Mean absorbance values, along with standard deviation (SD) and standard error (SE), were calculated for each group [11].

### 1.6 Statistical Analysis

**ANOVA and Post-hoc Tests:** The data obtained from the experiments were analyzed using SPSS software (Version 27, IBM, Armonk, NY, USA). One-way ANOVA was applied to assess the overall variance between formulations, followed by post-hoc tests to identify significant differences ( $p < 0.05$ ) between specific groups. Data were presented as means  $\pm$  standard errors (SE), and statistical significance was set at  $p < 0.05$ . These statistical analyses enabled the identification of significant differences between the effects of the different formulations. The application of ANOVA allowed for the assessment of overall variation among the groups, while post-hoc tests enabled a detailed examination of specific differences between individual formulations.

By employing these comprehensive materials and methods, we can evaluate the protective effects of liposomal vitamin C formulations compared to NL- Vitamin C, providing a detailed understanding of their performance in mitigating UV-induced cellular damage.

## 2 RESULTS

### 2.1 Cell Viability Assay

The assessment of cell viability elucidated distinct trends among the different formulations of vitamin C. LipoVITA<sup>®</sup> C PLUS exhibited the highest percentage of viability across all concentrations and UV exposure durations, as shown in Table 1 and Figure 1, followed by LipoVITA<sup>®</sup> C, as shown in Table 2 and Figure 2, compared with NL-Vitamin C demonstrating the lowest viability rates, as shown in Table 3 and Graph 3. This observation underscores the potential of liposomal encapsulation in enhancing the stability and efficacy of vitamin C formulations, results showed.

#### 2.1.1 The metabolic cytotoxicity with MTT assay

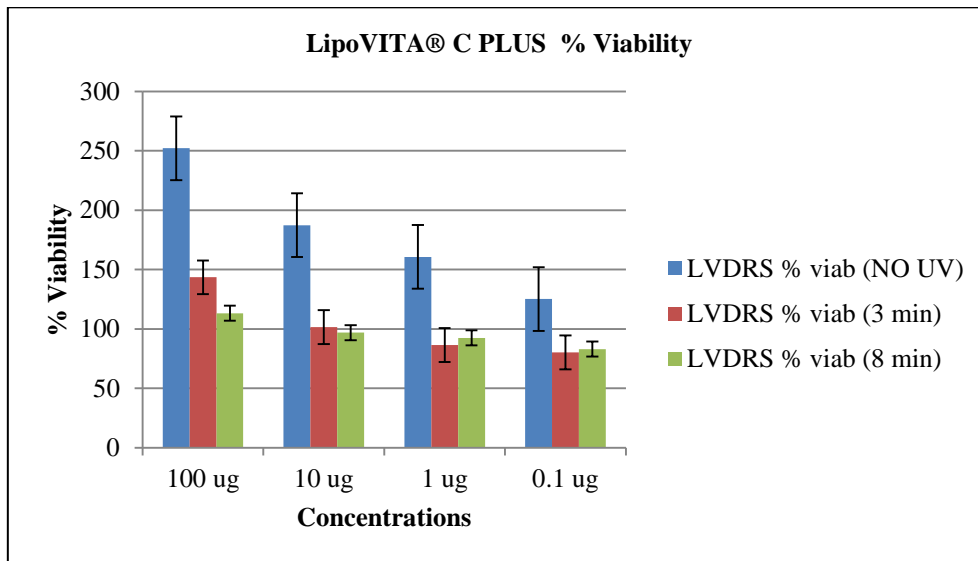
The metabolic cytotoxicity of LipoVITA<sup>®</sup> C PLUS on HSF cell viability was evaluated using MTT assay, see Table 1. Cytotoxicity was represented as a percentage of surviving fraction compared to untreated control cells. HSF cells were treated with LipoVITA<sup>®</sup> C PLUS at different concentrations ranging from (100- 0.1  $\mu$ M) with different conditions to ultraviolet rays for 48h. the cell

growth is not inhibited at any concentration indicating its safety.

**Table 1:** The mean percentage of the viable HSF cells after treatment with different doses of LipoVITA® C PLUS for 48h

Concentration of LV®C + μM	%HSF-cell viability ±SE (NO UV)	%HSF-cell viability ±SE (3 min UV)	%HSF-cell viability ±SE (8 min UV)
100	250.82 ±0.88	145.11 ±0.83	112.82± 0.86
10	185.34 ±1.15	102.58±0.57	95.83±0.56
1	161.61± 0.57	86.36±0.55	92.44±0.58
0.1	125.22±0.58	80.28± 0.56	82.96±0.57

Results are represented as (mean ± SE, n=3)



**Figure 1:** The mean percentage of the viable HSF cells after treatment with different doses of LipoVITA® C PLUS for 48h

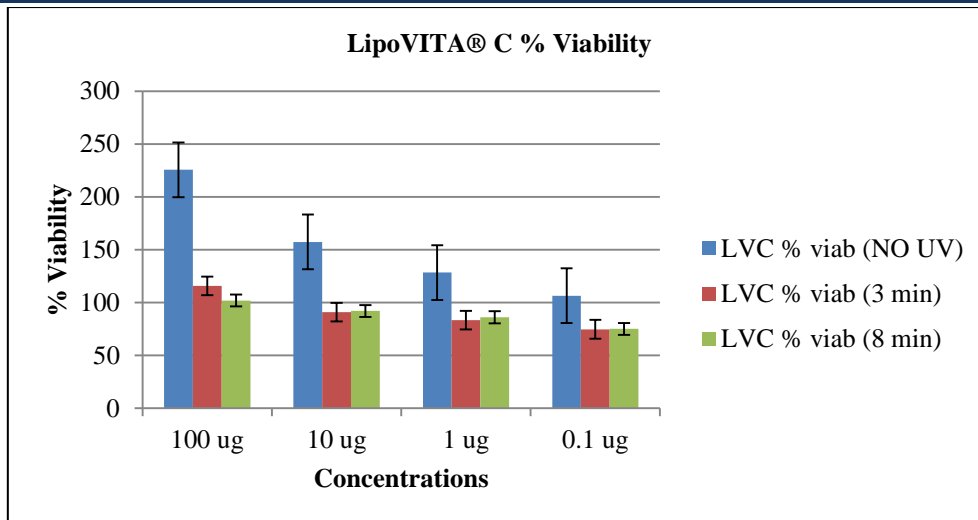
The metabolic cytotoxicity of LipoVITA® C on HSF cells viability was evaluated using MTT assay, see Table 2. Cytotoxicity was represented as a percentage of surviving fraction compared to untreated control cells. HSF cells were treated with

LipoVITA® C at different concentrations ranging from (100- 0.1 μM) with different conditions to ultraviolet rays for 48h. the cell growth is not inhibited at any concentration of LVC indicating its safety of use.

**Table 2:** The mean percentage of the viable HSF cells after treatment with different doses of LipoVITA® C for 48h

Concentration of LV®C + μM	%HSF-cell viability ±SE (NO UV)	%HSF-cell viability ±SE (3 min UV)	%HSF-cell viability ±SE (8 min UV)
100	224.87±2.33	118.06±1.20	103.89±1.15
10	128.44±0.88	92.84±0.87	91.97±0.57
1	128.77±0.88	82.45±0.57	86.07±0.86
0.1	106.39±0.58	75.68±0.33	75.01±0.56

Results are represented as (mean ± SE, n=3)



**Figure 2:** The mean percentage of the viable HSF cells after treatment with different doses of LipoVITA® C for 48h

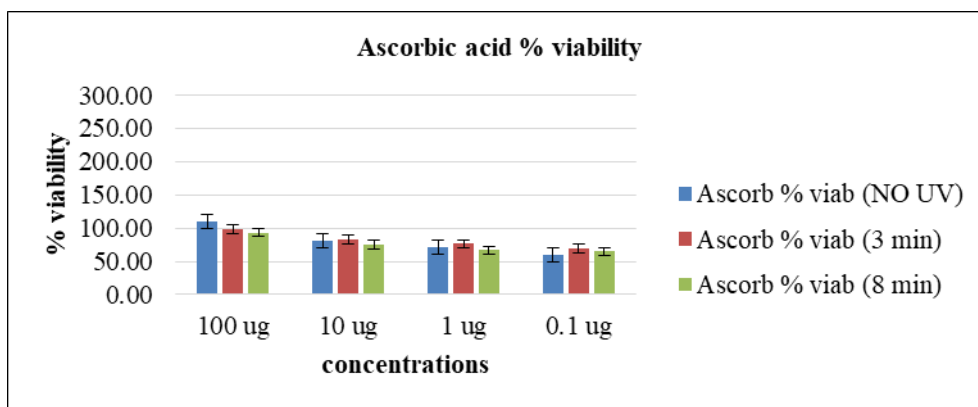
The metabolic cytotoxicity of ascorbic acid on HSF cells viability was evaluated using MTT assay; see Table 3 and Figure 3. Cytotoxicity was represented as a percentage of surviving fraction compared to untreated control cells. HSF cells

were treated with ascorbic acid at different concentrations ranging from (100- 0.1 μM) with different conditions to ultraviolet rays for 48h. the cell growth is not inhibited at any concentration of ascorbic acid indicating its safety of use.

**Table 3:** The mean percentage of the viable HSF cells after treatment with different doses of ascorbic acid for 48h

Concentration of LV®C + μM	%HSF-cell viability ±SE (NO UV)	%HSF-cell viability ±SE (3 min UV)	%HSF-cell viability ±SE (8 min UV)
100	110.07 ± 0.33	99.74 ± 0.57	95.04 ± 0.88
10	81.19 ± 0.58	82.54 ± 0.56	75.40 ± 0.50
1	71.19 ± 0.57	76.47 ± 0.88	65.62 ± 0.57
0.1	61.06 ± 0.54	69.54 ± 0.32	64.54 ± 0.59

Results are represented as (mean ± SE, n=3)



**Figure 3:** The mean percentage of the viable HSF cells after treatment with different doses of ascorbic acid for 48h

**2.1.2 Effect of Concentration**

A dose-dependent decrease in viability was observed for all treatments, highlighting the importance of concentration in determining the cytotoxic effects of the formulations. Notably, at a concentration of 0.1 μg/ml, LipoVITA® C PLUS maintained a viability of 82.96%, whereas NL-

Vitamin C exhibited a substantially lower viability of 64.54%. This disparity underscores the superior performance of liposomal formulations in maintaining cellular viability at lower concentrations.

**2.2 Effect of UV Exposure**

Duration the impact of UV exposure duration on cell viability varied across formulations. For instance, viability after 8 minutes of UV exposure was lower (64.54%) compared to 3 minutes (69.54%) for NL- Vitamin C at a concentration of 0.1 µg/ml. In contrast, LipoVITA® C PLUS demonstrated remarkable resilience, maintaining over 90% viability even after 8 minutes of UV exposure at the same concentration. This finding suggests the enhanced photoprotective properties of LipoVITA® C PLUS under prolonged UV exposure (Table 4, Graph 4. A, B, C).

Absorbance values for each treatment group (0 min, 3 min, and 8 min) were recorded and analyzed. The mean absorbance values decreased with increasing UV exposure duration, as shown in Table 5. As shown in Figure 5, absorbance values decreased significantly with increasing UV exposure time. In Figure 6, the relationship between absorbance and UV exposure is further highlighted with a regression line, showing a negative correlation ( $y = -0.2695x + 1.1557$ ,  $R^2 = 0.9404$ ), indicating a strong linear decrease in absorbance as UV duration increased.

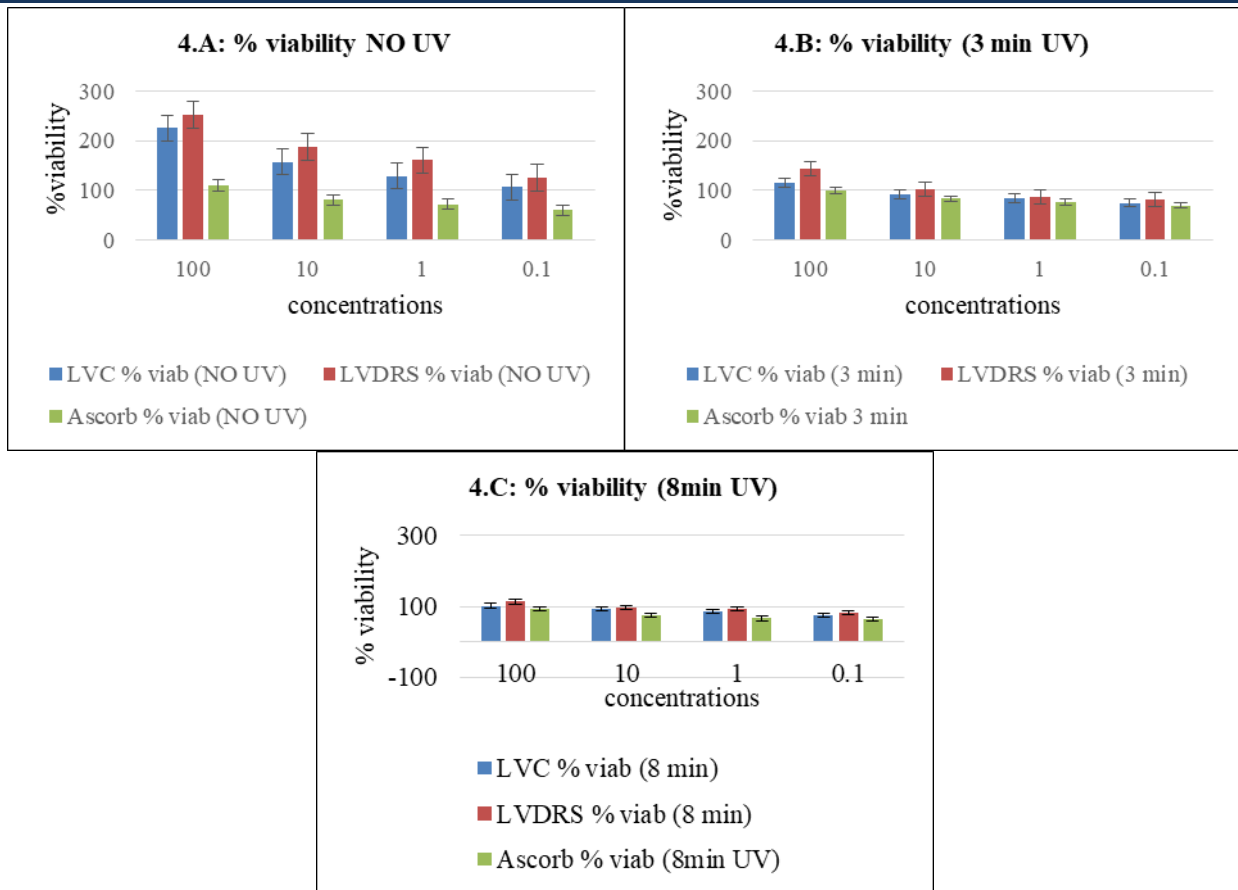
**Table 4:** Multiple comparisons of different drugs from 3 different experiments

Drugs	Groups	No UV	3 min UV	8 min UV
LipoVITA® C	No UV	NS	0.001	0.001
	3 min UV	0.001	NS	NS
	8 min UV	0.001	NS	NS
LipoVITA® C PLUS	No UV	NS	0.001	0.001
	3 min UV	0.001	NS	NS
	8 min UV	0.001	NS	NS
Ascorbic acid (NL-VC)	No UV	NS	0.001	0.001
	3 min UV	0.001	NS	NS
	8 min UV	0.001	NS	NS

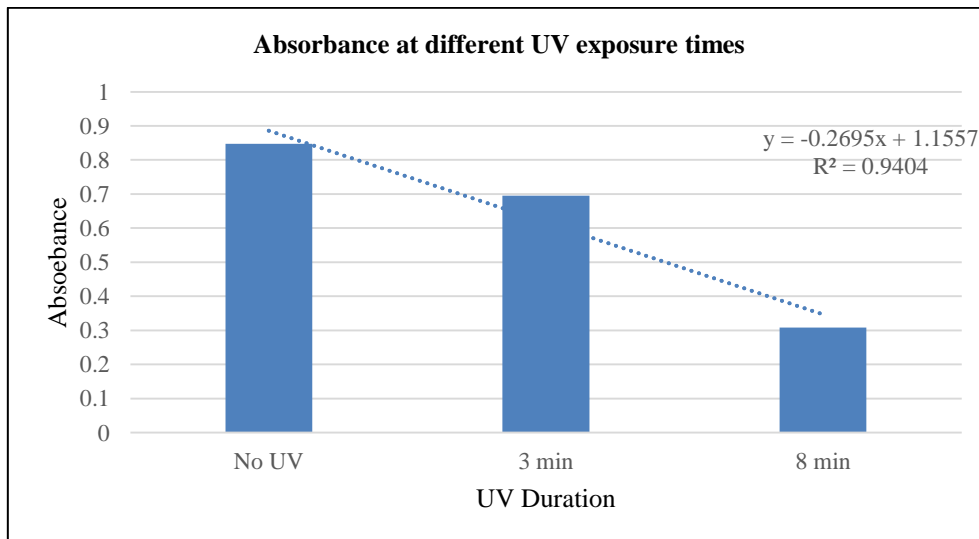
NS: nonsignificant while significant at  $\leq 0.05$  (one-way ANOVA) post HOC, Bonferroni test.

**Table 5:** Absorbance values with varying UV exposure durations.

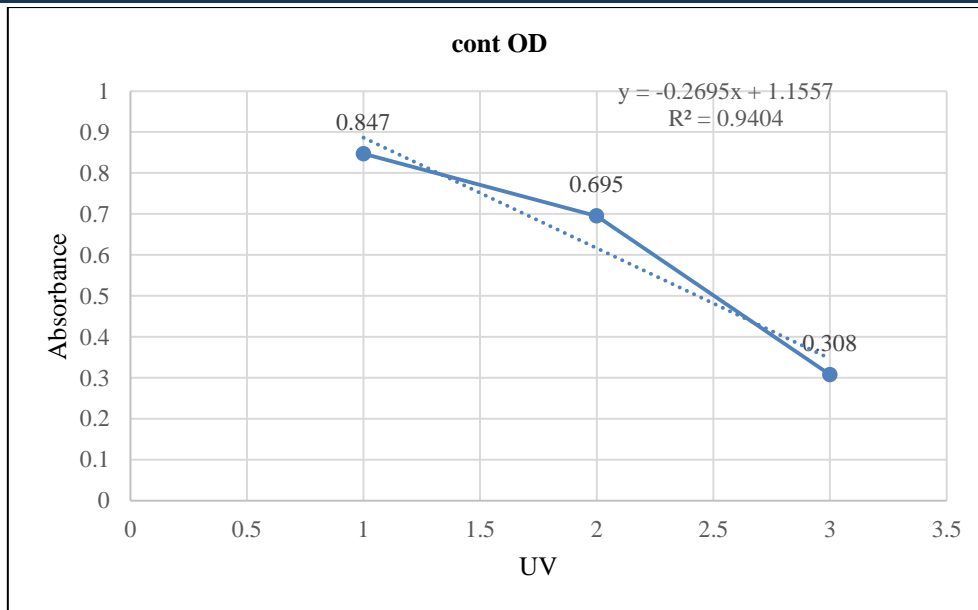
	Mean	±SD	±SE
Control (NO UV)	0.8470	0.0573	0.0165
Control (3 min)	0.6953	0.0617	0.0178
Control (8 min)	0.3076	0.1219	0.0352



**Figure 4:** Comparative Analysis of Cell Viability across Different Drug Concentrations and UV Exposure Durations. % Viability of cells with different drug concentrations without UV exposure (A). % Viability of cells with different drug concentrations after 3 minutes of UV exposure (B). % Viability of cells with different drug concentrations after 8 minutes of UV exposure (C)



**Figure 5:** Absorbance at different UV exposure times. The chart illustrates the decrease in absorbance with increasing UV exposure time. The trend line shows a clear negative correlation, with an  $R^2$  value of 0.9404, indicating a strong fit of the trend line to the data points.



**Figure 6:** Correlation between absorbance and UV exposure duration. This graph shows absorbance values at each UV exposure point with a trend line fitted to the data. The line graph confirms the reduction in absorbance with longer UV exposure, reinforcing the negative correlation.

**2.3 The IC50 Values**

The determination of IC50 values provided insights into the concentration thresholds required to achieve a 50% reduction in cell viability. IC50 values for each formulation were determined under both 3-minute and 8-minute UV exposures. LipoVITA® C PLUS showed the highest IC50 (100 µg/ml), suggesting higher photoprotective efficacy compared to LipoVITA® C and NL-

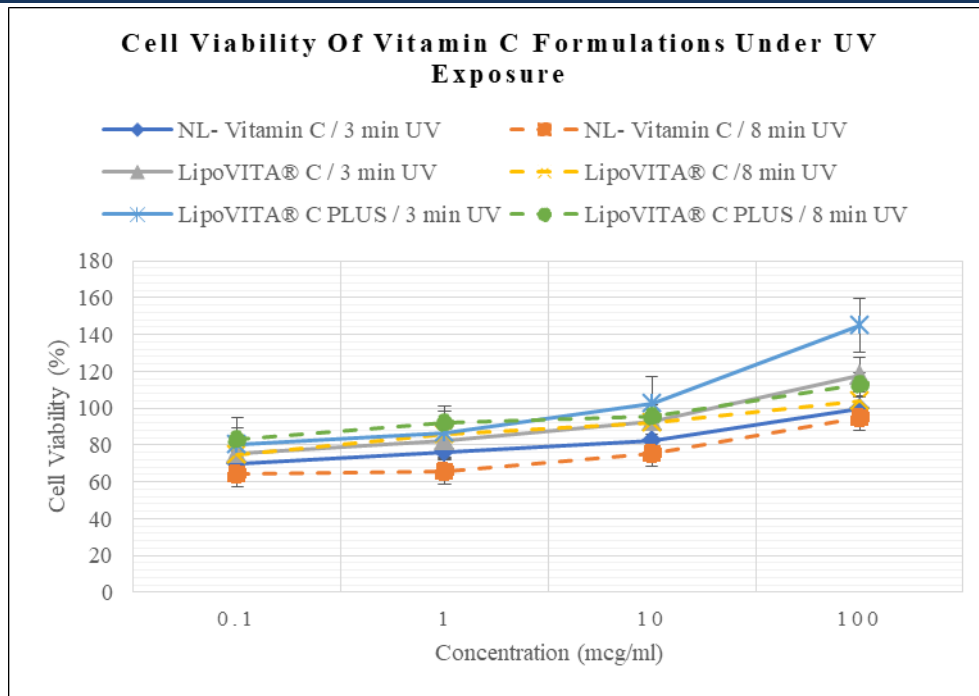
Vitamin C requirement for inducing cytotoxic effects compared to other formulations. Furthermore, LipoVITA® C PLUS maintained higher cell viability at lower concentrations, particularly under prolonged UV exposure. This finding underscores the potential safety profile of LipoVITA® C PLUS at lower concentrations, see Table 6 and Figure 7.

**Table 6:** Cell Viability Under UV Exposure at Various Concentrations

Concentration of LV® C + µM	Concentration (µg/ml)	Cell Viability (%) - 3 min UV	Cell Viability (%) - 8 min UV
<b>LipoVITA® C</b>	0.1	75.68	75.01
	1	82.45	86.07
	10	92.84	91.97
	100	118.06	103.89
<b>LipoVITA® C PLUS</b>	0.1	80.28	82.96
	1	86.36	92.44
	10	102.58	95.83
<b>NL- Vitamin C</b>	0.1	69.54	64.54
	1	76.47	65.62
	10	82.54	75.4
	100	99.74	95.04

Results are represented as (mean ± SE, n=3)





**Figure 7:** Cell Viability Under UV Exposure at Various Concentrations

### 3 DISCUSSION

#### 3.1 Effect of Liposomal Encapsulation

Liposomal encapsulation offers several significant advantages in enhancing the stability and bioavailability of vitamin C formulations. By encapsulating vitamin C within liposomes, the release kinetics can be precisely modulated, resulting in a prolonged serum half-life and improved cellular uptake. This sophisticated controlled release mechanism not only significantly enhances the efficacy of vitamin C but also minimizes its premature degradation. Consequently, liposomal encapsulation maximizes the therapeutic potential of vitamin C, ensuring sustained antioxidant activity and superior protection against oxidative stress. Additionally, this method facilitates targeted delivery to skin cells, optimizing the overall effectiveness of the formulation in topical skin applications. [Kenchappa, V. *et al.*, 2022].

#### 3.2 Superior Protection by LipoVITA® C & LipoVITA® C PLUS

The superior photoprotective efficacy of LipoVITA® C PLUS can be attributed to its multi-antioxidant formulation, which may exert additive or synergistic effects in combating UV-induced oxidative stress. The combination of antioxidants within the liposomal formulation enhances the overall antioxidant capacity, providing robust protection against UV-induced cytotoxicity and oxidative damage. Also, LipoVITA® C PLUS's higher IC<sub>50</sub> and cell viability suggest superior

photoprotection under UV-induced oxidative stress, likely due to its multi-antioxidant composition. This increased efficacy highlights its potential as a safe and effective formulation for topical or supplemental photoprotective applications [Każmierczak-Barańska, J. *et al.*, 2020].

#### 3.3 Mechanism of Protection & Anti-oxidation

Liposomal formulations offer a protective microenvironment that shields vitamin C from pre-systemic degradation and oxidative stress. Furthermore, the nanosized liposomes facilitate efficient intracellular delivery of active vitamin C to target organelles, such as mitochondria and nuclei, where it can exert its antioxidant effects. In contrast, NL- Vitamin C is more susceptible to oxidation and degradation in the extracellular milieu, limiting its bioavailability and efficacy [Caritá, A. C. *et al.*, 2022; Milano, G. *et al.*, 2022].

LipoVITA® C PLUS, with its advanced modified polymeric surface, offers additional benefits. The polymeric surface stabilizes the liposomes, enhances membrane interaction, and improves penetration and retention in the skin. It also provides controlled and sustained release of vitamin C, resulting in prolonged antioxidant activity and superior protection against UV-induced oxidative damage [Babbush, K. M. *et al.*, 2020].

In contrast, NL-Vitamin C is more susceptible to oxidation and degradation in the extracellular

milieu, limiting its bioavailability and efficacy. The enhanced stability and targeted delivery of liposomal formulations, especially LipoVITA<sup>®</sup> C PLUS, offer significant advantages over non-liposomal vitamin C in protecting the skin from oxidative stress and UV-induced damage [Łukawski, M. *et al.*, 2020].

### 3.4 Impact of Concentration and UV Exposure

The observed concentration-dependent effects and the enhanced resilience of liposomal formulations under prolonged UV exposure highlight the importance of formulation design in optimizing therapeutic outcomes. Lower concentrations of LipoVITA<sup>®</sup> C and LipoVITA<sup>®</sup> C PLUS were found to confer significant protection against UV-induced cytotoxicity, suggesting a potential dose-sparing effect. Moreover, the sustained efficacy of LipoVITA<sup>®</sup> C PLUS under prolonged UV exposure underscores its utility in mitigating the deleterious effects of environmental stressors on skin health.

The results indicate a negative correlation between UV exposure duration and absorbance values. This decrease in absorbance may be attributed to structural or chemical changes in the sample caused by prolonged UV exposure, potentially leading to degradation of absorbing compounds. The reduction in absorbance between each treatment group suggests that UV exposure significantly impacts sample integrity. This finding may indicate a threshold beyond which UV exposure causes notable degradation. Similar studies have reported a decrease in absorbance with increasing UV exposure, suggesting that this effect is consistent across different sample types. Such findings reinforce the idea that prolonged UV exposure can compromise the integrity of biological samples.

These findings underscore the importance of controlling UV exposure in experimental and industrial applications. Future studies should explore alternative exposure times to identify optimal conditions for preserving sample integrity [Mohamed, M. B. I. *et al.*, 2022].

### 3.5 Future Studies

Future studies should focus on the detailed characterization of liposomal formulations to better understand their bioavailability, retention, and permeability. This includes investigating the physicochemical properties of liposomes, such as size, surface charge, encapsulation efficiency, and release kinetics. Advanced techniques like

dynamic light scattering (DLS) and electron microscopy can be employed to precisely characterize these properties.

Additionally, studies should assess the retention and permeability of liposomal vitamin C in skin tissues over extended periods. This involves examining how well the liposomes penetrate different skin layers and how long they can retain vitamin C within the skin. Techniques such as fluorescence microscopy and skin diffusion assays (e.g., Franz diffusion cell) can be utilized to measure the depth and duration of vitamin C retention in the skin. Understanding these factors will provide valuable insights into optimizing liposomal formulations for enhanced therapeutic efficacy and longer-lasting skin protection.

### 3.6 Relevance to In vivo Efficacy

The physicochemical properties of LipoVITA<sup>®</sup> C PLUS, including its negatively charged and nanosized liposomes, are conducive to enhanced skin penetration and localization of vitamin C within skin cells and tissues. This localization facilitates the targeted delivery of antioxidants, thereby enhancing the topical anti-photoaging efficacy of LipoVITA<sup>®</sup> C PLUS. The findings from this study hold implications for the development of novel skincare formulations aimed at combating UV-induced skin damage and promoting skin health.

## CONCLUSION

This study comprehensively evaluated the protective effects of two liposomal vitamin C formulations against UV-induced cytotoxicity in human skin fibroblasts. LipoVITA<sup>®</sup> C and LipoVITA<sup>®</sup> C PLUS, featuring a multi-antioxidant liposomal system, conferred the strongest protection as evidenced by the highest cell viability across all treatment parameters. Both liposomal formulations performed significantly better than NL- Vitamin C in maintaining cellular health under increasing oxidative stress.

The enhanced efficacy of the liposomal vitamin C formulations can be attributed to their ability to shield vitamin C from degradation and facilitate its efficient intracellular uptake. This allows higher concentrations of active antioxidants to reach subcellular sites and combat UV-induced free radicals before damage occurs. In contrast, the NL- Vitamin C demonstrated inferior photoprotection due to its susceptibility to pre-systemic oxidation.

The results validate that liposomal encapsulation of vitamin C using optimized delivery systems like

LipoVITA<sup>®</sup> C PLUS, as an advanced modified polymeric surface liposome, maximizes its antioxidant defence capabilities and promotes cell viability. This has important implications for developing advanced topical formulations with enhanced skin protection and anti-aging properties. Overall, this study provides novel insights into the formulation factors modulating vitamin C efficacy and the biomolecular mechanisms by which liposomal delivery systems strengthen photoprotective outcomes.

## ACKNOWLEDGEMENTS

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