Liposomal-Vitamin C (LSL - VitCLip): Comparative Bioavailability Study.

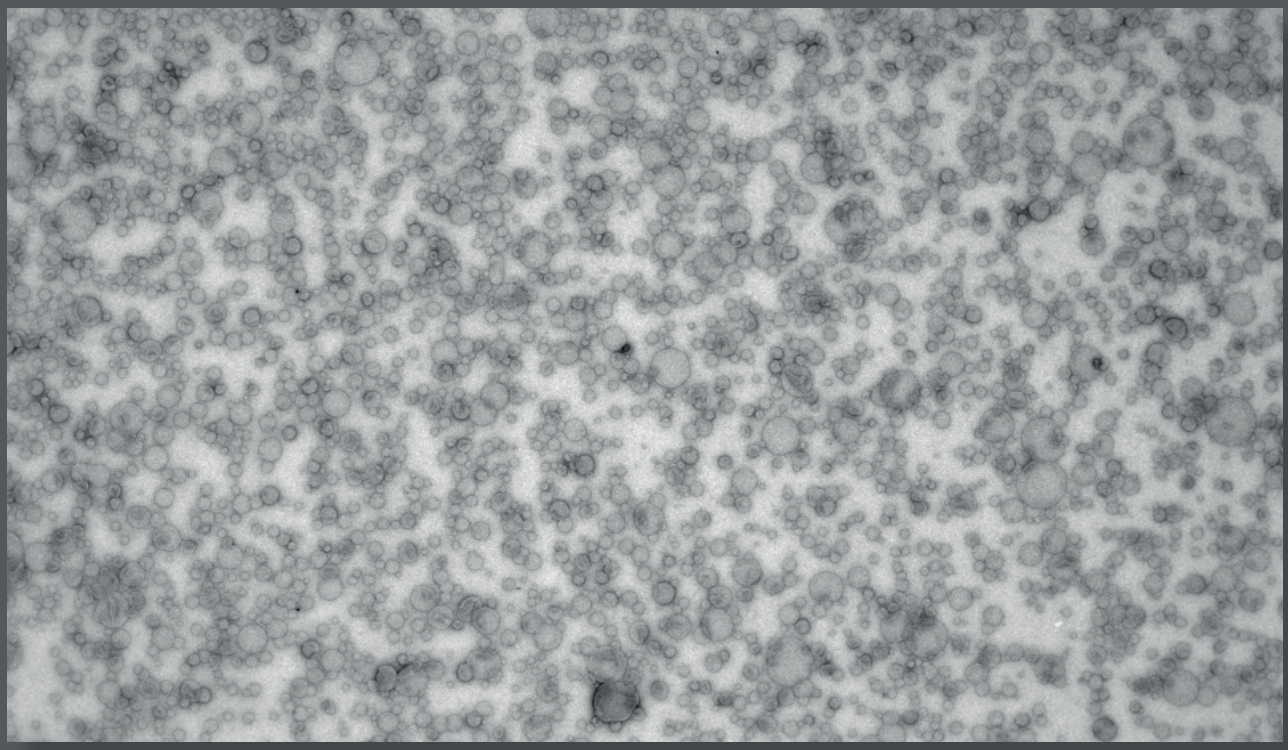
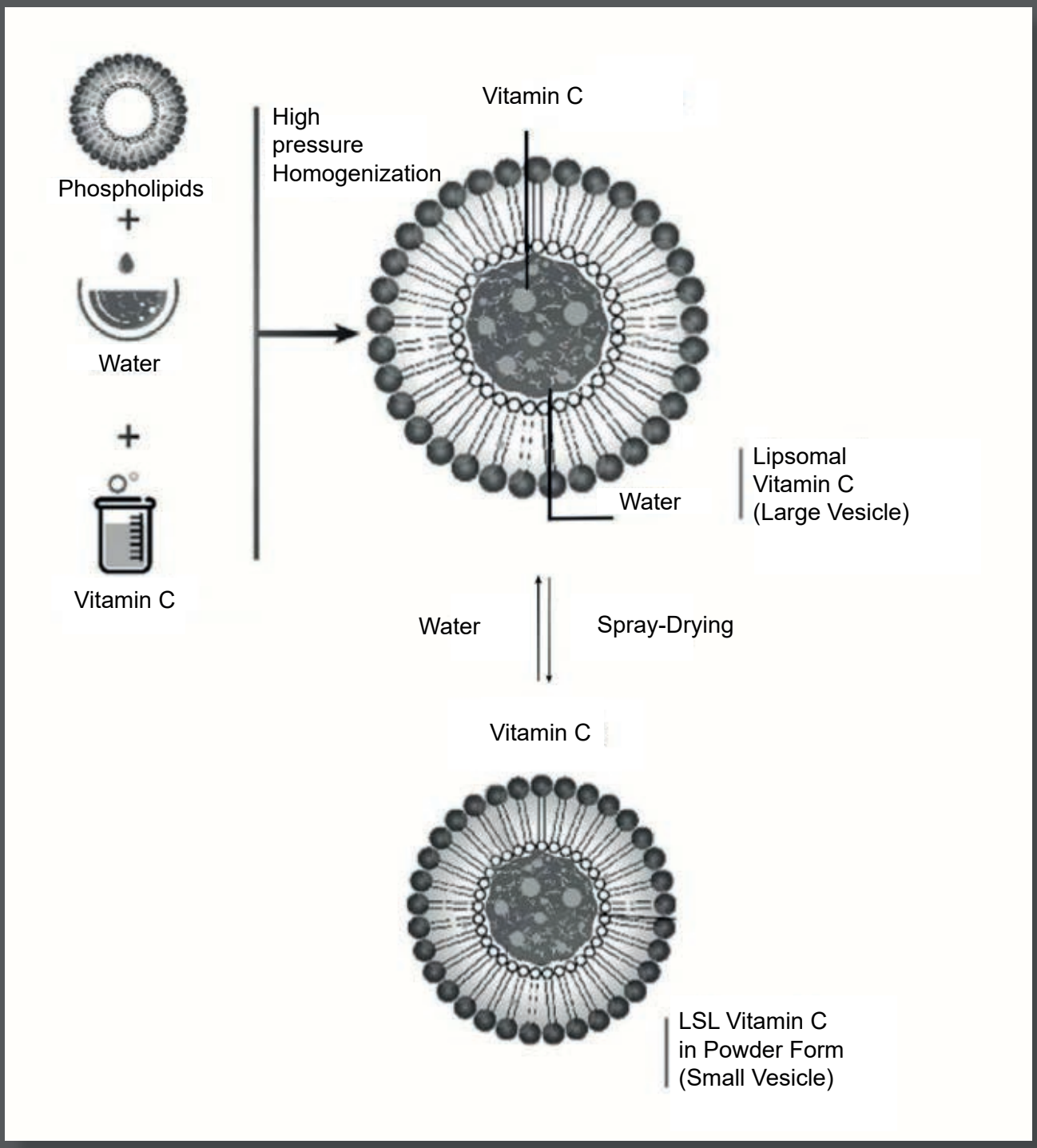


Figure 1. Transmission electron microscopy (HITACHI TEM SYSTEM x 1,0k Zoom - 1 HC -1 100 ky) of LSL-Vitamin C.

1. INTRODUCTION. The water-soluble vital substance Vitamin C (Ascorbic acid) is one of the best-known Vitamins today. Vitamin C is involved in numerous reactions in the human body, for example as an antioxidant and cofactor. Vitamin C is still being intensively researched. Almost every year, new mechanisms of action of this fascinating Vitamin are discovered. But Vitamin C is vulnerable to environmental settings (temperature, pH, light, and oxygen) and undergoes easily degradation to inactive products and loses its vitamin property. Therefore, new approaches are required to augment its stability. The objective of this study is to characterize LSL-VitClip, a liposomal Vitamin C in powder form, which was developed to optimize the stability but also the oral bioavailability of Vitamin C. The comparative study by single-dose, two-treatment, two-period shows that LSL-VitClip has an approximately 8.2-fold higher bioavailability than conventional, non-liposomal Vitamin C. Furthermore, the study shows a significantly extended duration of action of liposomal Vitamin C. The structural morphologies, surface morphologies and mean particle size were analyzed by transmission electron microscopy. Encapsulation efficiency of Vitamin C onto the phospholipids in percentages has been recorded with 79,23%.

To address the challenges associated with the oral bioavailability and therapeutic efficacy of Vitamin C, a variety of strategies have been employed by different groups. These strategies include microencapsulation and the utilization of liposomes and nanoparticles. Phospholipids, serving as suitable encapsulation matrices, consist of amphiphilic phospholipid bilayers that possess both hydrophilic and hydrophobic properties, making them effective carriers for a wide range of compounds. Furthermore, these lipid molecules are generally recognized as safe and biodegradable, facilitating enhanced transcellular transport through transient disruption of cellular lipophilic bilayers and potentially improving paracellular drug transport. The encapsulation of Vitamin C within liposomes not only enhances its stability and bioavailability but also enables controlled release within the gastrointestinal tract. Liposomal technology has significant potential to safeguard the functional characteristics of active molecules, increasing their stability and ensuring sustained release over a specific timeframe. Consequently, this approach contributes to an enhanced bioavailability of active molecules, ultimately promoting better outcomes. The characterization of Liposomal Vitamin C (LSL-Vitamin C) was conducted using transmission electron microscopy (TEM). Another crucial objective of this study was to assess the impact of liposomal Vitamin C on availability through an, single-dose, two-treatment, two-period investigation.

2. Development of Liposomes. The liposomal Vitamin C in powdered form was encapsulated within liposomal vesicles using LSL technology. This approach was employed to protect the functional attributes of Vitamin C. In this process, Vitamin C is effectively enclosed within the lipid bilayers of the liposomes, with the aqueous space forming the core of these liposomal structures. As part of the spray-drying procedure, the initially large vesicle liposomes undergo a transformation, contracting to smaller dimensions and becoming small vesicle liposomes.



Scheme 1. Schematic Representation of the Preparation of LSL-Vitamin C.

3. RESULTS AND DISCUSSION

3.1 Morphology by TEM. The surface characteristics and morphology of the powdered Vitamin C were examined using Transmission Electron Microscopy (TEM), as illustrated in Figure 1. The LSL product exhibited spherical shapes without any signs of aggregation, undersoring the stability of the formulation. The Vitamin C within the Liposomes displayed on smooth surface, indicating effective encapsulation of Vitamin C. The TEM images revealed a well-organized, circular liposomal structure with the active ingredient preserved, confirming the formation of a stable liposomal product. The images in Figure 2 display a distinct dark gray interface internally, surrounded by a well-defined transparent phospholipid layer. The darker inner portion was attributed to the bonding effect of the active ingredient. The TEM images of Vitamin C within the liposomal formulation demonstrated the creation of true liposomal products, characterized by a smooth surface and spherical shape, devoid of any accumulation.

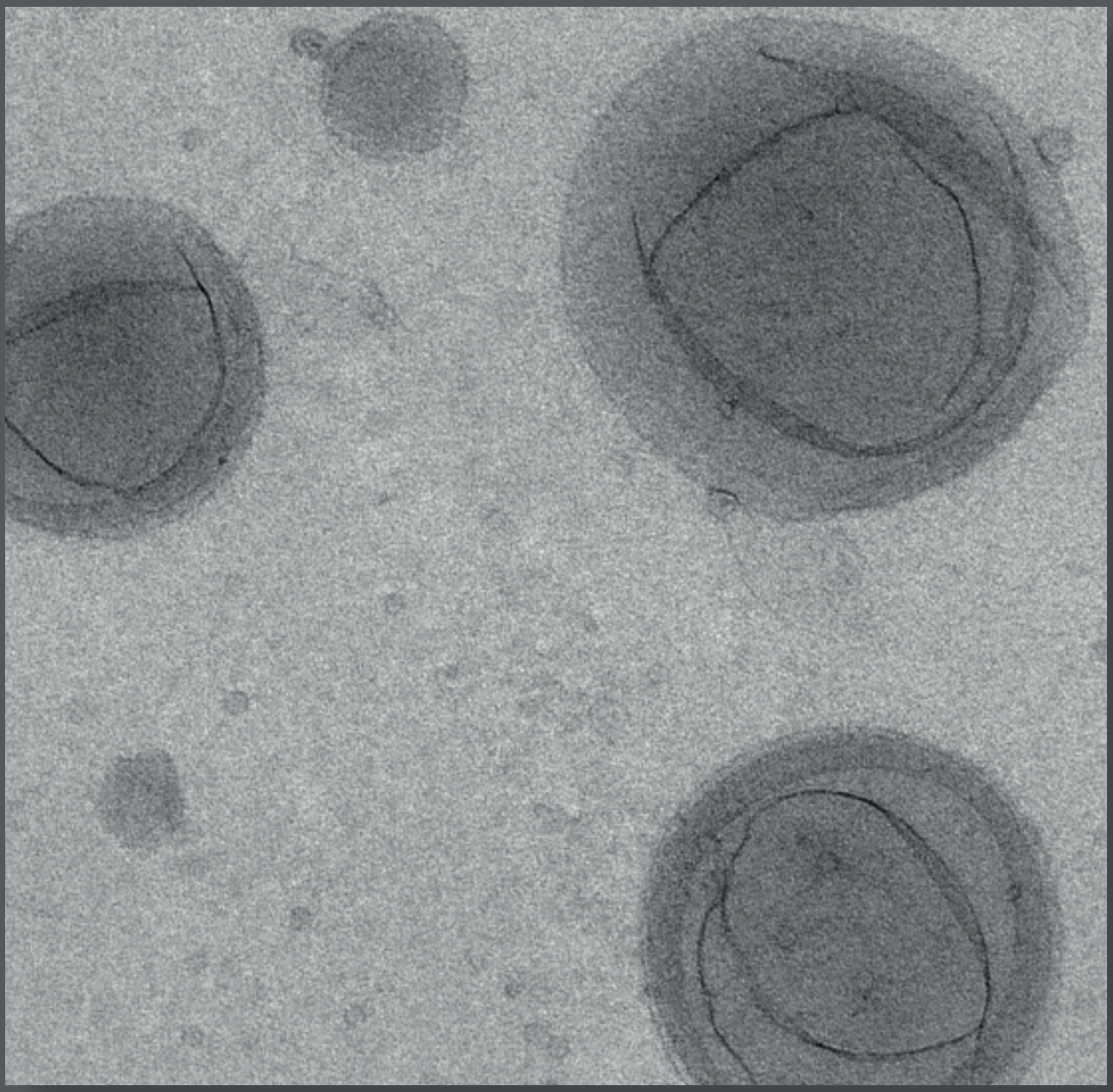


Figure 2. Transmission electron microscopy (HITACHI TEM SYSTEM x 40,0k Zoom - 1 HC -1 100 ky) of LSL-Vitamin C.

3.2. Encapsulation Efficiency (EE). The calculation of Encapsulation Efficiency (EE%) was performed following the procedure established by Parhizkar et al. First, known amounts of Vitamin C were dissolved in methanol and thoroughly mixed to create a colloidal solu- tion. Subsequently, this solution was subjected to centrifuga- tion for a duration of 15 minutes at a speed of 3000 revoluti- ons per minute (rpm) and at a temperature range of 5 to 10 degrees Celsius. The Vitamin C present in the supernatant (liquid above the sediment) was then quantified using High- Performance Liquid Chromatography (HPLC) to determine the content of unencapsulated Vitamin C.

The quantity of encap- sulated Vitamin C and the encapsulation efficiency (EE%) were calculated using the following equation: EE (%) = [(Total Vitamin C - Unencapsulated Vitamin C) / Total Vitamin C] × 100 Where: Total Vitamin C represents the initially added amount of Vitamin C. Unencapsulated Vit- amin C refers to the amount of Vitamin C found in the super- natant after centrifugation. This calculation allowed for the determination of how efficiently Vitamin C was encapsulated within the vesicles or particles of the formulation.

The calculation of the encapsulation efficiency of LSL – VitCLip in the phospholipids yielded an excellent result with a recorded percentage of
79,23. These findings unequivocally demonstrate the successful incorporation of Vitamin C into the lipid bilay- er, ensuring the preservation of its integrity in the powdered form.

3.3. Efficacy of LSL-Vitamin C and Pharmacokinetic Parameters. All participants who were enrolled in the research study completed the study without any dropouts. The oral administration of Vitamin C was well-tolerated, and no adverse events were reported. Baseline circulating concentrations of Vitamin C did not exhibit any significant differences between the treatment groups. The current study is a comparative study with 10 healthy volunteers. Half of the participants are male and half female, the age of the participants is between 20 and 60 years, none of the participants suffers from chronic diseases or was acutely ill at the time of the examinations. On the first day of the study, all subjects received the non-liposomal Vitamin C, one week later, on the second day of the study, the same subjects received the liposomal Vitamin C in the same dosage. Participants received their respective doses of Vitamin C while fasting. Blood samples were taken at the beginning of the examination (before administration of the preparation), as well as 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours and 24 hours after administration of the preparation. In the laboratory, the concentration of ascorbic acid in the blood was determined at each of the above time points.

3.4. Results The comparative study shows that liposomal Vitamin C has an approximately 8.2-fold higher bioavailabili- ty than conventional, non-liposomal Vitamin C. Furthermore, the study shows a significantly extended duration of action of liposomal Vitamin C. The pharmacokinetic parameters of Vitamin C concentrations, including mean Area Under the Curve (AUC) ± standard deviation (SD), maximum and mi- nimum absorbance at each time interval, and median con- centrations (all measured in mg/dL) for each formulation at different time intervals up to 24 hours, are provided in Table 1. Additionally, graphical representations depicting the mean concentrations of Vitamin C and Vitamin C over time are presented in Graphic 1.

Graphic 1: Mean plasma Vitamin C levels over time after 1000 mg dose liposomal Vitamin C powder (LSL-VitCLip™) and 1000 mg dose non-liposomal Vitamin C (powder form)

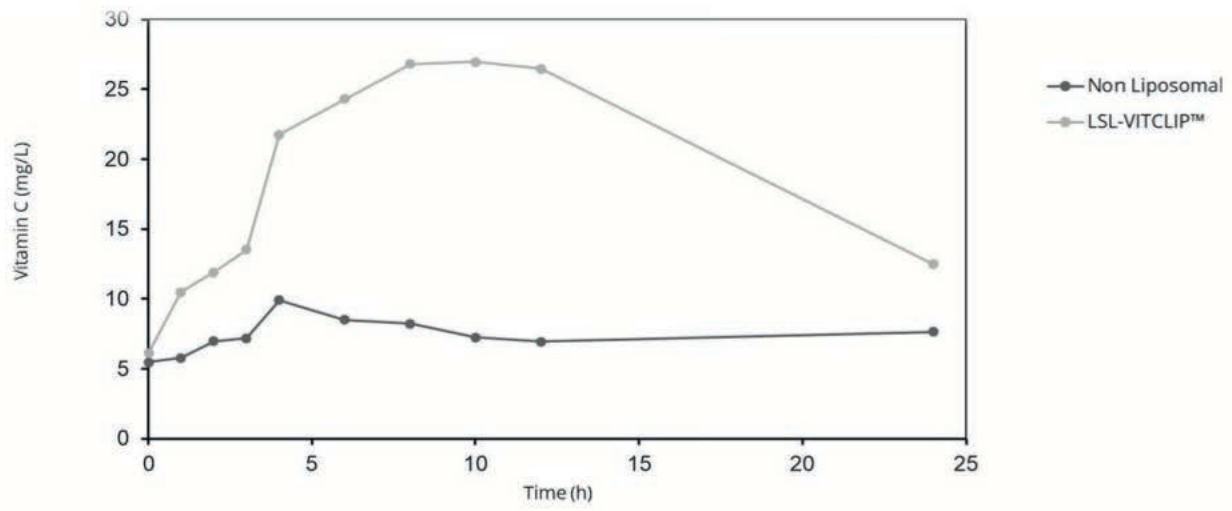


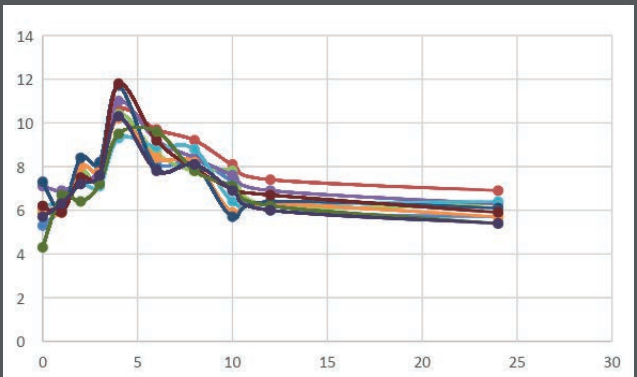
Table 1: DATA: Plasma vitamin C (ascorbic acid) levels were measured individually for each patient.

NON-LIPOSOMAL	8 Uhr	9 Uhr	10 Uhr	11 Uhr	12 Uhr	14 Uhr	16 Uhr	18 Uhr	20 Uhr	8 Uhr
Time	0	1	2	3	4	6	8	10	12	24
Patient 1	5,3	6,1	7,4	7,6	10,2	8,1	8,3	7,3	6	5,7
Patient 2	5,9	6,3	7,5	7,7	10,6	9,7	9,2	8,1	7,4	6,9
Patient 3	6	5,9	7,8	7,3	10,4	8,6	7,8	7,8	6,2	6,3
Patient 4	7,1	6,9	7,3	7,9	11	9,2	8,4	7,6	6,9	6,3
Patient 5	6,2	6,4	7,2	7,1	9,3	8,9	8,8	6,4	6,3	6,4
Patient 6	6,1	5,9	8	7,8	10,2	8,4	8,2	5,9	6,3	5,7
Patient 7	7,3	6	8,4	8,2	11,7	7,9	8,1	5,7	6,4	6,1
Patient 8	6,2	5,9	7,5	7,6	11,8	9,2	7,9	7	6,7	5,9
Patient 9	4,3	6,7	6,4	7,2	9,5	9,6	7,8	7,1	6,2	5,4
Patient 10	5,7	6,3	7,2	7,6	10,3	7,8	8,1	6,9	6	5,4

LSL-VITCLIP™	8 Uhr	9 Uhr	10 Uhr	11 Uhr	12 Uhr	14 Uhr	16 Uhr	18 Uhr	20 Uhr	8 Uhr
Time	0	1	2	3	4	6	8	10	12	24
Patient 1	6,2	9,8	11,3	13,7	22	25,3	25,7	26,2	26,4	11,9
Patient 2	5,3	10,9	11,2	13,2	22,7	24,5	26	26,3	24,3	12,6
Patient 3	7,1	10,3	12,4	13,7	21,3	26,1	26,4	26,9	27,2	13,5
Patient 4	6,9	10,7	12,1	12,5	21,4	22,1	26,3	27,4	26,1	12,7
Patient 5	5,9	11,2	11,9	13,1	21,7	24,2	26,9	27	26,2	13,5
Patient 6	5,9	9,6	13,2	13,4	21,5	23,7	26	27,1	26,3	11,4
Patient 7	6,3	10,7	11,2	14,9	22	28,3	28	27,7	26,8	10,5
Patient 8	6	10,6	11,7	12,4	22,8	22,1	27,3	27,1	28,4	13,4
Patient 9	6,1	11,3	11,2	14,8	21,8	21,3	28,4	26,5	25,7	12,7
Patient 10	5,5	9,8	12,7	13,6	20,3	25,4	26,9	27,2	27,1	12,6

Table 2 provides the comprehensive average pharmacokinetic parameters derived from plasma Vitamin C concentrations. Notably, the maximum plasma Vitamin C concentration (C_{max}) and the Area Under the Curve (AUC) for LSL-Vitamin C were notably higher, measuring approximately 26,94 mg/L and 318 mg/L, respectively. In contrast, for the standard Vitamin C group, these values were considerably lower at 10.50 mg/L for C_{max} and 39 mg/L for AUC. The AUC is considered the most reliable indicator of bioavailability since it quantifies the overall response over time, offering a more precise assessment of bioavailability.

The time taken to reach the maximum plasma concentration was approximately 4 hours for non-liposomal Vitamin C. LSL-Vitamin C reached this level after approximately 2 hours. Furthermore, the study shows a significantly extended duration of action of liposomal Vitamin C.



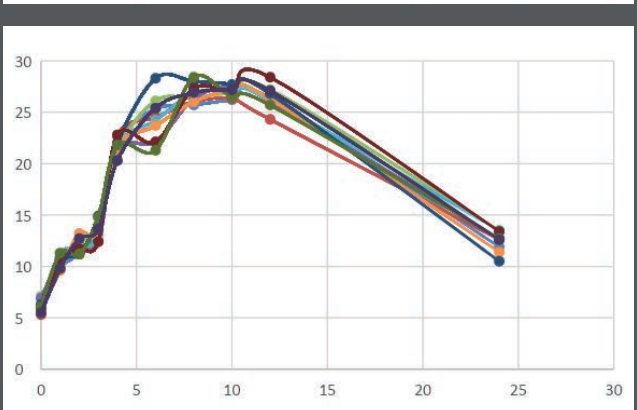


Table 2. Average PK Variables from Plasma Vitamin C of Vitamin C and LSL-Vitamin C.

	AA	LSL - AA
AUC	39	318
C_{max} (mg/L)	10,5	26,94
T_{max} (h)	4h	8h

It‘s important to note that drug absorption is influenced by several factors, including membrane permeability, as well as intestinal absorption, skin permeability, and the potential for a substance to act as either a substrate or inhibitor of P-glycoprotein (P-gp).

In the present study, Vitamin C was encapsulated within vesicles composed of phospholipids, forming a highly stable lipid bilayer within the powdered formulation. The results indicated that these phospholipid vesicles are expected to exhibit significant absorption.

Furthermore, P-glycoprotein (P-gp), a member of the ATP-binding transmembrane glycoprotein family found in various cells throughout the body, including those lining the intestine, plays a crucial role in pumping substrates against concentration gradients using ATP. The study results suggested that phospholipid vesicles, being substrates of the P-gp drug transporter, are likely to be transported from the intestinal tract, further supporting their potential for enhanced absorption and bioavailability.

To comprehensively examine the physicochemical properties of Vitamin C and gain insights into how these characteristics influence its in vivo distribution and behavior within formulations, a series of morphological studies were conducted. Through TEM analysis, it was observed that Vitamin C powder exhibited a spherical shape with no apparent aggregation, indicative of the formulation‘s stability. Furthermore, the formulation demonstrated uniformity in terms of shape and size. This indicated successful encapsulation and the formation of a genuine encapsulated product. Encouragingly, the mean percentage of Vitamin C encapsulated within the Liposomal vehicle was found to be 79.5%, suggesting the presence of a significant aqueous space within this FRP-based vehicle. This stable core, once dried, forms a highly stable core, which facilitates the accelerated delivery of Vitamin C. The results further revealed that the bioavailability of Vi- tamin C had increased compared to standard Vitamin C, resulting in higher plasma levels. This observation strongly suggests that a single oral dose of the liposomal-based Vitamin C formulation could elevate plasma levels of ascorbic acid (AA) to a maximum of 28,4 mg/L. It is possible that the de- encapsulation of the liposomal-based vehicle within the plasma environment leads to a sustained release of AA, thereby elevating plasma Vitamin C concentration. Consequently, the increased plasma Vitamin C concentration observed in the bioavailability study, along with enhanced distribution to other tissues, underscores the effectiveness of liposomal-based vehicles as a successful delivery system for Vitamin C.

Mechanistically, the significant absorption of Vitamin C in the presence of phospholipids from the gastrointestinal tract, aided by the P-glycoprotein (P-gp) transporter, likely contributes to an acceptable Vitamin C concentration in plasma. A suitable VDss is pivotal in supplying the drug to various body tissues. Generally, the ability to interact with pharmacological target proteins, such as receptors, channels, and enzymes, enables a drug to readily diffuse between plasma and tissues in its unbound (free) form. Furthermore, adequate metabolism, as forecasted by CYP-P450 enzymes, and excretion mechanisms help prevent drug accumulation in the body, reducing the risk of toxicity.

3. CONCLUSIONS In summary, this study focused on comparing the oral bioavailability and characterizing Vitamin C encapsulated within Liposomal vehicles in healthy adult human participants under fasting conditions. Microscopic images revealed a core-type structure, confirming the characteristic pattern of phospholipid vesicles with sizes ranging from 200 to 700 nm. Even after encapsulation, the vesicles maintained their spherical morphology. The encapsulation efficiency (EE%) was found to be 83.58.The results clearly indicated that the oral administration of Vitamin C using this Liposomal-based delivery system demonstrated 8,2 times higher bioavailability compared to standard Vitamin C. Notably, Vitamin C significantly improved key parameters such as C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ in comparison to nonliposomal Vitamin C.

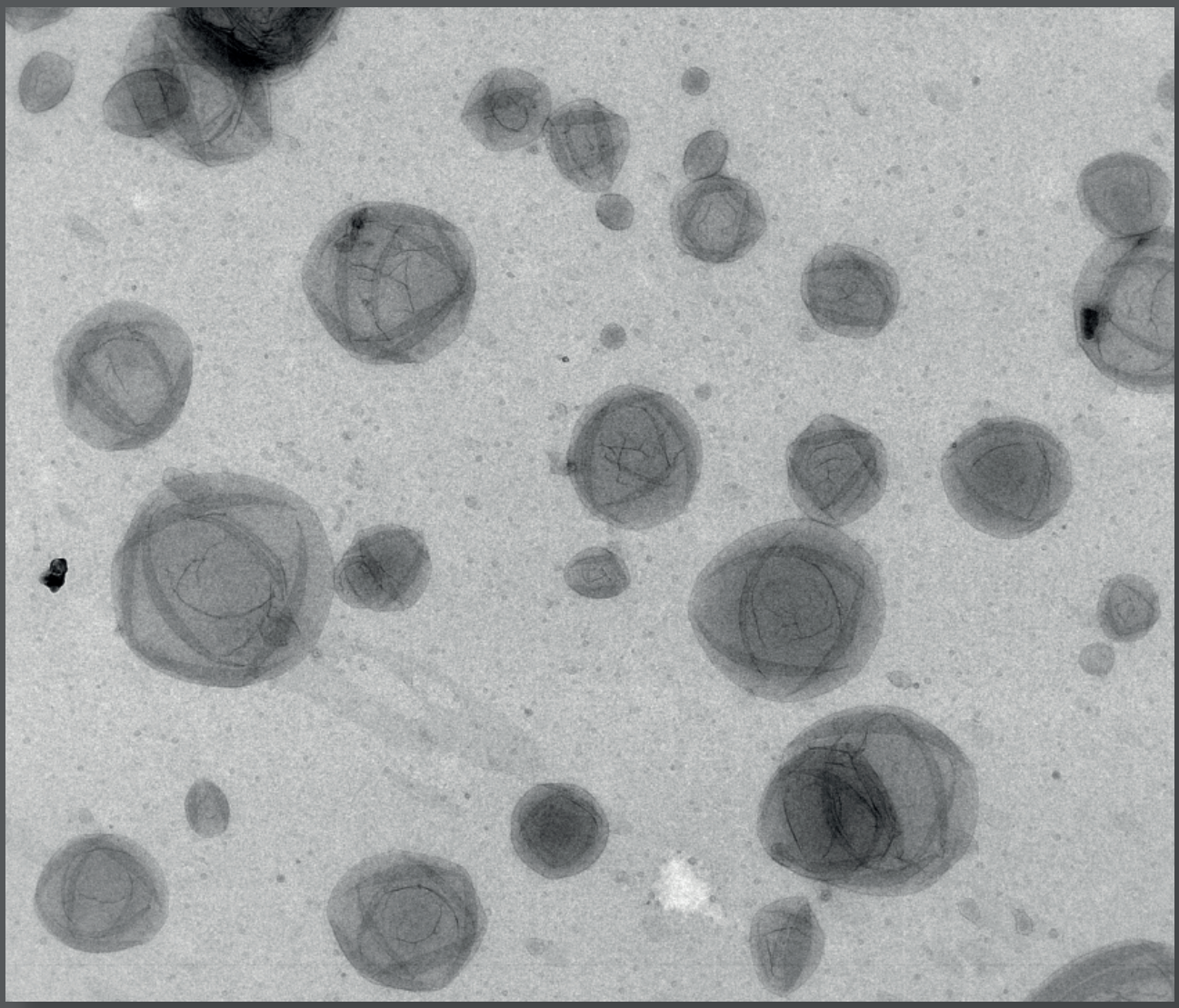


Figure 3. Transmission electron microscopy (HITACHI TEM SYSTEM x 15,0 k Zoom - 1 HC -1 100 ky) of LSL-Vitamin C.

4. MATERIALS AND METHODS.

4.1. Morphological Studies. To examine the surface morphology of LSL-Vitamin C phospholipid vehicles advan- ced microscopy techniques were employed. Transmission Electron Microscopy (TEM) was conducted using a HITACHI TEM SYSTEM. The phospholipid suspension was initially diluted with deionized H2O, and this diluted solution was then applied onto mesh grids. After removing any excess solution, the samples were subjected to analysis at an acceleration voltage of 100 kV. This allowed a detailed investigation of the sur- face morphology of LSL-Vitamin C.

$$EE\ (\%) = \left[\frac{\text{total AA content - unencapsulated AA content}}{\text{total AA content}}\right] x100$$

4.2. Participants for the Bioavailability Study. The study involved 10 healthy volunteers. Half of the partici- pants are male and half female, the age of the participants is between 20 and 60 years, none of the participants suffers from chronic diseases or was acutely ill at the time of the examinations. These par- ticipants willingly agreed to adhere to the study‘s protocol requirements, which was confirmed through written, infor- med consent. As part of their commitment to the study, participants committed not to use any medications, including vi- tamins and minerals, either during or prior to the study. The study had a total sample size of 10 participants. These parti- cipant re- ceived the non-liposomal Vitamin C on the first day of the study, one week later, on the second day of the study, the same subjects received the liposomal Vitamin C in the same dosage. The administration of doses was carried out at the study site by an authorized individual independently.

4.3. Ethics and Approvals. The study was conducted by Master Key Labs, located in Cologne, Germany. To ensure ethical compliance and oversight, all documents related to the study were thoroughly reviewed by an Institutional Review Board.