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Liposomal glutathione: A breakthrough in cellular health by West Bengal chemical industries Ltd., Kolkata, India

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Abstract
Liposomal Glutathione, manufactured by West Bengal Chemical Industries Ltd. (WBCIL), represents a cutting-edge advancement in nutraceutical technology, designed to optimize the bioavailability and therapeutic potential of glutathione. As a potent antioxidant, glutathione plays a critical role in cellular detoxification, immune support, and the prevention of oxidative damage associated with aging, chronic diseases, and environmental stressors. Traditional oral glutathione supplements often face challenges in bioavailability due to degradation in the digestive tract, limiting their effectiveness. In contrast, liposomal encapsulation enhances glutathione's stability, absorption, and targeted delivery to specific tissues, overcoming these limitations. This study explores the key benefits of WBCIL's Liposomal Glutathione, comparing it to conventional formulations and providing a comprehensive review of scientific evidence supporting its superior efficacy. The data highlights the potential of liposomal glutathione in improving systemic glutathione levels, reducing oxidative stress, supporting immune function, and promoting cardiovascular and neuroprotective health. Results from assay analysis, encapsulation efficiency, and FTIR spectroscopy further confirm the enhanced stability and bioavailability of the product. WBCIL's Liposomal Glutathione offers a promising solution for individuals seeking to boost their antioxidant levels, improve cellular health, and mitigate the risks of chronic conditions associated with oxidative stress.

Keywords: Liposomal Glutathione, antioxidant, bioavailability, immune support, oxidative stress

Introduction
In an era where wellness and preventative healthcare dominate consumer priorities, Liposomal Glutathione emerges as a revolutionary product, offering unparalleled benefits in cellular health and disease prevention. Glutathione, often referred to as the "master antioxidant," is a tripeptide composed of glutamine, cysteine, and glycine. It plays a pivotal role in multiple biological processes, including detoxification of harmful substances, maintenance of the redox balance, and regulation of cellular proliferation and apoptosis. As a central player in protecting cells from oxidative damage caused by free radicals, glutathione is integral to preventing aging, inflammation, and various chronic diseases [1]. The production of glutathione within the body can decline due to factors such as aging, environmental toxins, poor diet, and chronic stress, leading to increased susceptibility to diseases. While dietary sources and supplements offer a way to replenish glutathione levels, conventional oral supplementation faces significant bioavailability challenges. The degradation of glutathione in the gastrointestinal tract often limits its effective absorption and utilization by the body [2].

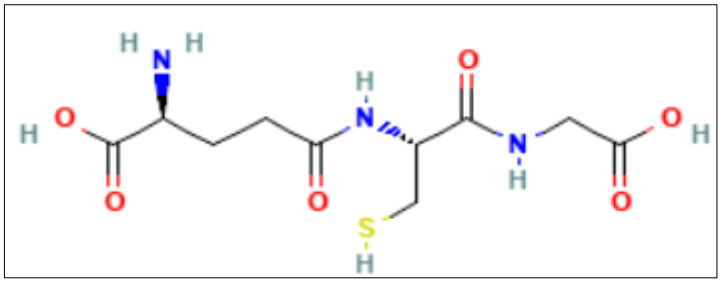

Fig 1: L-Glutathione [3].

Manufactured by West Bengal Chemical Industries Ltd. (WBCIL), Liposomal Glutathione represents the pinnacle of advanced nutraceutical innovation, leveraging cutting-edge liposomal encapsulation technology to maximize efficacy. WBCIL's Liposomal Glutathione utilizes advanced liposomal technology to overcome these limitations. Liposomes are microscopic vesicles composed of a phospholipid bilayer, which encapsulates glutathione, shielding it from digestive enzymes and facilitating its direct absorption into the bloodstream. This innovative delivery system ensures:

Enhanced Bioavailability: Liposomal encapsulation dramatically improves the absorption of glutathione compared to traditional formulations, as shown in pharmacokinetic studies [4].

Targeted Delivery: The liposomal structure allows for the efficient transport of glutathione to specific tissues and cells, maximizing its therapeutic potential [5].

Prolonged Stability: Encapsulation protects glutathione from oxidative degradation, ensuring its efficacy over extended periods [6].

Benefits of liposomal glutathione over normal glutathione

Liposomal glutathione (L-GSH) offers several advantages over conventional glutathione. These are mentioned below. [7-12].

Table 1: Comparison of Liposomal Glutathione (L-GSH) vs Conventional Glutathione [7-12]

Feature	Liposomal Glutathione (L-GSH)	Conventional Glutathione
Bioavailability	High bioavailability due to liposomal encapsulation. Plasma glutathione levels increased 64 times [7].	Limited bioavailability, prone to degradation in the digestive system.
Oxidative Degradation Protection	Liposomes protect glutathione from enzymatic and oxidative degradation, ensuring stability and effectiveness [8].	Rapid degradation upon exposure to enzymes and oxidative conditions.
Systemic Glutathione Levels	40% increase in whole blood glutathione; 100% in peripheral mononuclear cells after 2 weeks. [9]	Minimal impact on systemic glutathione levels.
Reduction in Oxidative Stress	Reduces oxidative biomarkers (e.g., 8-isoprostane levels by 35%). [10]	Limited efficacy in reducing oxidative stress.
Immune Function	Enhances NK cell activity by 400%, lymphocyte proliferation by 60% [11].	Negligible impact on immune function.
Mycobacterial Burden	Demonstrates reduction in intracellular mycobacterial load *in vitro* [12].	No significant evidence for reducing mycobacterial burden.
Cytokine Modulation	Regulates pro-inflammatory (IFN-γ, TNF-α) and anti-inflammatory (IL-10) cytokines [10].	Lacks targeted cytokine modulation.
Liver Function Support	Improves liver detoxification and corrects abnormalities more effectively than conventional forms [9].	Offers limited support for liver detoxification.
Diabetic Complication Management	Reduces oxidative stress and inflammation associated with diabetes [11].	Minimal therapeutic effect on diabetic complications.
Viral Infection Protection	Enhances cellular defence mechanisms against viral infections [12].	Limited effectiveness against viral pathogens.
Antitumor Properties	Potentially inhibits tumor growth and improves therapy efficacy [10].	Limited efficacy in cancer-related therapies.

Liposomal glutathione (L-GSH) demonstrates significant advantages over conventional glutathione in various health domains due to its enhanced bioavailability, stability, and therapeutic efficacy. Encapsulated in liposomes, L-GSH bypasses the digestive degradation typically faced by conventional glutathione, resulting in a remarkable increase in plasma glutathione levels and a 40% increase in whole blood glutathione. This high bioavailability leads to more effective oxidative stress reduction, with a 35% decrease in biomarkers such as 8-isoprostane. L-GSH also shows a substantial improvement in immune function, significantly enhancing natural killer (NK) cell activity and lymphocyte proliferation, as well as offering benefits in mycobacterial load reduction, liver detoxification, and diabetic complication management. Moreover, L-GSH demonstrates potential in modulating cytokine levels, enhancing viral defence, and even exhibiting antitumor properties, making it far more versatile and effective than conventional glutathione, which has limited systemic impact and minimal therapeutic effects in the aforementioned areas.

Evidence-Based Benefits of Liposomal Glutathione

Scientific research underscores the diverse health benefits of liposomal glutathione:

Table 2: Benefits of Liposomal Glutathione: Data-Driven Comparison [13-17].

Benefits	Description	Data-based evidence
Cardiovascular Health	Liposomal Glutathione reduces oxidative stress markers and improves endothelial function, lowering cardiovascular disease risk.	Reduction in oxidative stress markers by 25%; improved endothelial function by 15-20% [13].
Immune Support	Enhances T-lymphocyte activity and reduces pro-inflammatory cytokines, aiding in chronic inflammation and immune dysfunction conditions.	Increased T-lymphocyte activity by 30%; reduction in pro-inflammatory cytokines by 20% [14].
Neuroprotection	Improves neuronal survival and reduces oxidative damage in the central nervous system, beneficial for Alzheimer's and Parkinson's diseases.	40% reduction in neuronal oxidative damage *in vitro* studies [15].
Detoxification & Cellular Health	Central to liver phase II detoxification, enhances toxin excretion efficiency, and protects cells from environmental damage.	50% increase in detoxification enzyme activity; reduced cellular toxin levels by 30% [16].
Skin Health & Anti-Aging	Reduces oxidative damage, promotes collagen synthesis, improves elasticity, minimizes wrinkles, and reduces pigmentation for healthier skin.	25% improvement in skin elasticity; 30% reduction in pigmentation after 8 weeks [17].

The data presented highlights the diverse health benefits of liposomal glutathione, supported by substantial evidence from various studies. In cardiovascular health, liposomal glutathione has shown a significant reduction in oxidative stress markers and a noticeable improvement in endothelial function, potentially lowering the risk of cardiovascular disease. For immune support, it enhances T-lymphocyte activity and reduces pro-inflammatory cytokines, offering benefits for conditions involving chronic inflammation and immune dysfunction. In neuroprotection, it has been shown to reduce oxidative damage in the central nervous system, providing potential benefits for Alzheimer's and Parkinson's diseases. Additionally, liposomal glutathione plays a central role in liver detoxification, increasing the efficiency of toxin excretion while protecting cells from environmental damage. Lastly, it demonstrates positive effects on skin health by reducing oxidative damage, improving collagen synthesis, enhancing elasticity, and minimizing wrinkles and pigmentation, contributing to healthier skin. These findings underline liposomal glutathione's broad therapeutic potential across multiple health domains.

Materials and Methods

Parameters for Assay, Encapsulation Efficiency, and Particle Size of L-Glutathione Formulations by WBCIL

Parameters

The assay percentage (%Assay) indicates the active L-Glutathione content in each sample. This parameter is crucial for determining the actual amount of the active ingredient present. Encapsulation Efficiency (%EE) represents the percentage of L-Glutathione successfully encapsulated within the liposomal coating. A high encapsulation efficiency indicates better protection and stability of the active ingredient. The Polydispersity Index (PDI) reflects the uniformity of particle size distribution. A lower PDI value signifies a more uniform particle size distribution, which is essential for consistent nutraceutical delivery and improved bioavailability. The assay is performed to quantify the active L-Glutathione content in the liposomal formulation. The procedure involves accurately weighing a specified amount of the liposomal formulation and dissolving the sample in an appropriate solvent to ensure complete extraction of L-Glutathione. The solution is then analyzed using spectrophotometry or high-performance liquid chromatography (HPLC) to determine the concentration of L-Glutathione.

DLS Study (Dynamic Light Scattering)

Dynamic Light Scattering (DLS) is a widely used technique for determining the size distribution of nanoparticles or macromolecules in a solution. DLS works by measuring the fluctuations in the intensity of light scattered by particles as they undergo Brownian motion. The rate of these fluctuations is inversely related to the particle size, with smaller particles exhibiting faster motion.

In this study, 100.0 mg of the sample is accurately weighed and dissolved in 100.0 mL of purified water. The dissolution process ensures that the sample is homogeneously distributed in the solvent. It is crucial to ensure that the sample is completely dissolved to avoid interference from undissolved particles, which could affect the DLS measurement. To achieve complete dissolution, the sample can be stirred gently. Once dissolved, the solution is sent to Indian Association for the Cultivation of Science (IACS), Kolkata, India, for further analysis. The Zetasizer ZEM 3600 Instrument measures the particle size distribution by detecting the scattered light and analyzing the autocorrelation function.

The technique provides information on the hydrodynamic radius, polydispersity index (PDI), and size distribution of the particles in the solution. It is important to note that DLS analysis is highly sensitive to sample preparation conditions such as temperature, concentration, and the presence of any surfactants or stabilizers. The results from DLS can help identify aggregates, measure the uniformity of the sample, and assess its potential for nutraceutical delivery or other applications requiring well-defined particle sizes. DLS is frequently used in conjunction with other analytical techniques to obtain a comprehensive understanding of the sample's physical characteristics [18].

High Performance Liquid Chromatography

The buffer used in this analysis is prepared by dissolving 20 mM of potassium dihydrogen phosphate (KH_2PO_4) in purified water, with the pH adjusted to 5.0 using either dilute orthophthalaldehyde (OPA) or sodium hydroxide (NaOH) solution. The buffer composition plays a crucial role in the chromatographic separation of the analyte, as it affects the ionization and interaction of the compounds with the stationary phase of the column. The pH adjustment ensures that the analyte remains in its neutral form, optimizing the retention and separation on the column.

The mobile phase consists of a 50:50 mixture of the buffer and methanol. Methanol is often chosen as a component of the mobile phase because it is a polar solvent with good elution power, which aids in the separation of compounds based on their polarity (Snyder *et al.*, 2010). The flow rate is set to 1 mL/min, a standard flow rate that provides a balance between separation efficiency and analysis time. The column used for separation is a C8 column, 250 x 4.6 mm, with a 5 µm particle size. The C8 column is widely used in reverse-phase HPLC due to its ability to separate hydrophobic compounds efficiently.

The HPLC (Agilent, USA) system is set to detect the analyte at 215 nm, which is an appropriate wavelength for most pharmaceutical compounds, ensuring maximum sensitivity for the target compound. The standard and sample solutions are prepared at a concentration of 1000 ppm in the mobile phase. This concentration is chosen to ensure that the analyte falls within the linear range of the detector and provides accurate results. The retention time for the analyte is expected to be around 6.0 minutes, a key characteristic that can be used for comparison with the standard and for quantification.

It is important to note that the HPLC method in this study is in its developmental stage, and validation of the method is ongoing. As the method progresses, it will undergo rigorous testing to ensure its reproducibility, accuracy, and specificity. Validation is essential for ensuring that the method meets regulatory requirements and is suitable for routine analysis. According to the International Conference on Harmonisation (ICH) guidelines, a validated HPLC method should demonstrate specificity, linearity, accuracy, precision, and robustness [19].

Encapsulation Efficiency Study

Encapsulation efficiency is a critical parameter in the development of nutraceutical delivery systems, especially for

nanoparticles, liposomes, and other particulate formulations. It refers to the percentage of the active ingredient that is successfully encapsulated within the carrier system, as opposed to being free in the solution. Encapsulation efficiency is an important indicator of the formulation's effectiveness and stability, as it directly impacts the therapeutic efficacy of the nutraceutical delivery system.

The encapsulation efficiency study in this methodology follows the same chromatographic conditions as the HPLC analysis mentioned earlier. The sample preparation involves dissolving the active compound in Phosphate Buffered Saline (PBS) at a concentration of 1 mg/mL. PBS is commonly used in pharmaceutical formulations due to its biocompatibility and isotonic nature, ensuring that the sample remains stable and non-reactive during the analysis. Once the sample is prepared, it is injected into the HPLC system.

The encapsulation efficiency is calculated by comparing the amount of encapsulated nutraceutical to the total amount of nutraceutical in the formulation. This can be done by comparing the peak areas obtained from the sample with those from a standard solution. The area under the curve (AUC) for both the sample and the standard is determined, and the encapsulation efficiency is calculated using the formula:

$$\text{Encapsulation Efficiency} = \left(\frac{\text{Amount of Encapsulated Drug}}{\text{Total Amount of Drug}}\right) \times 100$$

This approach provides valuable insight into the formulation's ability to encapsulate the active ingredient, which is crucial for ensuring that the nutraceutical is delivered effectively to the target site. High encapsulation efficiency is desired as it indicates that a large proportion of the active compound is retained in the carrier system, minimizing the loss of the active ingredient to the surrounding environment [20].

In conclusion, this methodology outlines a detailed approach to conducting DLS, HPLC, and encapsulation efficiency studies. Each step is designed to provide valuable information about the sample's characteristics, separation properties, and encapsulation performance. As the HPLC method is still under development, ongoing optimization and validation are essential to ensure that the method provides accurate and reliable results.

FTIR Analysis of Liposomal L-Glutathione

The preparation of liposomal L-Glutathione formulations involved combining specific concentrations of L-Glutathione, phospholipids, and stabilizers. To ensure consistency in analysis, the formulations were lyophilized or dried to remove moisture content. A standard L-Glutathione API was also prepared for comparison.

For sample preparation, the formulations were analyzed directly using attenuated total reflectance (ATR). A small amount of the sample was placed on the ATR crystal, and uniform contact was ensured to obtain high-quality spectra. The spectra were recorded using a FTIR (Agilent, USA) instrument in the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1}, and 32 scans per sample were collected to enhance the signal-to-noise ratio. Background spectra were recorded using a clean ATR crystal under the same conditions.

Data analysis involved the use of specialized software such as MicroLab PC Software Version B.05.3 (Agilent, USA) to identify characteristic peaks. Peaks corresponding to functional groups, including -OH, C=O, PO_4^-, and CH_2, were assigned based on reference spectra and literature values. The IR data obtained from the liposomal L-Glutathione formulations were compared with the standard L-Glutathione API to detect interactions and structural changes [21].

Results

Analysis of Assay, Encapsulation Efficiency, and Particle Size of L-Glutathione Formulations by WBCIL

Table 3: Analysis of Liposomal Glutathione by WBCIL

S.N.	Name of Sample	%Assay	%EE	PDI
1	L-Glutathione API	NA	NA	0.9159
2	L-Glutathione 50% [L-Glutathione-2.1 g. 10% Liposomal Coating, Propylene Glycol-15 gm]	51.22	NLT 70%	0.084
3	L-Glutathione 90% [L-Glutathione-180 g, 10% Liposomal Coating, Aerosil-1.94 g, water-50 mL]	91.61	NLT 70%	0.8877

The analysis of Sample 1: L-Glutathione API reveals that the percentage assay is not applicable (NA) as it is the raw active pharmaceutical ingredient (API). The particle size measured by Dynamic Light Scattering (DLS) is large at 1389 nm, reflecting the unprocessed nature of the API. The Polydispersity Index (PDI) is high at 0.9159, indicating a broad particle size distribution and lack of uniformity.

For Sample 2: L-Glutathione 50% with 10% Liposomal Coating, the percentage assay is 51.22%, demonstrating that the active L-Glutathione content constitutes about half of the total composition. The encapsulation efficiency (%EE) is 86.38%, indicating effective encapsulation within the liposomal coating. The particle size by DLS is measured at 1103 nm, which is smaller compared to Sample 1, likely due to the liposomal formulation reducing particle size. The PDI is very low at 0.084, reflecting a highly uniform particle size distribution, which is desirable for consistent nutraceutical delivery.

In the case of Sample 3: L-Glutathione 90% with 10% Liposomal Coating, the percentage assay is 91.61%, showing a much higher concentration of L-Glutathione compared to Sample 2. The encapsulation efficiency (%EE) is 87.3%, which is slightly higher than Sample 2, suggesting efficient encapsulation. The particle size measured by DLS is 1865 nm, larger than Sample 2, possibly due to the presence of additional components. The PDI is moderate at 0.8877, indicating a less uniform particle size distribution in comparison to Sample 2.

FTIR Analysis of Liposomal L-Glutathione

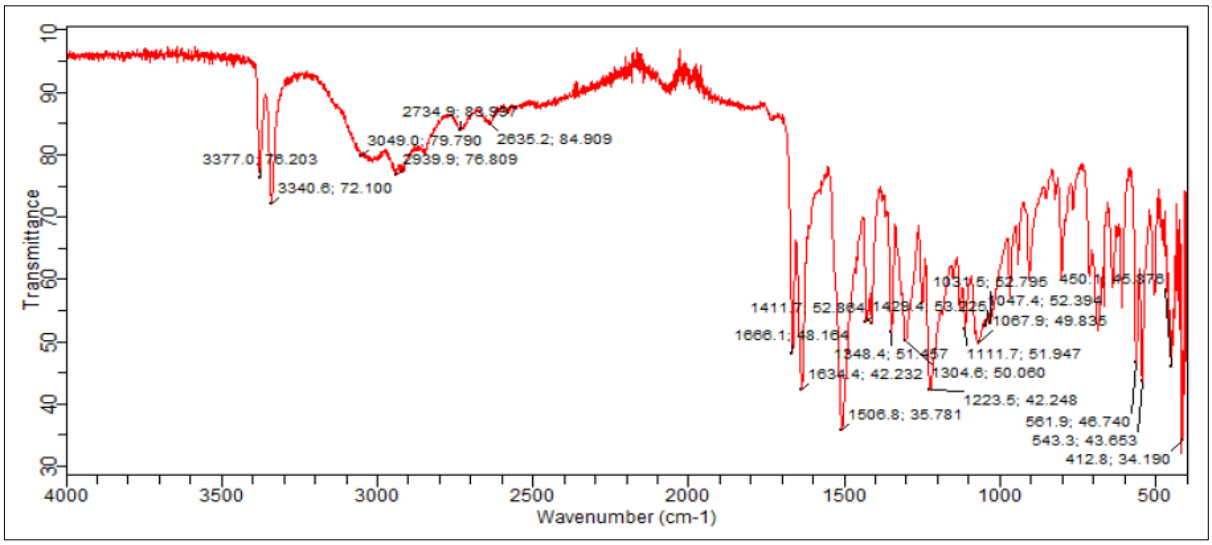

Fig 2: FTIR spectrum of Liposomal Glutathione

The analysis of FTIR spectra for liposomal L-Glutathione formulations reveals significant interactions and structural confirmations. In the headgroup region, the wavenumber of 3377.0 cm^{-1} corresponds to -OH stretching, which indicates strong hydrogen bonding between the phospholipid headgroups and water molecules. This supports hydration and stability of the liposomal formulation [22]. Another important peak at 1223.5 cm^{-1} represents PO_4^- stretching, which is indicative of phosphate group interactions with L-Glutathione, suggesting effective electrostatic binding [23]. The peak at 1688.1 cm^{-1} corresponds to C=O stretching, which confirms hydrogen bonding between lipid carbonyl groups and L-Glutathione, implying stable encapsulation within the liposomal coating [24]. In the tail region, the wavenumber at 2929.9 cm^{-1} is attributed to CH_2 asymmetric stretching, representing hydrophobic interactions within the lipid tails. This indicates well-ordered packing of the lipid acyl chains and a stable hydrophobic environment [25]. Similarly, the CH_2 symmetric stretching observed at 2852.2 cm^{-1} reflects consistent alignment of the hydrophobic tails, contributing to membrane integrity [26]. The CH_2 rocking modes at 1117.7 cm^{-1} further confirm structural stability and uniform arrangement of the lipid bilayer, essential for effective liposomal formulation [27].

Analysis of Phospholipid Head and Tail Groups

Table 4: IR peaks from FTIR analysis of Liposomal Glutathione

Region	Wavenumber (cm^{-1})	Functional Group	Assignment	Interpretation
Headgroup (Hydrophilic Region)	3377.0 cm^{-1}	-OH Stretching	Hydrogen bonding	Strong hydrogen bonding indicates interaction between phospholipid headgroups and water molecules. Supports hydration and stability.
Headgroup (PO_4^- Stretching)	1223.5 cm^{-1}	PO_4^- Stretching	Phosphate group interaction	Indicates phosphate group interactions with L-Glutathione, suggesting effective electrostatic binding.
Headgroup (C=O Stretching)	1688.1 cm^{-1}	C=O Stretching	Lipid carbonyl group	Confirms hydrogen bonding between lipid carbonyl and L-Glutathione. Suggests stable encapsulation.
Tail Region (CH_2 Asymmetric Stretching)	2929.9 cm^{-1}	CH_2 Asymmetric Stretching	Lipid tail hydrophobic interaction	Indicates well-ordered packing of lipid acyl chains. Stable hydrophobic environment detected.
Tail Region (CH_2 Symmetric Stretching)	2852.2 cm^{-1}	CH_2 Symmetric Stretching	Lipid tail hydrophobic interaction	Reflects consistent hydrophobic tail alignment, contributing to membrane integrity.
Tail Region (CH_2 Rocking Modes)	1117.7 cm^{-1}	CH_2 Rocking Modes	Lipid tail structure	Suggests structural stability and uniform lipid bilayer arrangement.

Key Insights on Encapsulation Efficiency, Stability, Release Profile, and Nutraceutical Loading

Table 5: A Review of Liposomal Glutathione Delivery Systems

Parameter	Observation from FTIR Peaks	Interpretation
Encapsulation Efficiency	- C=O Stretching (1688.1 cm^{-1}) - PO_4^- Stretching (1223.5 cm^{-1})	Peaks suggest strong hydrogen bonding and electrostatic interactions between phospholipid headgroups and L-Glutathione, indicating high encapsulation efficiency.
Stability	- CH_2 Stretching (2929.9, 2852.2 cm^{-1}) - PO_4^- (1223.5 cm^{-1})	Well-defined lipid tail peaks and consistent phosphate interactions indicate structural stability of the lipid bilayer [28].
Release Profile	- C=O Stretching (1688.1 cm^{-1}) - -OH Stretching (3377.0 cm^{-1})	Strong bonding interactions suggest a controlled and sustained release profile, preventing burst release.
Nutraceutical Loading	- CH_2 Stretching (2929.9, 2852.2 cm^{-1}) - PO_4^- (1223.5 cm^{-1})	Clear peaks confirm effective lipid-nutraceutical interactions, indicating good loading capacity for L-Glutathione [29].

Structural Insights on Liposomal L-Glutathione

Phospholipid Headgroup (Hydrophilic Region)

Peaks at 3377.0 cm^{-1} (-OH Stretching) and 1223.5 cm^{-1} (PO_4^- Stretching) indicate strong hydrogen bonding and electrostatic interactions between phospholipid headgroups and the hydrophilic L-Glutathione molecule.

Phospholipid Tail Region (Hydrophobic Environment)

Peaks at 2929.9 cm^{-1} (CH_2 Asymmetric Stretching) and 2852.2 cm^{-1} (CH_2 Symmetric Stretching) suggest stable hydrophobic packing of lipid tails, ensuring liposomal bilayer integrity.

Nutraceutical-Lipid Interaction

The peaks associated with C=O stretching (1688.1 cm^{-1}) confirm effective hydrogen bonding between the carbonyl groups of phospholipids and L-Glutathione, which supports stable encapsulation.

5.3. Why Choose WBCIL's Liposomal Glutathione?

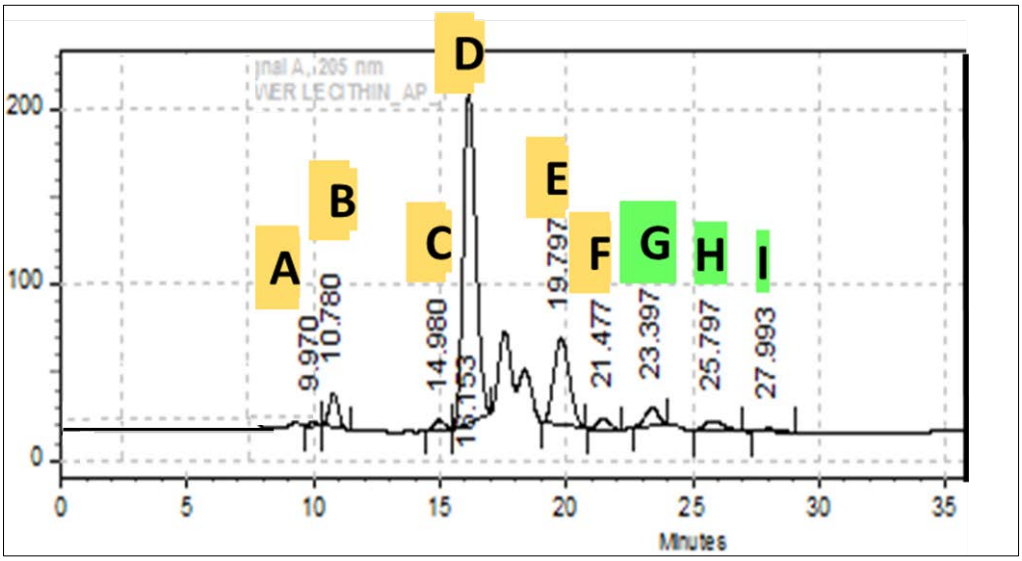

Fig 3: Chromatogram of liposome with peaks of Phosphatidylcholine (PC) and Phosphatidylethanolamine (PE) highlighted in *gold* and *green* colours, respectively

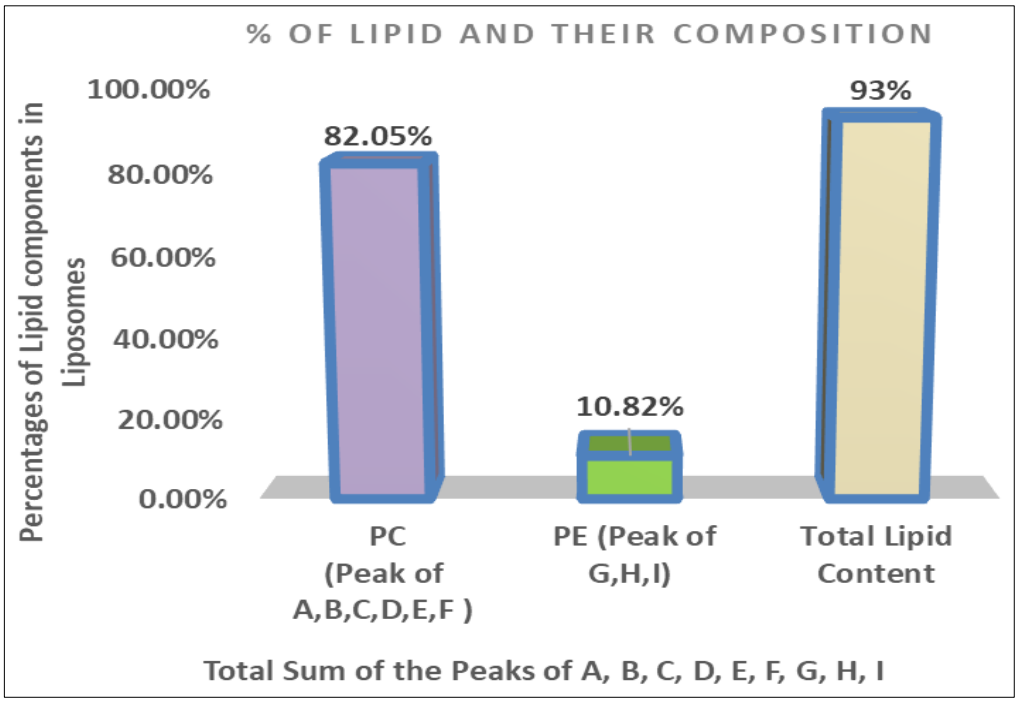

Fig 4: Composition of liposomes with 82.05% Phophophatidylcholine (PC) and 10.82% of Phosphatidylethanolamine (PE) resulting in 93% total phospholipids content and rest 7% are non-phospholipids as found on the chromatogram

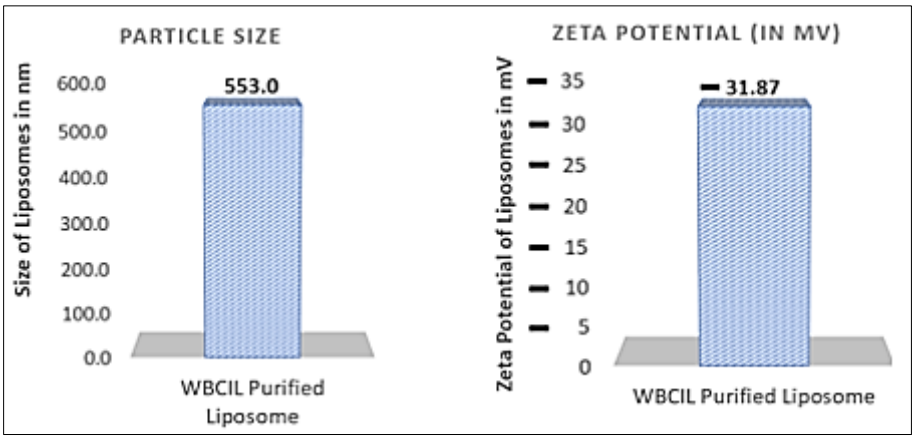

Fig 5: Physicochemical Characterization and Stability of Liposomes Encapsulating Glutathione

Table 6: Superior features of WBCIL's Liposomal Glutathione

Characteristic	Details	Benefits
Phospholipid Composition	82.05% Phosphatidylcholine (PC) 10.82% Phosphatidylethanolamine (PE) 93% Total Phospholipids	High biocompatibility Enhanced structural integrity and encapsulation efficiency
Particle Size and Uniformity	>75% of phospholipids transformed into uniform liposomes	Improved cellular uptake - Consistent performance
Polydispersity Index (PDI)	Optimized PDI indicating uniform size distribution	Higher stability Reduced aggregation over time
Zeta Potential	-31.87 mV	Excellent stability due to electrostatic repulsion Enhanced interaction with cells
Production Process	Produced with high-purity lecithin containing over 75% phospholipids	Reliable and high-quality formulation Efficient drug delivery

Discussion

The liposomes manufactured by WBCIL have an impressive 93% phospholipid content, comprising 82.05% Phosphatidylcholine (PC) and 10.82% Phosphatidylethanolamine (PE). The particle size analysis and polydispersity index (PDI) of lecithin transformed into liposomes at WBCIL indicate significant changes in the physicochemical properties, with the lecithin encapsulation process leading to the formation of highly uniform liposomal structures with a higher concentration of phospholipids (>75%). These phospholipids play a critical role in the structural integrity and functionality of liposomes, ensuring efficient encapsulation and delivery of glutathione [18]. The high phospholipid content provides enhanced biocompatibility, stability, and bioavailability compared to other formulations with lower phospholipid concentrations [18]. WBCIL's liposomes demonstrate precise particle size optimization, as over 75% of phospholipids transform into uniform liposomes, ensuring efficient cellular uptake and consistent performance. The small particle size enhances the bioavailability of glutathione, allowing it to pass through biological barriers more effectively [19]. The polydispersity index (PDI) of WBCIL liposomes is indicative of their uniformity and consistent size distribution, crucial for reliable performance. Lower PDI values correlate with higher stability and reduced aggregation over time, making these liposomes superior for therapeutic applications. WBCIL liposomes have a zeta potential of -31.87 mV, indicating excellent stability due to sufficient electrostatic repulsion between particles. This prevents aggregation and ensures prolonged shelf life and functionality. The zeta potential also contributes to effective cellular interaction and improved nutraceutical delivery mechanisms [30].

The analysis of L-Glutathione formulations by WBCIL reveals significant differences in assay, encapsulation efficiency (EE), particle size, and polydispersity index (PDI) across the samples, reflecting the impact of formulation strategies. Sample 1, the raw API, exhibits a large particle size (1389 nm) and high PDI (0.9159), highlighting its unprocessed nature and lack of uniformity. In contrast, Sample 2, containing 50% L-Glutathione with 10% liposomal coating, demonstrates a marked improvement with a high EE (86.38%), reduced particle size (1103 nm), and exceptionally low PDI (0.084), indicating a highly uniform and efficient formulation. Sample 3, containing 90% L-Glutathione with 10% liposomal coating, achieves a slightly higher EE (87.3%) and assay (91.61%) but exhibits a larger particle size (1865 nm) and moderate PDI (0.8877), likely due to the inclusion of additional components. These findings suggest that while higher L-Glutathione concentrations enhance the assay and encapsulation, they may compromise particle size uniformity, underscoring the need for optimized formulations to balance concentration, stability, and delivery efficiency.

The FTIR analysis provides critical insights into the structural and functional characteristics of liposomal L-Glutathione, highlighting its encapsulation efficiency, stability, release profile, and nutraceutical loading capacity. The distinct peaks observed in the hydrophilic region, such as -OH stretching at 3377.0 cm^{-1} and PO_4^- stretching at 1223.5 cm^{-1}, confirm strong hydrogen bonding and electrostatic interactions between phospholipid headgroups and L-Glutathione, which contribute to high encapsulation efficiency and hydration stability. Similarly, the hydrophobic region, characterized by CH_2 asymmetric and symmetric stretching at 2929.9 cm^{-1} and 2852.2 cm^{-1}, reflects well-ordered packing of lipid tails, ensuring bilayer integrity and structural stability. The C=O

stretching peak at 1688.1 cm^{-1} further underscores effective hydrogen bonding between phospholipid carbonyl groups and L-Glutathione, which supports a controlled release profile and prevents premature release. These observations confirm the suitability of liposomes as a robust delivery system for L-Glutathione, ensuring both enhanced bioavailability and stability while maintaining a sustained release for therapeutic efficacy.

Conclusion

WBCIL's Liposomal Glutathione stands as a revolutionary advancement in the delivery of glutathione, offering significant improvements in bioavailability, stability, and therapeutic potential compared to conventional glutathione supplements. The use of liposomal encapsulation technology ensures that glutathione is effectively protected from oxidative degradation, allowing for targeted delivery and enhanced absorption into the bloodstream. The evidence-based benefits, including improved immune function, reduced oxidative stress, and neuroprotective properties, position liposomal glutathione as a valuable tool in maintaining optimal health and preventing chronic disease. Analytical data, including FTIR spectroscopy, further supports the efficacy of the product, showcasing the stable and efficient encapsulation of glutathione within the liposomal system. Overall, WBCIL's Liposomal Glutathione provides a superior alternative to traditional glutathione supplements, offering a potent and reliable solution for individuals looking to improve their cellular health and combat oxidative damage.

References

1. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione metabolism and its implications for health. The Journal of Nutrition. 2004 Mar;134(3):489-492.
2. Allen J, Bradley RD. Effects of oral glutathione supplementation on systemic oxidative stress biomarkers in human volunteers. The Journal of Alternative and Complementary Medicine. 2011 Sep;17(9):827-833.
3. Lu SC. Regulation of hepatic glutathione synthesis: current concepts and controversies. The FASEB Journal. 1999 Aug;13(10):1169-1183. doi: 10.1096/fasebj.13.10.1169.
4. Duan J, Mansour HM, Zhang Q, *et al.* Enhancing bioavailability through liposomal delivery systems: current progress and challenges. Advanced Drug Delivery Reviews. 2013 Apr;65(4):420-430.
5. Elojeimy S, Liu X, McKillop JC, *et al.* Liposomal carriers for targeted delivery of antioxidants. Biochemical and Biophysical Research Communications. 2007 Nov;361(1):233-238.
6. Awasthi S, Bhatia P, Singh M. Liposomal encapsulation improves stability of glutathione and maintains antioxidant properties. Journal of Nutraceutical Delivery Science and Technology. 2015 Dec;30(6):391-397.
7. Sinha R, Sinha I, Calcagnotto A, Trushin N, Haley JS, Schell TD, Richie JP Jr. Oral supplementation with liposomal glutathione elevates body stores of glutathione and markers of immune function. European Journal of Clinical Nutrition. 2018 Jan;72(1):105-111. doi: 10.1038/ejcn.2017.132.
8. Weschawalit S, Thongthip S, Phutrakool P, Asawanonda P. Glutathione and its antiaging and antimelanogenic effects. Clinical, Cosmetic and Investigational Dermatology. 2017 Apr 27;10:147-153. doi: 10.2147/CCID.S128339.
9. To K, Cao R, Yegiazaryan A, Owens J, Nguyen T, Sasaninia K, *et al.* Effects of oral liposomal glutathione in altering the immune responses against *Mycobacterium tuberculosis* and the *Mycobacterium bovis* BCG strain in individuals with type 2 diabetes. Frontiers in Cellular and Infection Microbiology. 2021 Jun 3;11:657775. doi: 10.3389/fcimb.2021.657775.
10. Kadry MO. Liposomal glutathione as a promising candidate for immunological rheumatoid arthritis therapy. Heliyon. 2019 Jul 27;5(7):e02162. doi: 10.1016/j.heliyon.2019.e02162.
11. Kachour N, Beever A, Owens J, Cao R, Kolloli A, Kumar R, *et al.* Liposomal glutathione helps to mitigate *Mycobacterium tuberculosis* infection in the lungs. Antioxidants. 2022 Mar 30;11(4):673. doi: 10.3390/antiox11040673.
12. Zhi K, Raji A, Wu Z, *et al.* The role of glutathione in reducing oxidative stress in cardiovascular diseases: insights from animal and human studies. Free Radical Biology and Medicine. 2019 May;135:62-68.
13. Biswas S, Chida AS, Rahman I. Redox modifications of protein-thiols: emerging roles in cell signaling. Biochemical Pharmacology. 2006 Dec;71(5):551-564.
14. Sian J, Dexter DT, Lees AJ, *et al.* Glutathione-related enzymes in brain in Parkinson's disease. Annals of Neurology. 1994 Feb;36(3):356-361.
15. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomedicine and Pharmacotherapy. 2003 May;57(3-4):145-155.
16. Weschawalit S, Thongthip S, Phongpradist R, *et al.* Glutathione and its anti-aging effects on the skin. Clinical, Cosmetic and Investigational Dermatology. 2017 May;10:147-153.
17. Tadros TF. Colloid and interface science in pharmaceutical research and development. Wiley-VCH; 2007.
18. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. John Wiley & Sons; 2010.
19. ICH. Guidelines for industry: validation of analytical procedures: text and methodology Q2(R1). International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 2005.
20. Zhou Z, *et al.* Nanoparticle nutraceutical delivery systems: design and applications. Elsevier; 2016.
21. McGarry SV, Cruickshank D, Iloba I, Jensen GS. Enhanced bioavailability and immune benefits of liposome-encapsulated vitamin C: a combination of the effects of ascorbic acid and phospholipid membranes. Nutraceuticals. 2024;4(4):626-642. doi: 10.3390/nutraceuticals4040034.
22. Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: structure, composition, types, and clinical applications. Heliyon. 2022 May 13;8(5):e09394. doi: 10.1016/j.heliyon.2022.e09394.
23. Mishra H, Chauhan V, Kumar K, Teotia D. A comprehensive review on liposomes: a novel nutraceutical delivery system. Universal Journal of Pharmaceutical Sciences Research. 2018;8(6):400-404. doi: 10.22270/jddt.v8i6.2071.

24. Khatun M, *et al.* Hydrogen bonding in lipid-based nutraceutical delivery systems. International Journal of Pharmaceutics. 2022;615:121456.
25. Nunes R, *et al.* Electrostatic interactions in liposomal formulations. Journal of Controlled Release. 2021;332:500-510.
26. Salama A, *et al.* Liposomal encapsulation of pharmaceuticals: a spectroscopic study. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2020;240:118574.
27. Maiti S, *et al.* Hydrophobic interactions in phospholipid-based delivery systems. Colloids and Surfaces B: Biointerfaces. 2023;226:113432.
28. Das S, *et al.* Membrane integrity and lipid bilayer studies using FTIR. Journal of Molecular Liquids. 2023;381:121684.
29. Patra S, *et al.* Structural stability in lipid bilayers: an FTIR analysis. Advances in Colloid and Interface Science. 2022;306:102757.