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food & pharma solutions

Liposovit® Glutathione

Liposomal glutathione in powder form

HIGH STABILITY AND BIOAVAILABILITY
NON-ALLERGENICITY
WIDER APPLICATION POSSIBILITIES

STRONG ANTIOXIDANT PROPERTIES • SUPPORT OF THE IMMUNE SYSTEM AND DETOXIFICATION OF THE BODY
BRIGHTENED AND EVEN SKIN TONE • HEALTY AGING

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What is **Liposovit®-Glutathione**

Liposovit®-Glutathione is liposomal glutathione (γ-glutamylcysteinylglycine) in the form of a **homogeneous powder**. The hydrophilic space of liposomal vesicles encapsulates the biologically active L-isomer of reduced glutathione (GSH) (1), which is significantly superior in quantity in the cell to oxidized glutathione (GSSG) (2,3). The obtained liposomes underwent a drying process, which largely determined special properties of the product.

Glutathione

Glutathione plays a key role in physiological processes. It is a powerful antioxidant that reduces oxidative stress and maintains the body's redox balance[1] (4,5). It enables the maintenance of physiological levels (regeneration) of vitamins C and E as well as β-carotene (5,6). It also facilitates the conversion and removal of lipid peroxides and xenobiotics (pollutants, heavy metals and drugs) from the body (7). Its detoxifying action involves direct conjugation with undesirable substances or acting as a substrate in conjugation reactions (4). GSH is also involved in the regulation of gene expression, DNA synthesis and repair (5), and the regulation of cell proliferation and apoptosis (6). It enables the body to maintain optimal levels of cytokines (4) and boosts the immune system response (5) as well. An important feature of glutathione is its ability to brighten the skin. A change in complexion is possible, among other things, due to the reduction of tyrosinase (TYR)[2] activity, disruption of its transport to premelanosomes, and scavenging by GSH of free radicals and peroxides that contribute to the activation of the enzyme (8). Glutathione also "redirects" the melanin biopolymer biosynthesis pathway from brown-black eumelanin toward yellow-red pheomelanin, the pigment responsible for lighter skin colour (8,9).

Oxidative Stress

Oxidative stress is defined as the presence in the body of active oxygen species (free radicals and non-radicals) in excess of the body's antioxidant capacity. This imbalance between the excessive synthesis of oxidative compounds and the effectiveness of the body's antioxidant defence mechanisms plays an important role in the pathophysiology of a number of diseases, especially non-communicable diseases (10), including cancer (11). There is also growing evidence that ROS-induced oxidative stress is a major contributor to the ageing process (12,13) as well, which significantly reduces the quality of life extended by medical advances (14).

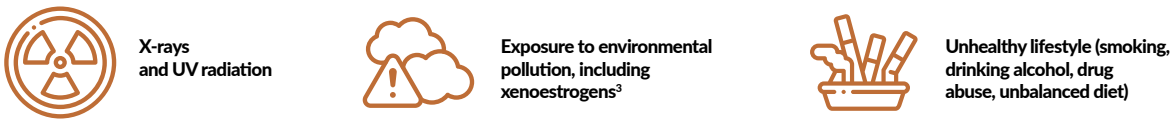

Fig 1. Main exogenous sources of ROS
Compiled based on ref.: 12,13 and 16

[1]Maintaining redox balance is important especially in the brain. This organ, due to the high consumption of oxygen taken up by the body, is particularly susceptible to the generation of reactive oxygen species (4).
[2]Tyrosinase (TYR) is a key enzyme in melanogenesis (9).
[3]Synthetic xenoestrogens, including bisphenols (especially Bisphenol-A, BSA), phthalates and parabens are classified as endocrine disrupting chemicals (EDCs). They can cause a number of health problems, such as neurodevelopmental disorders in children, fertility problems, hormone-dependent cancers and metabolic diseases. These substances can be found in plastics, pesticides, preservatives, pharmaceuticals (ethinylestradiol), personal care products or UV filters added to cosmetics (15).

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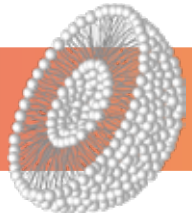

Liposovit®-Glutathione: for whom?

Liposovit®-Glutathione is particularly recommended for those:

- Struggling with stress and fatigue
- Living and/or working in a polluted environment (large urban areas, industrial districts)
- Exposed to tobacco smoke (passive and active smokers)
- Wishing to boost immunity and maintain proper liver function
- Caring about maintaining the proper condition and colour of the skin, including after sunbathing
- Seeking effective strategies to slow the ageing process, including maintaining and/or improving cognitive function and vigour.

Uniqueness of the **Liposovit®-Glutathione** technology

Because conventional lipid carriers in liquid suspensions may degrade when stored (17), **BART Research & Development Department** has devised an **innovative technology solution**, based, among others, on the drying of obtained liposomes. This solution:

- Significantly increases the storage stability of the final product compared to its liquid form
- Eliminates the need to maintain refrigerated temperatures during transport, distribution and storage of **Liposovit®-Glutathione**
- Eliminates the need to add preservatives, making the product safe for people with broadly defined food hypersensitivities
- Increases the range of possible applications of the product, adapting it for easy encapsulation and convenient use in powder mixtures, also with other products of the brand **Liposovit®**

As shown in our study, the drying process does not affect the quality and size of the formed liposomes (< 300 nm).

Distinctive features of **Liposovit®-Glutathione**

Liposovit®-Glutathione developed by us:

- Exhibits the typically higher absorption and bioavailability of liposomal forms
- Is characterized by a high concentration of reduced glutathione (min. 40%)
- Dissolves well in water
- Does not contain ingredients of animal origin, making it suitable for consumption by **vegetarians and vegans**
- Is free of GMOs and allergens
- Is biocompatible and non-toxic.

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Liposovit®-Glutathione: characteristics

Liposovit®-Glutathione comes in the form of a fine powder with a homogeneous structure and a white to cream colour. Its homogeneity was confirmed by observations under a Leica DM2500LED microscope (Figure 2).

Macroscopic image | **Microscopic image Magnification x 25** | **Microscopic image Magnification x 100**

Fig 2. Macroscopic and microscopic image of Liposovit®-Glutathione powder

The surface of **Liposovit®-Glutathione** powder was analysed with scanning electron microscopy (SEM), which allows for the imaging of materials and structures with submicron resolution (18).

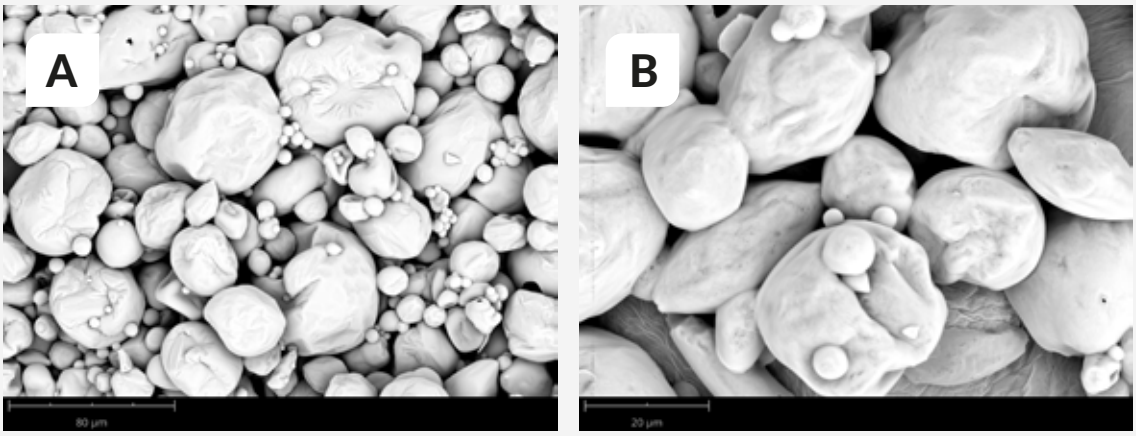

Fig. 3. Liposovit®-Glutathione powder particles in images obtained under the scanning electron microscope (Phenom XL, Phenom World BV). A. Magnification of 1000 x, B. Magnification of 3000 x.

Figure 3 shows that the surface of microcapsules made of a carbohydrate vesicle which encloses liposomal particles remained intact after the drying process. This confirmed the effectiveness of lipid carriers microencapsulation process and the existence of an additional barrier to protect liposome-enclosed reduced glutathione against adverse external factors.

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Visualizing the particles morphology is an important element in the assessment of liposome physical and chemical properties (19). Cryogenic electron microscopy (cryo-TEM) is the most useful tool to assess their shape and internal structure, including their lamellarity. With this technique you can examine the previously hydrated sample in a state most closest to its native condition (20).

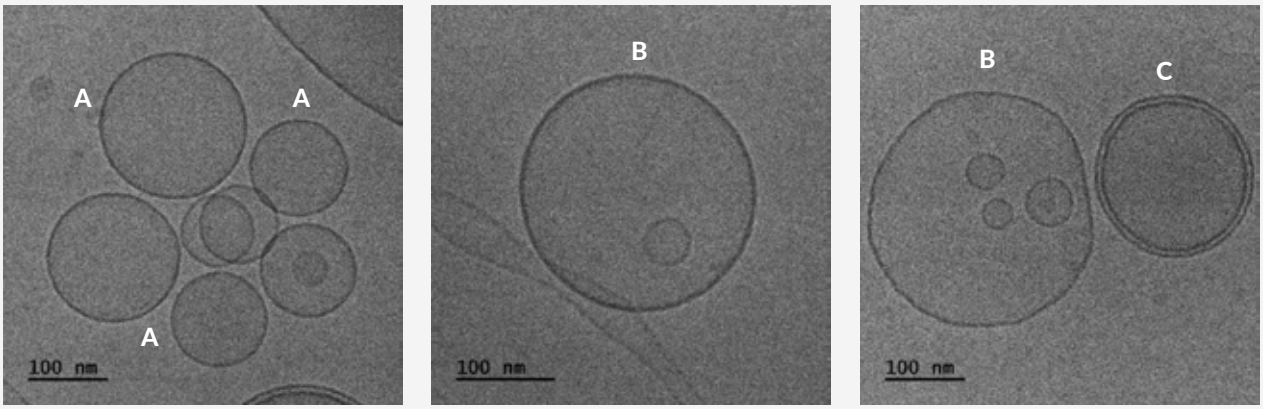

Fig. 4. Morphology of liposomes in Liposovit®-Gluathione, in images from the cryogenic electron microscope (Tecnai F20 X TWIN, FEI Company)
A. large unilamellar vesicle (LUV), B. multivesicular vesicle (MVV), C. oligolamellar vesicle (OLV)

Figure 4 shows liposomes contained in **Liposovit®-Glutathione** powder. This has confirmed the presence of smooth-surfaced spherical vesicles with intact continuous phospholipid bilayer.
The size of the obtained liposomes is also subject to our on-going checks as it significantly affects their effective action. Although the size of liposomes can range from 25 to 2,500 nm (21), the most effective liposomes are those characterized by small particle diameter and narrow particle size distribution (22).
The size of the liposomes and their particle size distribution were determined using the LUMiSizer® 651 particle size analyzer. Based on the results obtained for 3 independent measurements, carried out after the test batch samples were reconstituted in water and degassed, it was determined that the average size **Liposovit®-Glutathione** (D50) liposomes was **219 nm**, with a harmonic mean of 210 nm (Table 1).

Table 1. Results of particle size measurements in the powder form of Liposovit®-Glutathione

Quantiles	Particle size distribution parameters
10% of particles ≤ 129.0 nm	Median x50: 218.7 nm
16% of particles ≤ 135.8 nm	Harmonic mean: 210.2 nm
50% of particles ≤ 218.7 nm	Standard deviation: 149.1 nm
84% of particles ≤ 366.4 nm	Mode: 129.0 nm
90% of particles ≤ 464.8 nm	

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Uniform particle size distribution of liposomes in **Liposovit®-Glutathione** was also confirmed (Figure 5).

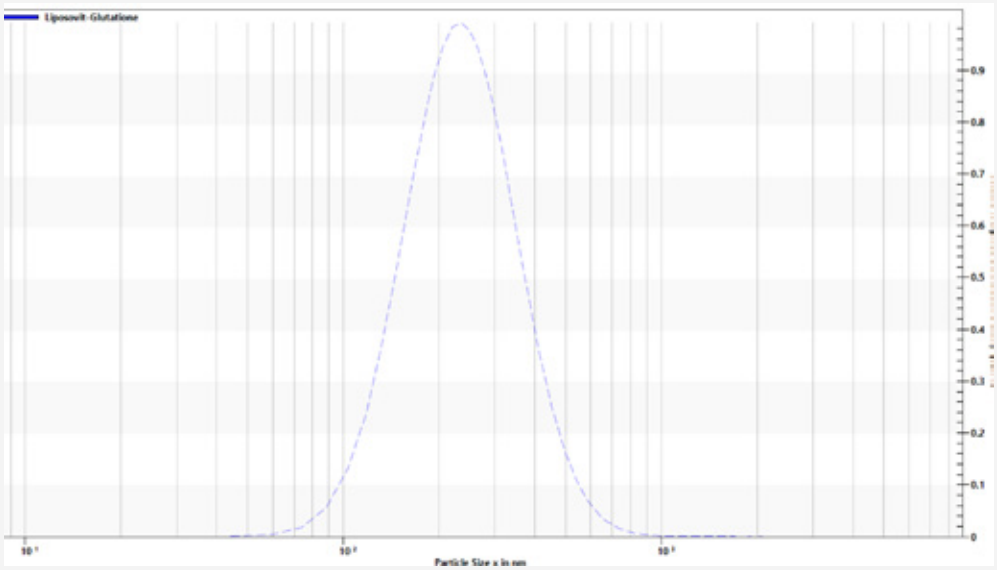

Fig. 5. Distribution of liposome sizes in powdered Liposovit®-Glutathione.

The expiration date of the product stored under the conditions indicated in the specification (temperature 5-25°C, humidity 25-70%) is 24 months.

Liposomal glutathione - current state of knowlege

Reduced L-glutathione is the subject of a number of *in vivo* and clinical studies.

In an animal model (Sprague-Dawley rats), the bioavailability of GSH encapsulated in positively charged hydrated proliposomes (300 mg/kg bw equivalent dose) was AUC 1782 ± 92 h x µg/mL and was statistically significantly higher than that of conventional GSH (300 mg/kg bw dose, AUC 1610 ± 79 h x µg/mL) (23).

The ability of liposomal vesicles to increase glutathione bioavailability and its antioxidant potential was also demonstrated in an animal model of diabetic nephropathy (rats). The liposomal form of GSH additionally effectively inhibited the activity of aldose reductase (AR), which converts glucose to sorbitol in the polyol pathway[4]. A cross-section of the animals' kidneys also showed that liposomal glutathione penetrated into these organs, resulting in lesion relief (24).

Oral administration of liposomal glutathione for 2 months to apolipoprotein E-deficient mice (E^0) significantly slowed the progression of atherosclerotic lesions. The observed effect was attributed to the antioxidant properties of liposomal GSH (reduction of oxidative stress in serum and macrophages) and its ability to inhibit cholesterol biosynthesis, lower serum cholesterol levels and stimulate the efflux transport of cholesterol from macrophages (26).

[4] The kidneys are among the organs in which sorbitol dehydrogenase is not produced. As a result, sorbitol reaches toxic levels in kidney tissues of diabetic patients, leading to their impaired function (25).

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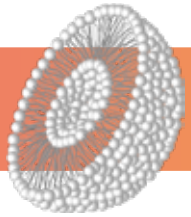

In a 4-week pilot study involving healthy non-smokers aged 50-80 years (n=12), the efficacy of liposomal GSH in liquid suspension form (doses of 500 and 1000 mg/day) was examined. The result of supplementation was an increase in blood GSH levels (the greater the lower the initial level of this tripeptide), a decrease in oxidative stress biomarkers (↓ GSSG + GSSP/GSH ratio, ↓ 8-isoprostane level[5]) and activation of the immune system (↑ lymphocyte proliferation; ↑ NK cell activity) (27).

In another experiment, 13-week supplementation with liposomal GSH, when compared to placebo, significantly contributed to lowering free radical levels and restoring the cytokine balance disrupted in people with HIV, i.e., increasing levels of IL-1β, IL-12, IFN-γ and TNF-α (Th1 cytokines) and lowering the levels of IL-10 and TGF-β (Th2 cytokines) (28).

In a randomized double-blind, placebo-controlled clinical trial, three-month supplementation with liposomal glutathione (1260 mg L-GSH in liquid suspension/day) of people with type two diabetes (glycated hemoglobin HbA1c levels ranging from 7-13%, age 18-56) led to (29):

- Significant reduction in levels of the pro-inflammatory cytokine IL-6 and malondialdehyde[6] (MDA) in serum, red blood cells (RBC) and PBMC
- Significant reduction in the level of GSSG in PBMC and erythrocytes, as well as significant increase in the level of GSH in RBC
- Significantly increased levels of IFN-γ, TNF-α, IL-2 and decreased levels of IL-10 compared to before supplementation.

The intervention with placebo (empty liposomes) did not result in the positive changes observed after application of liposomal GSH.

In addition, 3-month supplementation of liposomal glutathione augmented the immune system response of study participants to strains of *Mycobacterium bovis* and *Mycobacterium tuberculosis*, as demonstrated in an *in vitro* study using infected PBMC cells isolated from study participants (29).

Liposovit®-Glutathione: higher bioactivity and wider application possibilities

Liposovit®-Glutathione is a response to the needs of a wide range of consumers at risk of developing and/or increasing levels of oxidative stress in the body. The encapsulation of reduced glutathione in liposomes significantly improves its bioavailability. Thus, this potent non-enzymatic antioxidant more effectively assists the body in restoring the oxidant-antioxidant balance necessary to maintain good health and function, especially in senior age.

In addition, the powder form of **Liposovit®-Glutathione** places the product among the innovations that significantly expand the range of its possible applications in the manufacturing of dietary supplements.

[5] 8-isoprostane is a marker of lipid peroxidation
[6] Free malondialdehyde is a marker of oxidative stress

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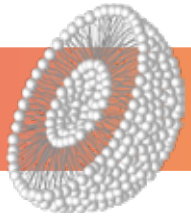

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