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Development of Nutraceutical and Nutritional Design Method for Liposomal Curcumin and Vitamin C Using Microstructural and Elemental Analysis

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Abstract
The biomolecular components of curcumin are potent inhibiting nutraceuticals with hydrophobic characteristics. Vitamin C, a water-soluble antioxidant, plays a crucial role in strengthening the body's immune system. Liposomal encapsulation enhances the solubility and bioactivity of curcumin, while also increasing the bioavailability of vitamin C. In this study, a functional nutraceutical and nutritional design method was developed using structural and elemental analysis of prepared liposomal curcumin and liposomal curcumin-vitamin C nanoparticles, utilising scanning electron microscopy. The microstructural images of the liposomal curcumin nanoparticles revealed their hydrophobic nature and adequate structural model. The liposomal curcumin-vitamin C displayed solid surface spherical particles in their dried forms. The peak patterns graph and data of the elemental constituents of the liposomal curcumin samples indicated high levels of carbon and oxygen, while the liposomal curcumin-vitamin C samples showed elemental carbon and oxygen, as well as minerals Mg, P, and K.

Keywords
Liposomal curcumin-vitamin C, electron microscopy, structural and compositional analysis, bioavailability

1 Introduction

Electron microscopy techniques are valuable tools for developing functional nutraceutical and nutritional design methods through structural and compositional analysis.[1] Nanotechnology applications are particularly useful for enhancing the therapeutic and dietary potentials of nutraceuticals and nutritional bioactives with limited solubility and stability through nano-encapsulation methods, which offer solubility, biopersistency, dispensability, biocompatibility, bioavailability, bioaccessibility, and biodegradability.[2]

Liposomes, known as phospholipid vesicles, have proven advantages in high nutraceutical and nutritional loading. They can incorporate larger amounts of functional nutraceuticals and nutrients by controlled release, thus enhancing their bioactivity and increasing bioavailability. Liposomal nanoparticles with large matrices are suitable biomaterials for the nano-entrapment of curcumin and vitamin C.[3] Studies have shown that the use of liposomes as drug vesicles does not interfere with the antioxidant activities of curcumin and vitamin C.[4] Curcumin is a natural bioactive nutraceutical with the active constituent diferuloylmethane, extracted from the *Curcuma longa* plant. It exhibits inhibitory power and potent multitherapeutic effects. Multifunctional curcumin bioconstituents mediate these multitherapeutic effects, making curcumin bioactives functional nutraceuticals and phytopharmaceuticals. They exhibit antioxidative, antimicrobial, antiviral, antispasmodic, antidiabetic, antifertility, anti-inflammatory, antihypertensive, immunomodulatory, lipid-lowering, anticarcinogenic, and chemoprotective activities.[5–10] Curcumin inhibits major human drug-metabolising cytochrome P450 enzymes (CYPs). Studies investigating the interactions between curcumin and CYPs have shown that curcumin inhibits CYP1A2, CYP3A4, CYP2D6, CYP2C9 and CYP2B6.[11] The lipid bilayer of liposomes produced in the presence of curcumin, vitamin D3, and even higher amounts of vitamin D3, did not adversely affect the stability of the phospholipid vesicles.[12] The preparation of liposomes containing curcumin for application in cancer therapy has been studied extensively. The results indicated that liposomal curcumin nanoparticles exhibited pro-apoptotic and inhibitory effects on the growth of cancer cells.[13]

Vitamin C, a basic nutritional component and a complete biochemical water-soluble complex, was first isolated in 1928 by Hungarian biochemist and Nobel laureate Albert Szent-Györgyi. In a series of early experiments on citrus plants, Szent-Györgyi discovered that plant browning could be caused by peroxidase, a plant enzyme active during oxidation. By adding citrus juice to peroxidase, the browning process could be stopped. In related experiments, Szent-Györgyi isolated the active substance, initially calling it hexuronic acid, which was later named ascorbic acid or vitamin C due its anti-scurvy properties.[14–19] Humans and other primates cannot synthesise vitamin C, and severe vitamin C deficiency leads to scurvy, a systemic disease affecting the skeletal, nervous, and hematopoietic systems with potentially fatal consequences. Szent-Györgyi subsequently discovered the Krebs cy-

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cle, also known as the citric acid cycle, highlighting the role of vitamin C in preventing scurvy.[20] As the human body cannot biosynthesise and store vitamin C complex endogenously, it must be obtained as an essential nutrient through the consumption of fruits, vegetables, and other dietary sources.[21]

The unique complete vitamin C complex includes ascorbic acid, ascorbinogen, hesperidin, rutin, bioflavonoids, factors K, J, and P, tyrosinase, and the minerals calcium and magnesium. The full bioactivity of vitamin C cannot occur if there is a deficiency in any of these associated components.[22] Vitamin C is a robust antioxidant and an essential nutrient for humans and animals. It has therapeutic effects on various infections, aids immune defence, dissolves free radicals, acts as a cofactor in biochemical reactions, and controls the bioprocesses of cell differentiation and proliferation.[23,24] Vitamin C strengthens the immune system, prevents scurvy, has anti-inflammatory effects, serves as a natural remedy for upper respiratory tract diseases such as the flu and colds, promotes wound healing, reduces the risks of developing cancer and cataract, increases estrogenic levels, lowers high blood pressure, reduces the risk of stroke, protects cardiovascular health, helps manage excessive blood cholesterol, is effective in the treatment of diabetes, supports kidneys function, and eradicates the risk factors that lead to the formation of kidney stones and assists their removal.[24–32]

While it is acknowledged that ascorbic acid alone cannot replicate all the functions of the vitamin C complex, it does exhibit compelling physiological effects as an antioxidant and scavenger of free radicals. This activity protects the functional components of the vitamin C complex from oxidation or degradation, and safeguards DNA, tissue, plant cell membranes, and the body from various oxidative damage.[33] Employing electron microscopy, spectroscopy, and diffraction techniques for structural and compositional analyses proves valuable in the development of methods for designing functional remedies. Encapsulation of vitamin C in chitosan microspheres was investigated using electron microscopy techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD). These studies determined that the release rate was influenced by the volume of the cross-linking agent.[34]Silicon is extremely hydroscopic and primarily exists as silica or silicate compounds, found in nature as hydrated crystalline aluminosilicates of zeolites. It is used as an adsorptive structure agent for higher molecules such as vitamins.

Therefore, the aim of this research was to develop a nutraceutical and nutritional design method for the structural characterisation and elemental analysis of dried liposomal curcumin and liposomal vitamin C nanoparticles using field emission gun scanning electron microscopy (FEG-SEM), and elaborate on the obtained results and their application.

2 Materials and methods

Lecithin, a naturally occurring mixture of phospholipids, is abundantly found in plant and animal tissue, and is an essential component in liposomal formulations. It is critical for maintaining the stability and functionality of these lipid-based vesicles. Plant-based lecithin derived from soybeans or sunflower seeds has been widely used in liposomal formulations due to its high phospholipid content, primarily phosphatidylcholine. The use of lecithin granules marks a significant advancement in the field of liposomal technology. The sonic method is one of the most widely used techniques for liposome preparation. Lecithin is hydrated with a water solution at around 60 °C, which is the lipid transition temperature necessary for smoother bilayer formation. This process, enhanced by vigorous shaking and potentially using sonication in an ultrasonic bath, facilitates the gradual transformation of the lipids into spherical structures known as liposomes. These carefully controlled steps are essential for ensuring the precise formation and integrity of the liposomal structures, crucial for their intended applications in various fields such as medicine and biotechnology.[35–38] A solution of lecithin granules was used to prepare liposomal curcumin and liposomal vitamin C samples by incorporating powdered curcumin and vitamin C complex in a WiseClean WUC-D10H ultrasonic unit with multiple sonic frequency cycles for higher cumulative quality absorption. The liposomal curcumin and liposomal vitamin C samples were dried at room temperature without additional preparation for electron microscopy image processing. The Quanta 250 FEG-SEM at Düzce University was used for highest resolution microstructural imaging and 'Energy Dispersive X-ray Analysis' (EDAX) elemental investigation of the liposomal curcumin and liposomal curcumin-vitamin C. The nutraceutical and nutritional syrups were crafted and prepared using an orange aroma infused with the specified blends as determined by this elemental analysis.

3 Results and discussion

This study conducted structural and elemental analysis through scanning electron microscopy to develop functional models for the design of nutraceutical and nutritional products, focusing on liposomal formulations of curcumin and curcumin combined with vitamin C nanoparticles, guided by related studies.[1–13] The dried samples of liposomal curcumin and liposomal curcumin-vitamin C were used to survey the purpose of developing a nutraceutical and nutritional design method. Microstructural images of the samples were provided for structural analysis of liposomal curcumin and liposomal curcumin-vitamin C.

Fig. 1 displays the microstructural images at a scale of 50 μm. The elemental peak pattern graph is presented in Fig. 2, and Table 1 provides the elemental constituents of the liposomal curcumin samples.

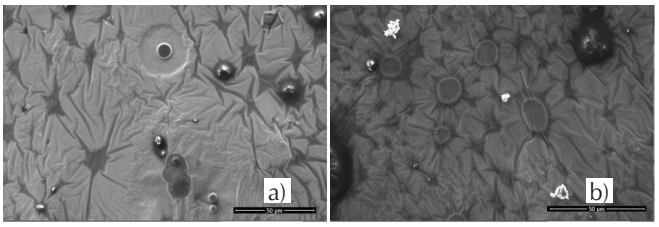

Fig. 1 – FEG-SEM images of liposomal curcumin (scale 50 μm)

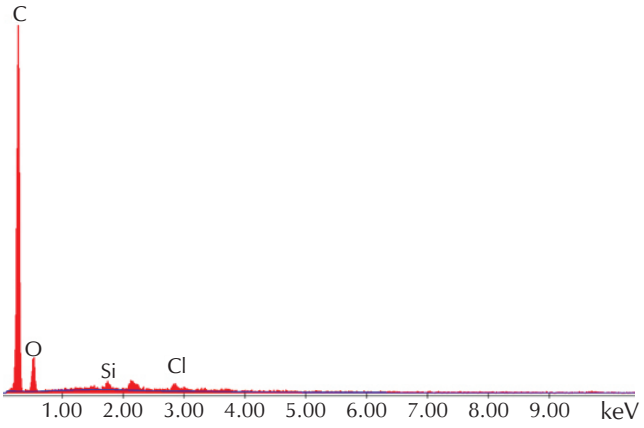

Fig. 2 – FEG-SEM peak patterns graph of liposomal curcumin

Table 1 – Elemental constituents of liposomal curcumin

EDAX ZAF QUANTIFICATION	
Element	wt %
C	76.73
O	21.89
Si	1.03
Cl	0.34
Total	100

Structural imaging, elemental peak pattern graphs, and elemental data for liposomal curcumin-vitamin C samples are presented in Fig. 3 at a scale of 50 μm. Fig. 4 presents the peak patterns graph, and Table 2 shows the elemental constituents.

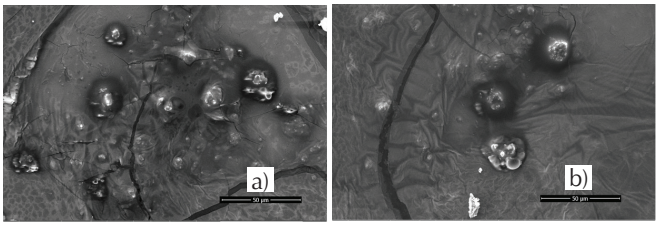

Fig. 3 – FEG-SEM images of liposomal curcumin-vitamin C (scale 50 μm)

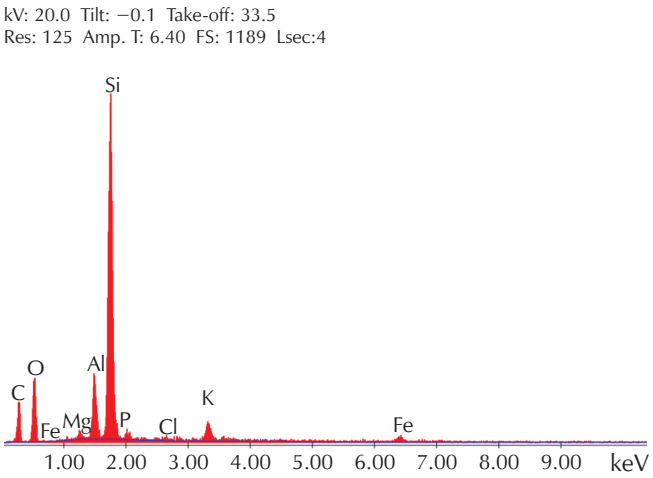

Fig. 4 – FEG-SEM peak patterns graph of liposomal curcumin-vitamin C

Table 1 – Peak patterns graph of the elemental constituents of liposomal curcumin-vitamin C

EDAX ZAF QUANTIFICATION	
Element	wt %
C	27.56
O	22.98
Mg	0.72
P	1.20
K	3.23
Fe	2.91
Si	34.73
Cl	0.60
Al	6.07
Total	100.00

The use of liposomes in encapsulation enhances the stability and bioactivity of nutraceutical and nutritional bioactives, which can be designed using scanning electron microscopy. The specific functional properties of curcumin as a nutraceutical can be determined through structural and compositional analyses. The microstructural images and elemental analysis of the liposomal curcumin and liposomal vitamin C formulations can be utilised in the development of design methods, as shown in Figs. 1–4 and Tables 1 and 2. The curcumin constituents, which possess significant inhibitory power include natural bioactives with hydrophobic properties. This is clearly presented in Fig. 1, which illustrates an imperfect sedimentary surface clarity, smooth oily, circular distributed hydrophobic physical structure, and an orderly illustrated particle image model. The refined peak patterns graph and data of the elemental constituents of the liposomal curcumin exhibited high carbon peaks with high carbon amount and oxygen element. The elemental constituent peak pattern analyses of liposomal curcumin revealed a high carbon and oxygen model. The liposomal

curcumin-vitamin C mixture components were methodologically formulated and characterised through electron microscopy analyses at a scale of 50 μm, as presented in Fig. 3, which displays the solid surface of the liposomal curcumin spherical particles. The elemental constituent peak pattern graph and data analyses of liposomal curcumin, as presented in Fig. 4 and Table 2, revealed carbon and oxygen elements, along with Mg, P, and K minerals.

The vitamin C component of liposomal curcumin was well characterised and methodologically formulated for the production of liposomal curcumin-vitamin C complex. This study clearly demonstrates the analogous relationships between the carbon and oxygen components of liposomal curcumin and liposomal-vitamin C formulations. The trace amounts of silica, chloride, aluminium, and iron, detected in the compositional analysis in Fig. 4 and Table 2, were recognised as the binding additive impurities found in the materials used in this study. The elemental component analysis indicated that no other unexpected compounds were observed in this study, and no perceptible harmful or biologically unsafe materials against food and drug regulations were determined.

4 Conclusions

The FEG-SEM analysis was used to reveal the nanoscale structural images and elemental components of liposomal curcumin and liposomal curcumin-vitamin C, facilitating the development of nutraceutical and nutritional design methods through structural characterisation and formulation analysis. The microstructural images of the liposomal curcumin showed oily surfaces and hydrophobic fine circular structures, while the elemental analysis results presented a high carbon and oxygen elements model. The liposomal curcumin-vitamin C microstructural analyses revealed solid surface spherical particles, and the elemental constituents included carbon and oxygen, as well as Mg, P, and K minerals. The novelty of this work lies in the combined analysis of structural images and elemental components using contemporary techniques. The outcomes of this study are projected to serve as a model for the formulation of the characteristic liposomal curcumin-vitamin C containing nutraceutical and nutritional products.

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SAŽETAK

Razvoj metode nutraceutskog i nutritivnog dizajna za liposomski kurkumin i vitamin C primjenom mikrostrukturne i elementarne analize

Yakup Ermurat

Biomolekularne komponente kurkumina snažni su inhibirajući nutraceutici s hidrofobnim karakteristikama. Vitamin C, antioksidans topljiv u vodi, ima ključnu ulogu u jačanju imunološkog sustava organizma. Liposomska inkapsulacija poboljšava topljivost i bioaktivnost kurkumina te ujedno povećava bioraspoloživost vitamina C. U ovoj studiji razvijena je funkcionalna metoda nutraceutskog i nutritivnog dizajna primjenom strukturne i elementarne analize pripremljenih nanočestica liposomskog kurkumina te liposomskog kurkumina i vitamina C. Mikroprikazi strukture nanočestica liposomskog kurkumina otkrili su njihovu hidrofobnu prirodu i odgovarajući strukturni model. Nanočestice liposomskog kurkumina i vitamina C, u suhoj formi, su sferične čestice čvrste površine. Elementarna analiza uzoraka liposomskog kurkumina ukazala je na visoke razine ugljika i kisika, a kod uzoraka liposomskog kurkumina i vitamina C na ugljik, kisik, magnezij, fosfor i kalij.

Ključne riječi
Liposomski kurkumin-vitamin C, elektronska mikroskopija, analiza strukture i sastava, bioraspoloživost

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