Application of Low Voltage Electron Microscopy in Multimodal Analysis of Nanoparticles in Food Matrices

Introduction

Nanotechnology is becoming increasingly important in various industries, particularly in the cosmetics and food sectors. Despite their widespread use, ensuring the safety and accurate quantification of both inorganic and organic nanoparticles remains a challenge, especially with the introduction of new regulations governing their use in food products. As regulatory measures take effect, the need for effective monitoring grows, driving the development of suitable analytical techniques for their detection and control. This study focuses on the application of a low-voltage transmission electron microscope (LVEM) equipped with energy-dispersive spectroscopy (EDS) to detect and characterize nanoparticles in food products [1, 2, 3].

Regulatory Perspectives on Nanomaterials in Food

To understand the broader context of this analytical need, it is necessary to consider the regulatory landscape shaping the requirements for nanoparticle monitoring in the food sector.

The use of nanomaterials in food has raised concerns about their potential impact on human health, prompting regulators worldwide to implement specific safety measures. Despite the widespread nanoparticles usage, the impact of NPs presence in food remain insufficiently understood.

A major challenge lies in establishing safety standards and developing reliable techniques to detect and quantify NPs in complex food matrices food. Thus, with upcoming legislative changes, there is an urgent need for advanced analytical techniques to enable reliable analysis to support regulatory efforts and ensure compliance and consumer safety.

In many countries, including the U.S., Canada, and Australia, nanomaterials are typically evaluated under existing food safety laws. While this approach ensures some level of oversight, the absence of detailed nano-specific regulations often creates gaps in risk assessment and monitoring [3].

In contrast, the EU has taken a more targeted approach. Regulation (EU) 2015/2283 mandates pre-market approval, risk assessment, and labeling for food nanomaterials [4]. In 2022, the EU revised its nanomaterial definition to include materials with 50% or more particles in the 1–100 nm range [5]. However, this update sparked controversy, with some advocating for a lower threshold of 10% to improve consumer protection [6, 7].

EFSA's 2021 guidance emphasizes the detailed characterization of nanomaterials, focusing on size, aggregation, and solubility [8]. As the international debate on the regulation of nanomaterials in food continues, one thing is clear: accurate characterization methods are essential for both monitoring and enforcement. Analytical tools like LVEM are not only vital for regulatory compliance but also for fostering scientific understanding to inform future legislative decisions and innovations in the food industry.

The Role of Nanotechnology in Food Products

The integration of nanotechnology into food products has brought both opportunities and challenges. Nanoparticles (NPs) offer benefits such as extended shelf life, improved taste, enhanced appearance, and increased nutritional value . However, concerns remain about health risks, unclear regulations and the need for better detection methods. Balancing safety and innovation remains a key challenge.

Among the most common nanoparticles are titanium dioxide (TiO_2), silicon dioxide (SiO_2), and silver (Ag), each serving a specific function. Titanium dioxide (E171) is a whitening agent found in products like chewing gum, candies, and dessert decorations, improving their appearance. However, safety concerns have led to restrictions in some countries, such as those in the European Union, due to potential health risks.

Another representative of NPs in the food industry is silicon dioxide (E551), which is widely used as an anti-caking agent to prevent clumping in powdered products such as instant drinks, nutritional supplements, and spice mixes. It also helps ensure a uniform distribution of flavorings and colorants. Studies have confirmed the presence of silica nanoparticles in various food products, highlighting their role in maintaining texture and consistency.

Silver (E174) is another additive commonly used in food products. Last but not least, silver (E174) is also added to food products. It is primarily used for decorative purposes, such as in silver-colored sugar pearls. In meat products, silver is valued for its antibacterial properties, which help inhibit bacterial growth and enhance preservation. While nanoparticles offer various benefits in food production, their potential health risks continue to be the subject of ongoing research.

Experimental Verification of Nanoparticles in Food Products

To verify the presence of nanoparticles in real-world food matrices, we selected commercially available decorative food additives that are commonly used as primary ingredients in the production of final food products. These included *Silver Sugar Pearls – Cake*, *Shimmering Deco Colored Lustre Dust*, and *Sugarflair Extra White Semi-Liquid Coloring.* [Figure 1].
The choice was motivated by their role as base components into which nanomaterials are deliberately added and which are subsequently incorporated into a wide range of consumer food items.

Sample preparation was intentionally simplified to preserve the native morphology of the particles and reflect realistic analytical conditions. Portions of the products were suspended in either distilled water or ethanol to extract and disperse the potential nanomaterials. The suspensions were then drop-cast onto carbon-coated copper TEM grids and air-dried. To improve dispersion and reduce particle agglomeration, selected samples underwent ultrasonic treatment prior to deposition.

No staining, filtration, or additional chemical modification was applied. This simplified approach allowed for direct imaging and characterization of the nanoparticles in a form closely resembling their state within the original product, enabling reliable evaluation of their morphology, size, and distribution within the food-related matrix.

Figure 1: Food products used in the experimental part (from left to right): Silver Sugar Pearls – Cake; Shimmering Deco Colored Lustre Dust; Sugarflair Extra White Semi-Liquid Coloring.

Methods

Although LVEMs are well known, particularly for high-contrast imaging of samples composed of light elements, the new setup of the LVEM 25E—often referred to as an "all-in-one" device—has expanded its application areas due to its versatility.

This instrument offers a comprehensive portfolio of imaging modes: Transmission Electron Microscopy (TEM) operating at an accelerating energy of 25 keV, Scanning Transmission Electron Microscopy (STEM) operating at 10 keV or 15 keV, and Scanning electron microscopy (SEM) with detection of backscattered electrons (BSE) operating at 15 keV. All transmission modes are capable of bright field and dark field imaging.

The versatility of the instrument is supported by analytical modes, including energy dispersive spectroscopy (EDS) for elemental analysis with mapping capability, and electron diffraction (ED) for crystallography. The combination of all of these modes makes LVEM 25E an optimal solution for multimodal imaging.

This multimodality opens new doors in materials science and, thanks to its user-friendly design, makes the instrument highly suitable for routine nanoparticle characterization. Its ease of use and analytical capabilities make it particularly valuable for applications such as detecting and analyzing nanoparticles in food products, an area of growing research interest.

Results

Nanoparticles were successfully detected in all tested food samples, in accordance with the substances listed

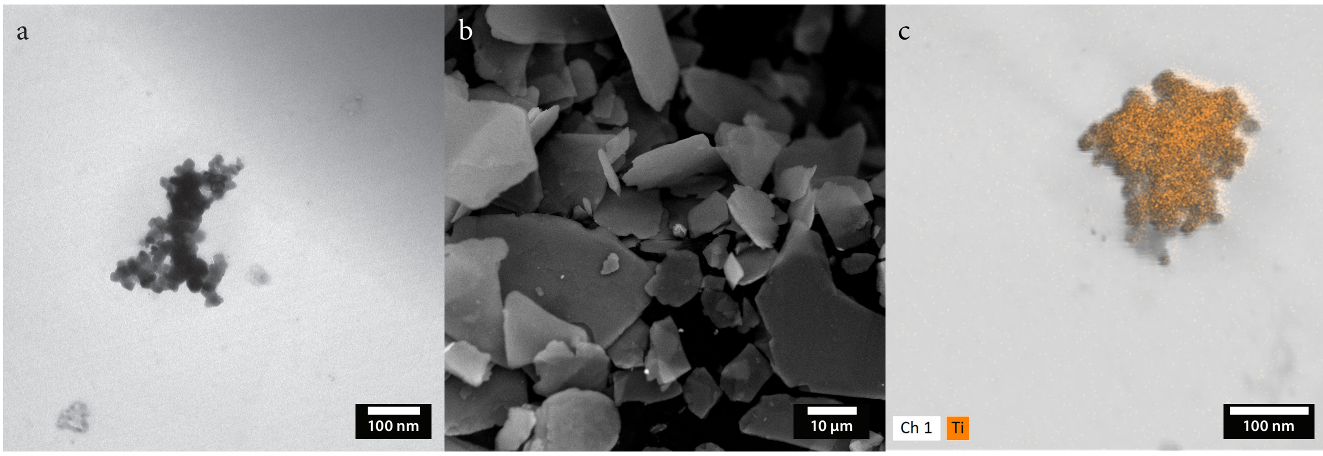

Figure 2: Analysis of Shimmering Deco Colored Lustre Dust performed using the LVEM 25E microscope. The presence of titanium dioxide nanoparticles (E171), declared in the product's composition, was confirmed through elemental mapping using STEM EDS (c). The particles were visualized in TEM mode (a), while the surface morphology of the native powder form was observed using SEM mode (b). The LVEM 25E enabled all these complementary imaging and analytical techniques within a single instrument.

in their ingredient declarations. Using LVEM 25E equipped with energy-dispersive X-ray spectroscopy (EDS), the nanoparticles were not only visualized but also chemically confirmed, reliably distinguishing them from the complex background of the food matrix.

In each sample, EDS analysis provided clear elemental identification of the nanoparticles, verifying their presence and composition with high specificity. When necessary, a combination of imaging modes was employed to obtain complementary structural information. TEM imaging was used for visualizing the smallest nanoparticles, while SEM with BSE detection provided insight into the bulk structures and surface morphology. For rapid elemental verification, standard TEM-EDS proved highly effective due to its high signal-to-noise ratio and fast peak acquisition. For precise particle-level identification, EDS mapping in STEM mode was applied to transparent samples, and SEM-EDS mapping was used for bulk samples [Figure 2].

In all food products tested, the nanoparticles declared by the manufacturer were confirmed. Notably,

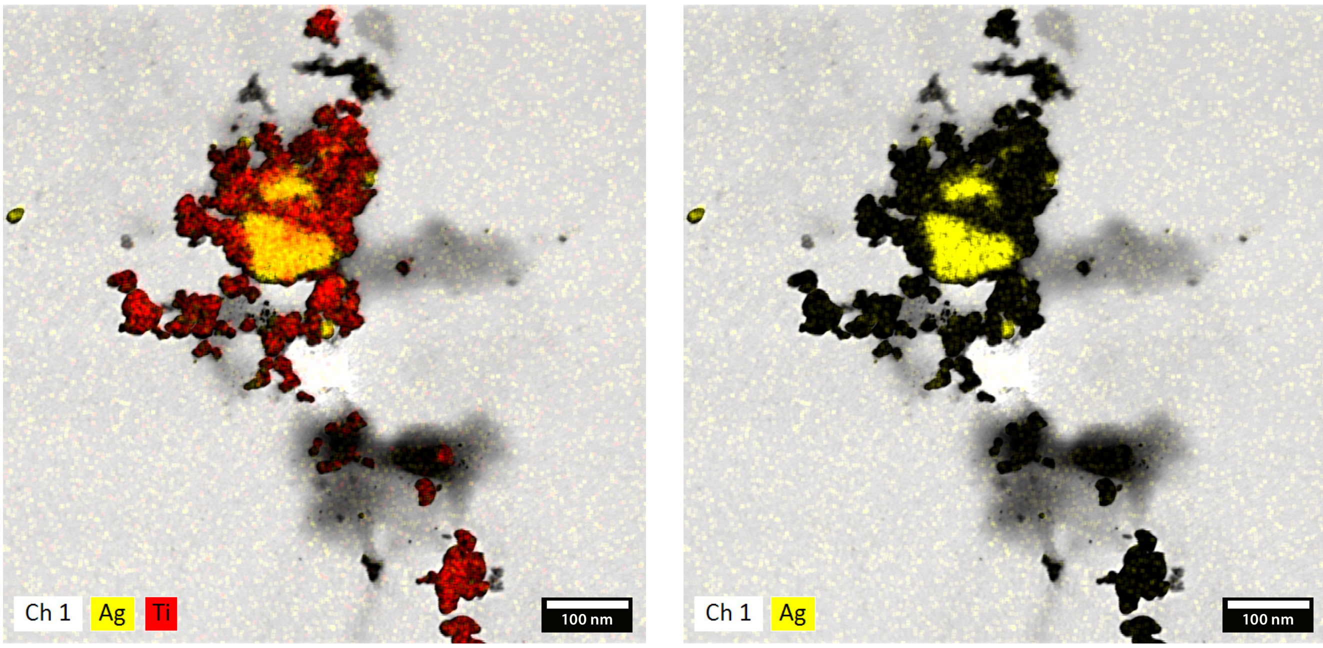

Figure 3: EDS STEM elemental mapping (LVEM 25E) confirming the presence of silver (yellow) and titanium (red) nanoparticles in Silver Sugar Pearls – Cake. While silver presence was expected, titanium was also detected despite not being listed among the ingredients.

in the case of the *silver sugar pearls*, in addition to silver—which was expected—titanium was also detected, even though it was not listed among the ingredients and is no longer permitted for use in food applications. This unexpected finding highlights the sensitivity of the method and raises regulatory concerns [Figure 3].

Overall, the results demonstrate that LVEM-based methods combined with EDS analysis allow for the effective and reliable detection of nanoparticles in complex food samples. This approach offers both rapid screening and detailed compositional analysis, making it highly suitable for both research and regulatory applications.

Conclusion

Detection of nanoparticles in food is essential to ensure health safety, meet regulatory requirements, and maintain quality control. The results of this study clearly demonstrate that the LVEM 25E, equipped with EDS, provides an effective, fast, and accessible tool for nanoparticle characterization in food products. Its multimodal capability and user-friendly operation make it an excellent candidate for implementation in both research laboratories and routine regulatory and monitoring facilities. Notably, the detection of titanium in silver sugar pearls—despite it not being listed among the ingredients and no longer permitted for use in food—underscores the high sensitivity of this method and highlights its potential to identify unauthorized substances, raising important regulatory concerns.

References:

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