

# Detection, Stability & Safety Evaluation of Titanium Dioxide (TiO<sub>2</sub>) Nanoparticles in Commercial Beverage

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## ABSTRACT

Titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) are widely used in the food and beverage industry as whitening, brightening, and opacifying agents. In commercial beverages, these nanoparticles are often added to improve appearance, texture, and product stability. However, growing concerns regarding nanoparticle toxicity, bioaccumulation, and long-term human exposure have attracted significant scientific attention. The present research paper focuses on the detection, stability analysis, and safety evaluation of titanium dioxide nanoparticles in commercial beverages. Various analytical techniques such as UV-Visible spectroscopy, Dynamic Light Scattering (DLS), Scanning Electron Microscopy (SEM), Transmission Electron Microscopy (TEM), X-ray Diffraction (XRD), and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) are commonly employed for nanoparticle characterization and quantification. Stability studies investigate the influence of pH, temperature, storage duration, and beverage composition on nanoparticle aggregation and sedimentation behavior. Safety evaluation involves assessment of cytotoxicity, oxidative stress, genotoxicity, and gastrointestinal absorption.

**Keywords:** Titanium dioxide nanoparticles, commercial beverages, nanoparticle detection food safety, toxicity

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## INTRODUCTION

Nanotechnology has emerged as an important field in food science and technology. Nanoparticles possess unique physicochemical properties due to their extremely small particle size, large surface area, and enhanced reactivity. Among various engineered nanoparticles, titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) are extensively used in food products, pharmaceuticals, cosmetics, and beverages.

Titanium dioxide is commonly used as a food additive under the code E171. It acts as a whitening and brightening agent in confectioneries, dairy products, chewing gums, sauces, and beverages. In commercial beverages, TiO<sub>2</sub> nanoparticles improve opacity, color consistency, and shelf appearance. Due to their nanoscale dimensions (1–100 nm), these particles exhibit improved dispersion and optical properties compared to bulk titanium dioxide.

Despite their industrial benefits, concerns have arisen regarding the safety of TiO<sub>2</sub> nanoparticles. Recent toxicological studies suggest that nanoparticles may cross biological barriers, accumulate in tissues, and induce cellular toxicity. Continuous consumption through beverages may increase human exposure, especially among children and adolescents. The present study discusses the detection methods, stability behavior, and safety assessment of titanium dioxide nanoparticles in commercial beverages.

### **Titanium Dioxide Nanoparticles(TiO<sub>2</sub>-NPs)**

Titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) are among the most widely used engineered nanomaterials in the food, pharmaceutical, cosmetic, and beverage industries. Due to their unique optical, chemical, and physical properties, these nanoparticles are extensively utilized as whitening agents, opacifiers, stabilizers, and coloring additives. In commercial

beverages, titanium dioxide nanoparticles improve product appearance, brightness, texture, and shelf stability. Their nanoscale size provides enhanced surface activity and better dispersion compared to conventional bulk titanium dioxide.

Titanium dioxide exists mainly in three crystalline forms:

1. Anatase
2. Rutile
3. Brookite

Among these forms, anatase and rutile are the most commonly used in food and beverage applications. Anatase possesses strong photocatalytic activity and excellent UV absorption capability, whereas rutile exhibits higher chemical stability and lower photoreactivity. Brookite is comparatively less stable and rarely used commercially.

TiO<sub>2</sub> nanoparticles possess several important physicochemical properties that make them suitable for industrial applications. These include:

- High refractive index
- Excellent whiteness and brightness
- Strong UV absorption capability
- Chemical and thermal stability
- Corrosion resistance
- Low toxicity in bulk form
- Low production cost

Because of these characteristics, titanium dioxide nanoparticles are commonly incorporated into commercial beverages, confectioneries, dairy products, protein supplements, chewing gums, sauces, and processed food products.

The nanoscale size of TiO<sub>2</sub> particles usually ranges between 1 and 100 nanometers. At this size, the particles exhibit increased surface area and enhanced reactivity, which contribute to improved optical and stabilizing functions in beverages. However, the same nanoscale properties that provide industrial benefits may also raise concerns regarding toxicity, biological interaction, and long-term health effects.

### **Material and Methods of Study**

Titanium dioxide nanoparticles may enter beverages through various intentional and unintentional sources. The major sources include:

#### **Processing Equipment**

Industrial beverage manufacturing equipment may contain titanium dioxide-coated surfaces or components. During processing, cleaning, or mechanical abrasion, nanoparticles can contaminate beverages.

#### **Contamination During Manufacturing**

Cross-contamination during production, transportation, or storage may introduce TiO<sub>2</sub> nanoparticles into beverages unintentionally. Poor quality control and inadequate purification systems may further contribute to contamination.

Commercial beverages that may contain detectable levels of titanium dioxide nanoparticles include:

- Milk and dairy beverages
- Fruit concentrates and juices
- Protein supplements
- Energy drinks
- Flavored soft drinks
- Nutritional beverages
- Sports drinks

The concentration of TiO<sub>2</sub> nanoparticles varies depending on product formulation, manufacturing practices, and storage conditions.

#### **Detection of Titanium Dioxide Nanoparticles**

Detection and characterization of titanium dioxide nanoparticles are essential for quality control, regulatory compliance, and toxicological evaluation. Due to the complex composition of beverages, accurate detection requires advanced analytical techniques capable of identifying nanoparticle size, morphology, concentration, and crystalline structure.

## UV–Visible Spectroscopy

UV–Visible spectroscopy is a simple, rapid, and cost-effective analytical method used for the preliminary identification of titanium dioxide nanoparticles in beverages. It is based on the interaction between ultraviolet or visible light and nanoparticles suspended in solution.

### Principle

Titanium dioxide nanoparticles strongly absorb ultraviolet light in the wavelength range of approximately 250–400 nm due to electronic excitation. When UV radiation strikes TiO<sub>2</sub> nanoparticles, electrons in the valence band absorb energy and move to the conduction band, producing characteristic absorption peaks.

The absorption behavior depends on:

- Particle size
- Crystal structure
- Surface properties
- Concentration of nanoparticles

Anatase TiO<sub>2</sub> generally exhibits absorption peaks around 320–350 nm, whereas rutile particles absorb at slightly different wavelengths.

### Procedure

The detection of TiO<sub>2</sub> nanoparticles in beverages using UV–Visible spectroscopy involves several important steps:

#### Sample Preparation

Commercial beverage samples are first prepared for analysis by removing interfering substances.

Steps include:

- Degassing carbonated beverages
- Dilution with distilled water
- Filtration or centrifugation to remove large particles
- Sonication to disperse nanoparticle aggregates

Proper sample preparation is essential for obtaining reliable absorbance measurements.

#### Spectrophotometric Analysis

Prepared samples are transferred into quartz cuvettes and analyzed using a UV–Visible spectrophotometer. The instrument scans the sample across a wavelength range typically between 200 and 800 nm.

Characteristic absorbance peaks observed within the UV region indicate the presence of titanium dioxide nanoparticles.

#### Data Interpretation

The absorbance spectra are compared with standard TiO<sub>2</sub> nanoparticle reference spectra. Variations in peak intensity and wavelength help estimate:

- Nanoparticle concentration
- Particle aggregation
- Optical stability
- Crystalline behavior

The Beer–Lambert law is often applied to correlate absorbance with nanoparticle concentration.

#### Advantages of UV–Visible Spectroscopy

UV–Visible spectroscopy offers several benefits for nanoparticle analysis:

##### 1. Rapid Analysis

The method provides quick results within a short period, making it suitable for routine screening of commercial beverages.

##### 2. Cost-Effective

Compared to advanced microscopy and spectrometric techniques, UV–Visible spectroscopy is relatively inexpensive and requires minimal operational cost.

##### 3. Non-Destructive Technique

The analysis does not significantly alter or destroy the beverage sample, allowing further characterization using additional techniques.

##### 4. Simple Operation

The instrumentation is easy to operate and does not require highly specialized technical expertise.

### 5. Preliminary Screening Capability

UV–Visible spectroscopy is useful for preliminary identification before advanced confirmatory analysis such as TEM or ICP-MS.

#### Limitations of UV–Visible Spectroscopy

Despite its advantages, UV–Visible spectroscopy has several limitations in nanoparticle detection.

##### 1. Limited Specificity

UV absorption peaks may overlap with pigments, proteins, vitamins, and other compounds present in beverages, making it difficult to confirm nanoparticle identity with complete certainty.

##### 2. Interference from Beverage Components

Colored beverages, sugars, preservatives, and flavoring agents may interfere with spectral measurements and reduce analytical accuracy.

##### 3. Inability to Determine Exact Morphology

UV–Visible spectroscopy cannot provide direct information regarding particle shape, surface morphology, or crystalline structure.

##### 4. Difficulty in Detecting Low Concentrations

Very low concentrations of TiO<sub>2</sub> nanoparticles may not produce detectable absorbance signals.

##### 5. Aggregation Effects

Nanoparticle aggregation may alter absorbance behavior and produce inaccurate results.

#### Importance of TiO<sub>2</sub> Nanoparticle Detection

Monitoring titanium dioxide nanoparticles in beverages is important for several reasons:

- Ensuring consumer safety
- Maintaining product quality
- Compliance with food safety regulations
- Toxicological risk assessment
- Evaluation of nanoparticle stability
- Detection of contamination sources

With increasing public concern regarding nanoparticle toxicity, reliable analytical methods are essential for regulatory monitoring and scientific investigation.

#### Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

ICP-MS is highly sensitive for titanium quantification.

##### Advantages

- Trace-level detection
- High sensitivity
- Quantitative analysis

This method is widely used for regulatory and toxicological studies.

#### Stability Evaluation of TiO<sub>2</sub> Nanoparticles

Stability analysis is essential because nanoparticle behavior changes during storage and consumption.

#### Factors Affecting Stability

##### 1. pH

Beverage acidity significantly influences nanoparticle aggregation.

- Low pH may increase particle dissolution.
- Neutral pH favors stable dispersion.

Acidic fruit beverages often promote nanoparticle agglomeration.

##### 2. Temperature

Temperature affects nanoparticle kinetics and stability.

- High temperatures accelerate aggregation.

- Cold storage improves dispersion stability.

Storage conditions therefore influence nanoparticle behavior.

### 3. Ionic Strength

Minerals and salts present in beverages affect electrostatic interactions.

High ionic concentration may reduce repulsive forces, leading to aggregation and sedimentation.

### 4. Storage Duration

Long-term storage may alter nanoparticle size and dispersion.

Observed changes include:

- Particle sedimentation
- Agglomeration
- Reduced optical stability

### 5. Presence of Organic Molecules

Sugars, proteins, and polysaccharides interact with nanoparticles.

These biomolecules may:

- Stabilize nanoparticles
- Form nanoparticle-protein corona
- Influence bioavailability

## Safety Evaluation of TiO<sub>2</sub> Nanoparticles

The safety assessment of TiO<sub>2</sub> nanoparticles is an important area of research.

### 1. Cytotoxicity

Studies suggest TiO<sub>2</sub> nanoparticles may damage cells under certain conditions.

#### Mechanism

- Generation of reactive oxygen species (ROS)
- Oxidative stress induction
- Lipid peroxidation

Excess ROS may damage proteins, lipids, and DNA.

### 2. Genotoxicity

Genotoxic effects involve damage to genetic material.

#### Reported Effects

- DNA strand breaks
- Chromosomal abnormalities
- Mutation induction

Several in vitro studies have demonstrated potential genotoxic effects of TiO<sub>2</sub> nanoparticles.

### 3. Gastrointestinal Toxicity

Since beverages are consumed orally, gastrointestinal exposure is significant.

#### Potential Effects

- Intestinal inflammation
- Altered gut microbiota
- Increased intestinal permeability

Nanoparticles may penetrate intestinal barriers and enter systemic circulation.

### 4. Bioaccumulation

Long-term exposure may lead to accumulation in organs such as:

- Liver
- Spleen
- Kidney
- Brain

Animal studies suggest chronic exposure may increase tissue deposition.

### 5. Immunotoxicity

TiO<sub>2</sub> nanoparticles may influence immune responses.

Reported findings include:

- Cytokine release
- Inflammatory response
- Immune cell activation

Further studies are needed to clarify long-term immune effects.

### Regulatory Perspectives

Different countries have adopted varying regulations regarding TiO<sub>2</sub> nanoparticles in food products.

#### European Union

The European Food Safety Authority concluded that titanium dioxide can no longer be considered completely safe as a food additive due to uncertainties regarding genotoxicity.

Several European countries have restricted or reassessed the use of E171 in food products.

#### United States

The U.S. Food and Drug Administration permits limited use of titanium dioxide in food products under specified concentration limits.

#### India

In India, food additive regulation is supervised by the Food Safety and Standards Authority of India. Continuous monitoring and updated toxicological guidelines are required for nanoparticle-containing beverages.

### Experimental Approach for Research Study

#### Sample Collection

Commercial beverages may include:

- Soft drinks
- Milk-based beverages
- Energy drinks
- Fruit juices
- Protein beverages

Samples should be collected from different manufacturers.

#### Sample Preparation

- Degassing of carbonated beverages
- Filtration and centrifugation
- Dilution using distilled water
- Digestion for elemental analysis

#### Analytical Workflow

The analytical workflow for the detection and characterization of titanium dioxide nanoparticles (TiO<sub>2</sub>-NPs) in commercial beverages involves a combination of spectroscopic, microscopic, and elemental analysis techniques. These methods provide comprehensive information regarding nanoparticle presence, size, morphology, crystalline structure, and concentration.

##### 1. Preliminary Screening by UV–Visible Spectroscopy

The first step involves preliminary screening using UV–Visible spectroscopy. Beverage samples are diluted, filtered, and analyzed within the wavelength range of 200–800 nm. TiO<sub>2</sub> nanoparticles exhibit characteristic absorption peaks between 250–400 nm due to electronic transitions. This method is rapid, non-destructive, and useful for initial nanoparticle identification. It also helps evaluate optical stability and possible aggregation behavior.

##### 2. Particle Size Analysis Using Dynamic Light Scattering (DLS)

After preliminary detection, Dynamic Light Scattering (DLS) is employed to determine particle size distribution and hydrodynamic diameter. DLS measures fluctuations in scattered light caused by Brownian motion of nanoparticles suspended in liquid. This technique provides valuable information regarding nanoparticle aggregation, dispersion stability, and colloidal behavior in beverage matrices.

##### 3. Morphological Characterization Using SEM/TEM

Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used for detailed morphological analysis. SEM provides surface imaging and particle distribution patterns, whereas TEM offers high-resolution

visualization of particle size, shape, and internal structure. These techniques help distinguish nanosized particles from bulk titanium dioxide aggregates.

#### **4. Crystal Analysis by X-Ray Diffraction (XRD)**

XRD analysis identifies the crystalline phases of titanium dioxide nanoparticles, such as anatase and rutile. The diffraction patterns generated by crystalline structures provide information regarding crystal size, purity, and structural arrangement.

#### **5. Quantification Using ICP-MS**

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is finally used for sensitive quantification of titanium content. This technique detects trace levels of nanoparticles with high precision and accuracy, making it suitable for toxicological and regulatory studies.

#### **Challenges in Detection**

The detection and characterization of titanium dioxide nanoparticles in commercial beverages present several analytical and methodological challenges. The complexity of beverage formulations often interferes with accurate nanoparticle identification and quantification.

Commercial beverages contain sugars, proteins, fats, preservatives, pigments, flavoring agents, and minerals. These components may interfere with analytical signals, causing difficulties in nanoparticle separation and characterization. Colored beverages especially complicate spectroscopic measurements due to overlapping absorbance spectra.

TiO<sub>2</sub> nanoparticles are often present at very low concentrations, making their detection difficult. Trace-level analysis requires highly sensitive instruments such as ICP-MS and advanced sample preparation procedures. Minor analytical errors may significantly affect quantitative accuracy.

Nanoparticles tend to aggregate or agglomerate during storage, sample preparation, or instrumental analysis. Aggregation changes particle size distribution and optical properties, leading to inaccurate characterization results. Variations in pH, ionic strength, and temperature further influence aggregation behavior.

Each analytical technique has certain limitations. UV-Visible spectroscopy lacks specificity, DLS cannot distinguish irregular particle shapes accurately, and microscopy techniques require expensive instrumentation and technical expertise. Sample preparation for SEM and TEM may also alter nanoparticle structure.

Currently, there is no universally accepted standardized protocol for nanoparticle detection in beverages. Differences in extraction methods, analytical parameters, and reporting standards make comparison between studies difficult. This lack of harmonization affects reproducibility and regulatory assessment.

These challenges highlight the need for advanced analytical methodologies, improved instrumentation, and internationally standardized testing procedures for accurate nanoparticle evaluation.

#### **Future Perspectives**

The growing use of titanium dioxide nanoparticles in food and beverage industries necessitates the development of improved analytical, toxicological, and regulatory approaches. Future research should focus on enhancing detection accuracy and ensuring long-term consumer safety.

One of the major future priorities is the establishment of internationally standardized analytical protocols for nanoparticle detection and characterization. Standardized methods would improve reproducibility, reliability, and comparability among scientific studies and regulatory assessments.

Further nano-toxicological investigations are required to understand the long-term biological effects of TiO<sub>2</sub> nanoparticles. Studies focusing on cellular uptake, oxidative stress, DNA damage, bioaccumulation, and intestinal absorption are essential for accurate risk evaluation.

Future technologies may enable real-time monitoring of nanoparticles during beverage manufacturing and storage. Advanced nanosensors and portable analytical devices could help detect contamination rapidly and improve industrial quality control systems.

Research should also focus on developing safer alternative whitening and stabilizing agents with lower toxicity risks. Natural pigments and biodegradable nanomaterials may serve as promising substitutes for titanium dioxide nanoparticles. Long-term epidemiological studies are necessary to evaluate chronic human exposure and possible health consequences associated with continuous consumption of nanoparticle-containing beverages. Such studies would provide stronger scientific evidence for regulatory decision-making.

Future regulatory frameworks should specifically address nanoparticle-related food safety concerns. Risk assessment models must consider nanoparticle size, surface chemistry, bioavailability, and cumulative exposure. Artificial intelligence (AI) and machine learning technologies may further revolutionize nanoparticle analysis by assisting in spectral interpretation, particle classification, toxicity prediction, and automated data processing. Integration of advanced computational tools with nanotechnology research may significantly improve food safety monitoring and toxicological assessment in the future.

## CONCLUSION

Titanium dioxide nanoparticles are extensively used in commercial beverages due to their whitening and stabilizing properties. However, increasing scientific evidence suggests possible health risks associated with chronic exposure to TiO<sub>2</sub> nanoparticles. Accurate detection and characterization techniques such as UV–Visible spectroscopy, DLS, SEM, TEM, XRD, and ICP-MS play a crucial role in evaluating nanoparticle presence and behavior. Stability studies reveal that pH, temperature, ionic strength, and storage conditions significantly influence nanoparticle aggregation and dispersion. Safety evaluation indicates potential cytotoxic, genotoxic, gastrointestinal, and immunological effects. Regulatory agencies worldwide are reassessing the safety of TiO<sub>2</sub> nanoparticles in food products. Therefore, comprehensive toxicological studies, strict regulatory guidelines, and continuous monitoring are essential to ensure consumer safety. The integration of advanced analytical technologies with toxicological research will help establish safer food and beverage systems in the future.

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