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Chapter 1

Titanium Dioxide as Food Additive

Marie-Hélène Ropers, Hélène Terrisse,
Muriel Mercier-Bonin and Bernard Humbert

Additional information is available at the end of the chapter

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Abstract

Titanium dioxide is a white metal oxide used in many food categories as food additives to provide a whitening effect. If its use complies with the five specifications including synthesis pathway, crystallographic phase, purity, amount and innocuousness, all other parameters are not defined and were hardly documented. However, in the last 3 years, two studies have deeply characterized food-grade TiO_2 and converged to the fact that the size distribution of food-grade TiO_2 spans over the nanoparticle range (<100 nm) and the surface is not pure TiO_2 but covered by phosphate and eventually silicon species or aluminium species, which modify the surface chemistry of these particles. Until now, this material was considered as safe. However, the toxicological studies later to the last re-evaluation by the European Food Safety Agency reveal some concerns due to the ability of TiO_2 particles to alter the intestinal barrier. This reinforces the idea to go on reinforcing the risk assessment about food-grade TiO_2.

Keywords: TiO_2, anatase, food, E171, food additive, food safety, intestinal barrier

1. Introduction

Titanium dioxide (TiO_2) is a transition metal oxide with two main applications as either pigment or photocatalyst, in many sectors including buildings (self-cleaning windows, cements, paints and anti-fouling paints), paper industry, cosmetics (sunscreens and tooth paste), pharmaceutics (tablets), food (colouring agent) and others (air-purification system, rubbers, inks and ceramics). Pigmentary applications are by far the most important application of TiO_2. The interest in TiO_2 lies in the scattering of visible light controlled by its high refractive index and its granulometry (size and shape). These requirements also apply for food where TiO_2 provides a whitening effect. Although this compound has been used for a very long time

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in food products (with a recent re-approval for a permitted use in food by the European Food Safety Agency), the use of titanium dioxide in food has risen some concerns in Western populations due to the presence of nanoparticles, that is, particles having one or more external dimensions in the size range of 1–100 nm. This review chapter targets an audience of practicing researchers, academics and PhD students, who are interested in the food applications of this compound and the reasons of controversy.

2. Use of TiO_2 in foods: function, properties and safety

Titanium dioxide is a food additive without any nutritive value and added in processed foods to provide a whitening effect. It was first approved for use in food by the United States Food and Drug Administration (FDA) in 1966, then by the European Union in 1969, on the basis of the Codex Alimentarius of the Food and Agriculture Organization/World Health Organization (FAO/WHO). When used as a food colouring, it is labelled as E171 in Europe or INS171 in USA. In other fields, it is also called titanium white, Pigment White 6 or CI 77891. Time to time, it was re-evaluated for minor revisions of specifications in 2006, 2009, 2010 and 2012. In particular, the European Union decided in 2006 to allow the crystalline structure rutile in food in addition to the former authorized form anatase (COMMISSION DIRECTIVE 2006/33/EC of 20 March 2006). Then, it was subjected to an in-depth evaluation in 2016 (EFSA 2016).

2.1. Food categories with permitted use of TiO_2

The food colours of Group II, including titanium dioxide, are authorized in most food categories,[1,2] such as (i) dairy products and analogues (flavoured fermented milk products and some creams), (ii) cheese and cheese products such as unripened cheese (Mozzarella, Codex Stan 262-2006 or fresh cheese, Codex Stan 221-2001), edible cheese rind, whey cheese, processed cheese, cheese products and dairy analogues including beverage whiteners, (iii) edible ices, (iv) confectionary (chewing gum, decorations, coatings and non-fruit-based fillings), (v) surimi and similar products and salmon substitutes, (vi) seasonings and condiments, mustard, soups and broths and sauces, and (vii) food supplements (Official Journal of the European Union, No 1129/2011). This list, despite its length, is in fact not exhaustive and the whole list with some restrictions of use is available on specialized websites.

Titanium dioxide was actually identified in chewing gums [1–3], confectionary [4, 5], sauces and dressings [5], non-dairy creamers [2, 5] and in dietary supplements [6]. According to a database collecting the details of new products (278,705) introduced on the market in 62 of the world's major economies, the use of TiO_2 increased constantly until 2014, representing a labelling on more than 3500 foods or drinks (Mintel GNPD database cited by the European Food Safety Agency EFSA [7]). If TiO_2 is found in only 1.3% of new products, it is nevertheless found in 51% of gums, 25% of stick, liquid and sprays, 21% of mixed assortments, 10% of

[1]https://webgate.ec.europa.eu/foods_system/.
[2]http://www.fao.org/gsfaonline/additives/index.html.

pastilles, gums, jellies and chews and 10% of lollipops [7]. Chewing gums and confectionary, including pastilles, gums, jellies and chews, are the most widely concerned food categories, both in number of products labelled with TiO_2 per category and in number of new products available on the market. Cakes and pastries represent a second category of importance. This scenario has to be regularly refined as the composition of food products may evolve [8, 9].

2.2. Levels of consumption

The amount of TiO_2 consumed in the USA on a daily basis was estimated around 0.2–0.7 mg of TiO_2 per kg of body weight per day (mg/kg bw/d), while the UK and German populations consume around 1 mg TiO_2/kg bw/day [4, 10]. These data were refined for all food categories, subpopulations and exposure scenarios in Netherlands [11, 12], in Germany [10] and in Europe [7]. For example, the estimate of the median long-term exposure to titanium dioxide (E 171) ranges from 0.5 (upper limit 1.1 mg/kg bw/d) for elderly adults to 1.4 mg/kg bw/d (upper limit 3.2 mg/kg bw/d) for children in Netherlands [12], close to the estimate in Germany [10].

Whatever the scenario of exposure and methodological choices, the biggest consumers of TiO_2 are children (3–9 years) and teenagers (10–17 years) [4, 7, 10–12]. In the scenario exposure of EFSA, the contribution of chewing gums is weak in comparison to other confectionary including breath-refreshing microsweets, or sauces, salads and savoury-based sandwich spreads [7]. In the study based on the Dutch National Food Consumption Survey, the products most contributing to TiO_2 intake for young children (2–6-year-olds) are confectionary (sweets, chocolate products and chewing gums) and fine bakery wares (biscuits). For 7–69-year-olds and elderly (70+), the same food items are identified but in a different decreasing order: chewing gums, coffee creamers, sauces, then fine bakery wares. As 10 food items most contributing to TiO_2 intake represent 55%, we must keep in mind that TiO_2 intake is spread over many products, chewing gums contributing by only a few percentage points more than other food categories [11]. In a similar study performed in Germany, the food products that contribute the most to the total titanium intake by adults are savoury sauces, dressings, soft drinks and cheese (more than 75%) [10]. In addition to food products, tablets such as medicine and food supplements contain TiO_2 up to 3.6 mg/g [13], resulting in a higher total daily intake of TiO_2.

2.3. Specifications of TiO_2 for food applications

In addition to the respect of the permitted use in the above-mentioned food categories, the powder introduced in these food products must respect five criteria, namely synthesis pathways, structure, purity, amounts and, certainly, absence of toxicity (Commission Regulation (EU) No 231/2012 and Joint FAO/WHO Expert Committee on Food Additives (JECFA) [14]). Firstly, these criteria are described according to the recommended specifications, then they are commented and discussed with literature data.

2.3.1. Synthesis: sulphate and chloride processes

Depending on the desired crystalline phase, titanium dioxide is produced by either the sulphate or the chloride process. The anatase phase of titanium dioxide can only be made by

the sulphate process, while the rutile phase of titanium dioxide can be obtained from both processes but the chloride process is more sustainable and provides crystals with a narrower particle size distribution than the sulphate process [15].

Briefly, in the sulphate process, sulphuric acid is used to digest the ilmenite ore ($FeTiO_3$ or FeO/TiO_2) into iron(II) sulphate and titanium salt ($Ti(SO_4)_2$). Iron(II) sulphate is removed from the liquor after dilution and crystallization/filtration to yield only the titanium salt ($Ti(SO_4)_2$) in the digestion solution. Then, some microcrystals of anatase are introduced into the liquor which is then hydrolysed under carefully controlled conditions to produce crystals of anatase. These are subsequently filtered, washed, calcined and micronized [13, 15, 16]. The chloride process, which generates rutile crystals, consists of a chlorination of the ore into titanium and iron chlorides which are then separated by distillation. Titanium chloride is then treated to remove impurities and oxidized in a controlled flame reactor to yield TiO_2 rutile crystals with the desired size [15, 16]. In addition, titanium dioxide may be coated with small amounts of alumina and/or silica to improve the technological properties of the product, which are described as blocker for photocatalytic activity [14].

Certain rutile grades of titanium dioxide as platelet form are produced using mica as a template. The specific properties of this pigment (interference colour) are controlled by the thickness of the coated titanium dioxide layer and by the coating process [13].

2.3.2. Crystallographic structure

Currently, E171 forms consist essentially of pure anatase and/or rutile. Until 2006, only the anatase form was authorized for food applications. Rutile has been authorized to replace anatase in food products especially in film coatings for food supplement tablets and foodstuffs [13]. In both anatase and rutile structures, the basic building block consists of a titanium atom surrounded by six oxygen atoms (**Figure 1**). The structures differ by the distortion and assembly of the octahedra [17]. In rutile, these octahedra are connected via their corners and edges (**Figure 1**) and the unit cell dimensions are $a = b = 4.587$ and c = 2.953 Å. For anatase, the octahedra are linked via edges and planes forming a unit cell with $a = b = 3.782$ and c = 9.502 Å (**Figure 1**). In each structure, the two bonds between the titanium and the apical oxygen atoms are slightly longer than the others (1.983 and 1.946 Å in the rutile structure, 1.966 and 1.937 Å in the anatase structure). Moreover, a sizeable deviation from a 90° bond angle was observed in anatase (92.6 and 102.3 Å, **Figure 1**).

Although both forms are authorized in foods, the characterization of samples in American and European laboratories shows that anatase is the predominant crystalline structure found in food applications [1, 3, 4, 18–20]. For example, five out of six chewing gums contained TiO_2 as anatase and only one contained a mixture of anatase and rutile [1]. Thus, the sulphate process seems to be predominant for obtaining pigmentary TiO_2 for food applications.

In the bulk structure, the titanium cations have a coordination number of 6 meaning the oxygen anions have a coordination number of 3 resulting from the trigonal planar coordination (**Figure 1**). But at the surface, anions and cations are said to be 'coordinatively unsaturated'. The lowly coordinated cations (Ti_{5c}) thus act as Lewis acids (electron pair acceptor) and are able to interact with electron donors like H_2O. Similarly, twofold-coordinated O atoms

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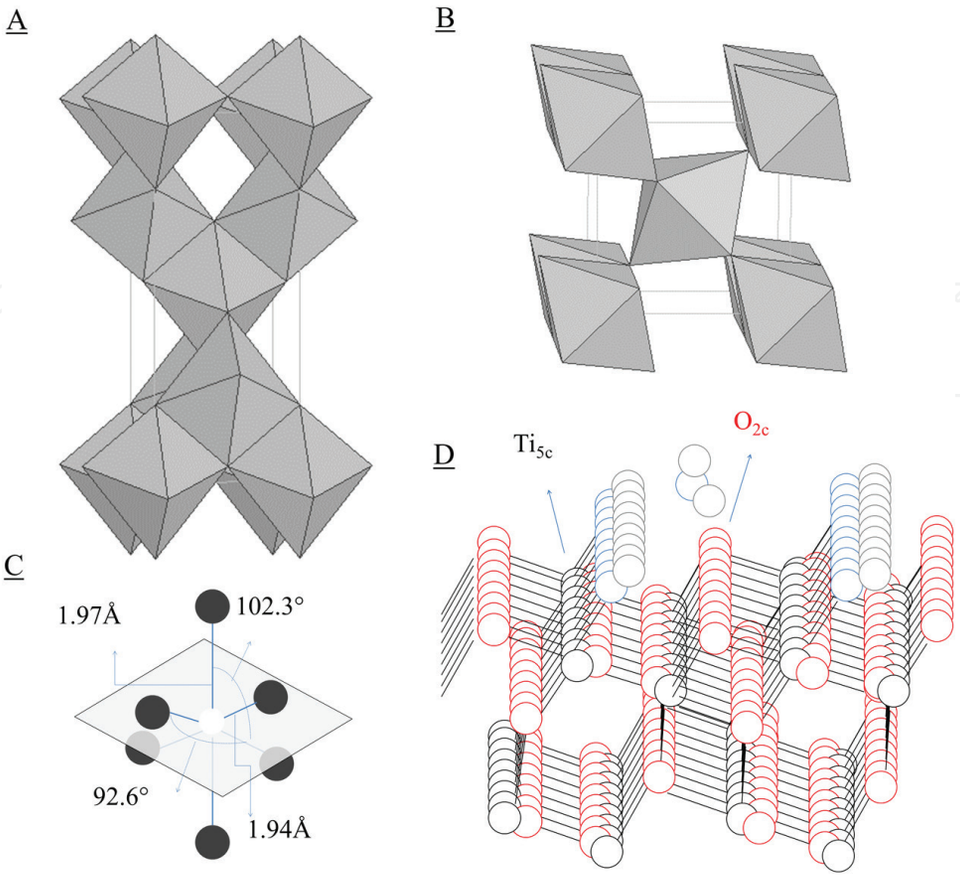

Figure 1. Bulk structures of (A) anatase, (B) rutile with (C) bond lengths and angles of the octahedrally coordinated Ti atoms in anatase, arranged from Diebold [17] and (D) arrangement of atoms on the (101) surface of anatase after adsorption and dissociation of water with Ti (grey filled balls), O from TiO_2 structure (empty balls), O from water (big hatched balls) and H from water (small hatched balls).

(O_{2c}) and named bridging oxygen atoms are Lewis base sites and are able to interact with electron acceptors like H^+. Thus, once the oxide surface is exposed to moisture present in the atmosphere, it becomes fully covered with adsorbed water and hydroxyl groups. Molecularly adsorbed water in vacancies partly dissociates to form two kinds of hydroxyl groups: (1) terminal hydroxyls which are adsorbed onto Ti_{5c} sites (TiOH) and (2) bridging hydroxyls which result from protonation of O_{2c} atoms (Ti_2OH) [21]. Surface hydroxyl groups are able to behave as Brønsted acid or base sites when TiO_2 particles are dispersed in water.

2.3.3. Purity

In Europe as well as in the USA, the content in titanium dioxide must be no less than 99.0% on an aluminium oxide and silicon dioxide-free basis (Commission Regulation (EU) No 231/2012) and the amount of alumina and/or silica must not exceed 2%. The investigated samples complied with these specifications [18–20, 22]. Additionally, the Commission specifies that the loss on drying must be lower than 0.5% (105°C, 3 h) and the loss on ignition must represent less than 1.0% (800°C) on the dried basis. The acid-soluble substances must represent less than 0.5% (less than 1.5% for products containing alumina or silica) and the water-soluble

matter must represent less than 0.5%. For impurities soluble in 0.5 N hydrochloric acid, their amount must be lower than 1 mg/kg for arsenic, cadmium and mercury, lower than 2 mg/kg for antimony and lower than 10 mg/kg for lead. These specifications are very similar to those given by JECFA [14].

2.3.4. Amounts

In Europe, titanium dioxide is authorized *at quantum satis*, whereas it is used in the USA in the limit of 1% by weight of food. Although no maximum use level is specified for this additive in Europe, it shall be used in accordance with the good manufacturing practices (GMPs), that is, at a level not higher than is necessary to achieve the intended technical effect. This decision was motivated by the fact that TiO_2 was considered as an inactive ingredient in human food, and that neither significant absorption nor tissue storage following the ingestion of TiO_2 was possible. In its last report, the Panel of EFSA concluded that definitive and reliable data on the reproductive toxicity of E 171 are not yet available to enable the Panel to establish an acceptable daily intake (ADI) [7].

The quantification of TiO_2 in commercial products indicates that chewing gums are the food products richest in titanium dioxide [2, 4]. They contain between 0.7 and 5.4 mg Ti/g of food. The next category is sweets with 0–2.5 mg Ti/g food, followed by pastry with 0–0.5 mg Ti/g food [2]. In the report of EFSA, including more numerous food categories and data provided by industry, the highest maximum level in TiO_2 is in decorations, coatings and fillings [7] with 20 mg TiO_2/g food which corresponds to 12 mg Ti/g food, a little bit above the maximum level reported for chewing gums (16 mg TiO_2/g, i.e., 9.6 mg Ti/g food). Considering the mean use level, it is a little bit higher in processed nuts (3.8 mg Ti/g food) than in chewing gums (3.4 and 2.8 mg Ti/g food, depending on manufacturers), food supplements (2.8 mg Ti/g food) and salads and savoury-based sandwich spreads (2.5 mg Ti/g food).

2.3.5. Innocuousness of TiO_2

Since the early 1960s, TiO_2 is considered as safe for use in food. Since this time, some authors called this fact into question [23]. In the recent re-evaluation of titanium dioxide (E171) as food additive [7], the EFSA Panel estimated that the absorption of orally administered TiO_2 particles, including micro- and nano-sized (less than 3.2% by mass) fractions, was negligible, reaching at most 0.02–0.1% of the administered dose. They also indicated that no adverse effect resulting from the eventual accumulation of the absorbed particles was expected, based on the results of long-term studies which did not highlight any toxicity up to the highest administered dose. The lowest value found in the literature for the no-observed adverse effect levels (NOAEL) was 2250 mg TiO_2/kg bw/d.

2.4. Other physicochemical properties of food-grade TiO_2

Titanium dioxide is insoluble in water, hydrochloric acid, dilute sulphuric acid and organic solvents. It dissolves slowly in hydrofluoric acid and hot concentrated sulphuric acid. It is almost insoluble in aqueous alkaline media (COMMISSION DIRECTIVE 2008/128/EC).

The physicochemical characteristics of particles, including morphology (spherical and cylindrical), size (smaller or larger than <100 nm), surface charge (negative, neutral or positive), structure (crystallinity), agglomeration (aggregates, agglomerates and primary particles) and surface composition, are assumed or demonstrated to play a role in nanoparticle uptake through the gut [24]. Therefore, the five criteria detailed before have to be completed by a deeper characterization of food-grade TiO_2, which unfortunately received much less attention.

2.4.1. Content in nanoparticles and size distribution

Considering the food use of TiO_2 as whitening agent, the size distribution is expected to be centred on a mean pigment size of 250 nm to obtain an optimal effect [25]. However, the mean size of food-grade TiO_2 is actually rather comprised between 106 and 145 nm and the size distribution spans between 30 and 300 nm [4, 18, 19, 26, 27] or 60 and 300 nm [2]. For example, several size distribution spans and mean sizes are reported in **Figure 2**. Overall, they span between 30 and 300 nm. In these batches, the fraction of nanoparticles (<100 nm) ranged from 17 to 36%. In the whole set of samples investigated in the literature, the nanoparticle size distribution expressed in number was always smaller than 50%. In chewing gums, this fraction mounts to 43.7% [1].

To determine the exposure scenario, the equivalent mass of NPs is more interesting. According to several studies, the mass (wt%) of nanoparticles present in E171 ranges between 0.31 and 12.5% [7, 10, 11, 18]. This explains some discrepancies in the different exposure to TiO_2 nanoparticles in the literature and, for example, the factor of 10 in the estimate of NP

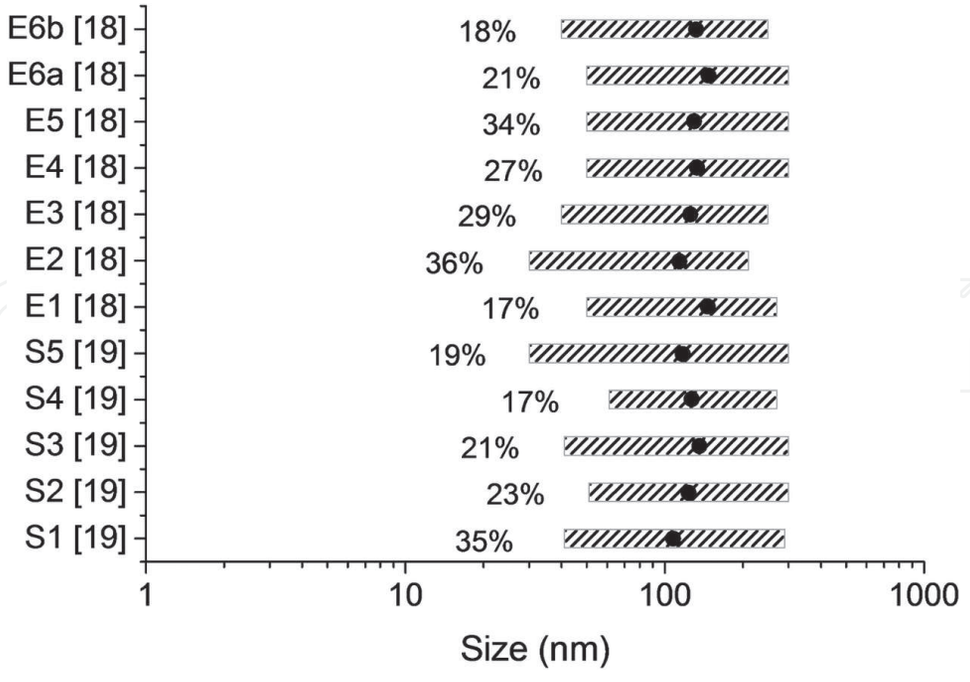

Figure 2. Size distribution (dashed rectangles), mean size (black dots) and percentage of nanoparticles in number (%) of food-grade TiO_2 particles characterized by (E) Dudefoi et al. [18] and (S) Yang et al. [19]. The mean sizes of the distribution vary between 106 and 145 nm.

consumptions between the study by Rompelberg et al. [11] who considered 0.31% of NPs and the evaluation of EFSA [7] who used a weight ratio of 3.2%.

2.4.2. Specific surface area

The specific surface area (SSA) of a material is defined as the total surface area of the material per unit of mass. It is reversely proportional to the size of the particles: the smaller the size of a material, the higher its specific surface area and its reactivity with the environment. The SSA is usually determined from the volumetric adsorption isotherms at 77 K of nitrogen gas followed by the Brunauer-Emmett-Teller (BET) adsorption treatment (the so-called N_2-BET isotherm) assuming a multilayer of adsorbates. The specific surface area of food-grade TiO_2 ranges between 8.6 and 10.7 m^2/g [18, 20] with an average of 9.3 m^2/g. These values are quite low in comparison to anti-caking agents, for example, which are around 200 m^2/g. This hints that TiO_2 offers a low contact surface with its environment.

2.4.3. Surface chemical composition

The surfaces of food-grade TiO_2 were found to be mainly covered by hydroxyl groups [18], phosphate groups [18, 19] and potassium ions [18]. Some phosphate groups may not be tightly bound to the surface and be released after washing [19]. In a few cases, TiO_2 was covered by silica [18] and alumina [19], thus modifying the surface chemistry.

2.4.4. Surface potential

As mentioned previously, surface hydroxyl groups, which behave as Brønsted acid or base sites, confer a charge to the particle surface. When TiO_2 particles are dispersed in an aqueous medium, this charge is mainly determined by two phenomena: protonation/deprotonation of surface hydroxyls controlled by pH and adsorption of electrolyte ions [28]. An electrostatic potential, exponentially decaying away from the surface, is associated to the overall charge distribution in the interfacial region. The experimental determination of this potential, called zeta potential, is generally performed by electrophoretic mobility measurements. All models converting electrophoretic mobility into zeta potential consider ideal spherical particles, which is a delicate assumption in the case of TiO_2 due to the formation of agglomerates with non-spherical particles (subsequent section). An improved model exists to convert electrophoretic mobility measurements to zeta potential values taking into account the effect of the agglomerate size and surface conductance of TiO_2 [29]. Zeta potential values depend not only on the parameters controlling the surface charges, namely, the nature of the medium where TiO_2 particles are dispersed (pH, ionic strength and adsorbed species [20]) but also on the primary particle size [29, 30] and the crystallographic face [31]. The point where the zeta potential is zero defines the isoelectric point (IEP).

The isoelectric point of food-grade TiO_2 samples measured by electrophoretic mobility measurements was found between 3 and 4 for most samples (**Table 1**), far below the classical value for anatase. Such a difference is interpreted by the presence of phosphate groups on the surface of TiO_2 particles [18, 19] or by silica coating [18], which decrease the isoelectric point

Reference	[18]	[20]	[19]
Experimental conditions	Ultrapure water, without fixing ionic strength	Ultrapure water, without fixing ionic strength	KNO_3 10^{-2} mol.L^{-1}
IEP	4.0 ≤ pH ≤ 4.2	pH = 5.1	3.2 ≤ pH ≤ 4.0
ζ at pH 7	−42 to −50 mV	−35 mV	−42 to −50 mV

Table 1. Isoelectric point (IEP) and zeta potential at pH 7 of various food-grade TiO_2 (E171) dispersed in water, without any protein.

towards lower pH values. It is interesting to note that the isoelectric point of a food-grade sample measured through electroacoustic measurements gave a value of 5.1 [20], close to the classical data for anatase. For all these samples, the zeta potential of their suspensions varies between −35 and −45 mV at a physiological pH value. Faust et al. compared the zeta potential of a food-grade TiO_2 and an extract of chewing gum, and observed that the gum extract presented a largely more negative potential (−45 mV at pH 7) than food-grade TiO_2 (−20 mV at pH 7), which may be due to coating of TiO_2 in chewing-gum formulation [26].

2.4.5. Agglomeration

The dispersion state of particles in aqueous solution is governed by the surface chemistry of the oxide and depends on the composition of the dispersion medium (pH, ionic strength, nature of electrolyte and presence of proteins). Traditionally, zeta potential measurements are used to assess the stability of colloidal dispersions: the higher the zeta potential absolute value, the more stable the dispersion. Around the IEP or when ionic strength is high in solution, the system is unstable and agglomeration of particles occurs, leading to settling of the suspension. It is thus important to consider agglomeration in the experimental medium, as this may alter the size of the particles which will be 'seen' later by the organism after ingestion.

In usual conditions of pH and ionic strength, TiO_2 particles tend to form large-sized agglomerates (particles relatively loosely bound) which settle after a few hours, partially due to the large density of TiO_2 (3.9 g/cm^3 for anatase as powder). For neutral pH values (around 6–7) and in the absence of any salt, E171 particles present agglomerates with a diameter of 200–400 nm, in agreement with the largely negative-measured zeta potential. When pH becomes closer to IEP, the measured diameter is larger than 1 μm, which is the sign of agglomeration due to low electrostatic repulsions [18].

Once particles are agglomerated or aggregated, they do not fragment easily and are difficult to disperse as primary particles. Ultrasound sonication can be used to break the agglomerates prior to zeta potential and size measurements, providing ultrasounds do not alter the surface chemistry of the material [32]. The hydrodynamic diameter of E171 particles dispersed in ultrapure water (pH not mentioned) and bath sonicated (for 5–30 min) comprises between 120 and 400 nm [19, 26]. Another possibility to stabilize the suspension and avoid agglomeration of particles consists in adding a dispersant which is able to cover the particles and create steric hindrance between them [33]. Bovine serum albumin (BSA) was typically used to stabilize E171 TiO_2 particles, in combination with ultrasound sonication (30 min),

leading to a mean hydrodynamic diameter of 150 nm [4]. In solutions added with salts (NaCl and $NaHCO_3$), E171 particles dispersed by sonication presented a moderate stability, with a particle size of agglomerates remaining between 360 and 390 nm for at least 2 h. The same experiment conducted with P25 sample showed rapid and extensive aggregation of the particles [4].

2.4.6. Specificities of food-grade TiO_2

Food-grade TiO_2 powders are finally characterized by a low specific surface area (around 10 m^2/g), a pure crystalline anatase phase (sometimes traces of rutile), a low isoelectric point (around 4.1 in water) related to the phosphate found at its surface, a mean size of 140 nm with a distribution spanning from 30 to 300 nm and a fraction of nanoparticles comprised between 17 and 36%. For toxicological studies, including toxicity assessment by oral exposure, another kind of TiO_2, called P25, is commonly used as it is considered as a reference material [34]. This compound is characterized by 100% NPs, a mean size of 23 nm, a specific surface area of 50 m^2/g, a mixture of anatase and rutile grains (85/15) and an isoelectric point at pH 6.5 [18, 19]. In **Figure 3**, some physical and chemical properties of E171 and P25 samples, extracted from two studies [18, 19], are reported.

The P25 samples clearly distinguish from E171 samples by all parameters taken into account. A peculiar sample of E171, rich in rutile phase, is observed as well. E171 TiO_2 being strongly different from the reference material P25, we thus concluded that P25 does not appear to be the most suitable reference material for toxicity studies by ingestion [18]. It is, moreover, not the most relevant material to represent the nanoparticle fraction of E171.

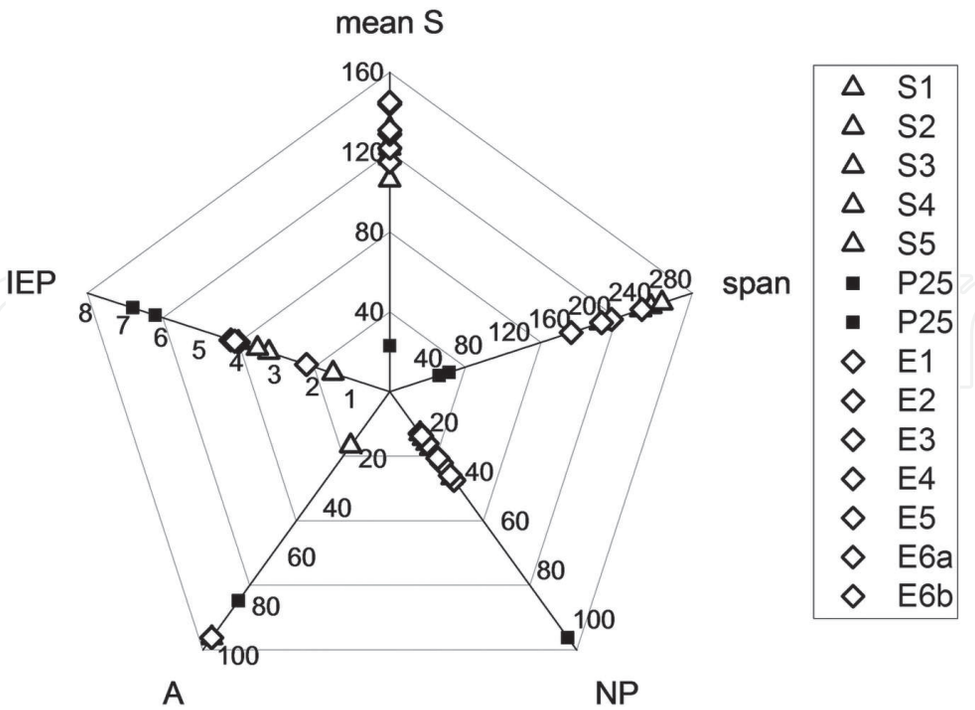

Figure 3. Physical and chemical parameters describing E71 and P25 forms of TiO_2, namely the content in NPs, isoelectric point (IEP), the mean size of the distribution deduced by transmission electron microscopy (mean S) and the span of the particle size distribution (span). Data come from references [8, 19].

2.5. Fate of TiO_2 after ingestion

Among the different routes of exposure to TiO_2, the oral uptake route remains the less documented. Once ingested, TiO_2 particles pass through the digestive tract, starting with the port of entry, the oral cavity followed by the gastrointestinal tract, comprising oesophagus, stomach, small and large intestines and rectum (**Figure 4**).

During the transit through the digestive fluids, TiO_2 particles were not metabolized and were found to be mainly agglomerated, mediated by proteins and electrolytes [35, 36], but according to some studies, a small fraction is still in the nanosized range [35, 37, 38]. The low absorption of TiO_2 and reversely the high percentage of titanium dioxide excreted from the body in faeces [39, 40] were believed to be the proof of any adverse effect. However, the recent data on the intestinal compartment call this belief into question. Indeed, the intestinal barrier, which involves epithelium, mucus and microbiota in its luminal side (**Figure 4**), provides a physical, chemical and biological line of defence for the host, probably through an orchestrated manner [41, 42]. Taken together or independently, these three partners exhibit some alterations due to the presence of TiO_2 particles, which are briefly reported from the microbiota to the epithelium.

2.5.1. TiO_2 in interaction with the intestinal microbiota

The effects of TiO_2 on the gut microbiota composition and metabolic activity in animal models or humans are largely unknown, whereas the intestinal microbiota contributes actively to the maintenance of host homeostasis. Indeed, it plays a key role in the gut, fulfilling protection, maturation and production functions. In particular, it acts as a barrier against pathogens, preventing their implantation, and participates in xenobiotic metabolism [43].

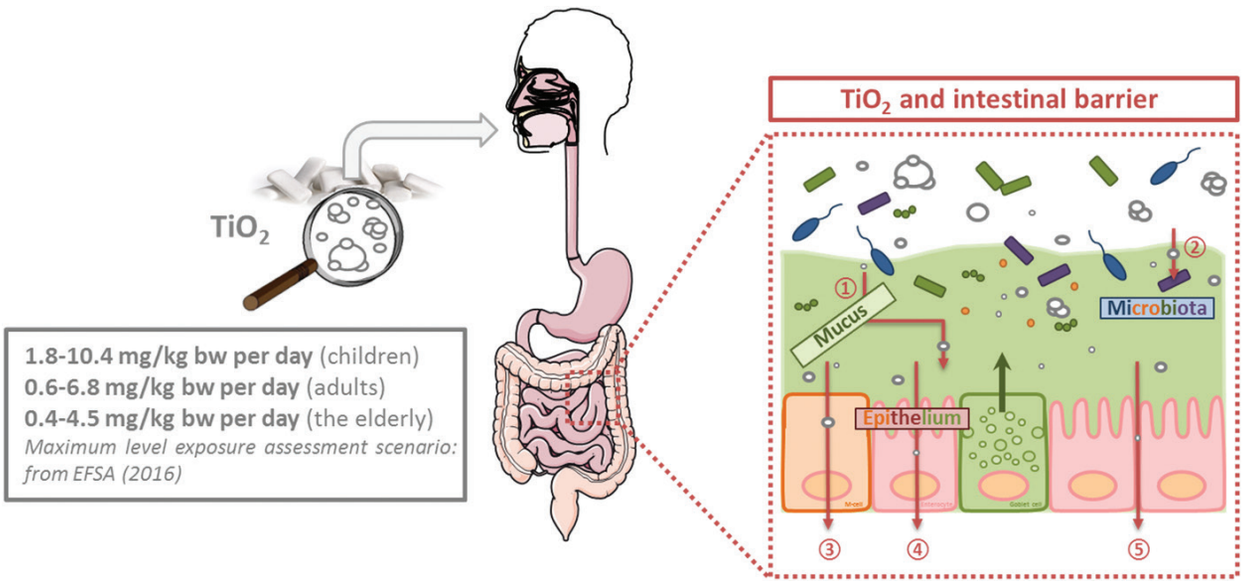

Figure 4. Schematic representation of the fate of TiO_2 within the digestive tract illustrating potential mechanisms by which ingested nanoparticles interact with the intestinal barrier; (1) mucus; (2) microbiota; (3–5) epithelium with (3) internalization and active transport to Peyer's patch lymphoid follicles by M-cells, (4) transcellular transport and (5) paracellular transport through intercellular tight junctions (intercellular space between adherent epithelial cells). The unrestricted migration through foci of damaged epithelium is not represented. For the sake of clarity, scheme is not to scale.

Studies reported to date were mainly focused on the antibacterial activity of TiO_2 nanoparticles in *in vitro* pure cultures using *Escherichia coli* as the bacterial representative [44–46]. Such an activity is generally associated with the photocatalytic effects of TiO_2, although increasing experimental evidence also demonstrated TiO_2-mediated cell alterations without UV illumination [50, 51]. Taylor et al. [47] investigated the *in vitro* exposure of a gut microbial community from a healthy donor to three different types of metal oxide nanoparticles, including TiO_2, in a model colon. Such exposure-induced changes in the phenotypic traits of the gut community, including short-chain fatty acid production (particularly for butyric acid), cell hydrophobicity, sugar content of extracellular polymers, cell size and electrophoretic mobility. In a further study, Waller et al. [48] evaluated the impact of food-grade TiO_2 (vs industrial-grade TiO_2) on the composition and phenotype of a human gut microbiota. An inhibition of the control-induced shift in microbial composition from Proteobacteria to Firmicutes phyla was observed. TiO_2 exposure also resulted in a lower value of the colonic pH (~pH 4) as compared to the control (>5). Additionally, similar trends in microbial community hydrophobicity and electrophoretic mobility were obtained between control and food-grade exposures. Interestingly, different microbial responses were observed with the industrial-grade form, underlying the significance of physical and chemical properties of TiO_2 in intestinal homeostasis.

2.5.2. TiO_2 in interaction with the intestinal mucus

Mucus is the viscoelastic gel that lines and protects the intestinal epithelium. It is secreted continuously along the whole intestine by specialized goblet cells in the epithelium (**Figure 4**), and is present in larger amounts in the colon than elsewhere. Mucus was long considered to act as a 'simple' physical barrier, but it is now known to have other key functions essential for the preservation of intestinal homeostasis [49–51], including (i) lubrication of the epithelium, facilitating the progress of material along the digestive tract, (ii) maintenance of a stable microenvironment at the epithelial surface, (iii) protection of the epithelium through the presence of immune system molecules and (iv) provision of an ecological niche for the intestinal microbiota.

Interactions between TiO_2 and intestinal mucus are far from being understood. Variable capacities for absorption and transport of TiO_2 nanoparticles have been described *in vitro* [52], depending on whether epithelial cells are cultured alone or in the presence of mucus-secreting goblet cells. In fact, Caco-2 cells in monoculture only displayed low levels of intracellular nano-TiO_2 accumulation after 24-h exposure, whereas the same treatment in Caco-2/HT29-MTX mucus-producing co-culture led to 50 times higher levels of accumulation [52]. In ex vivo studies on porcine buccal mucosa [36, 38], TiO_2 nanoparticles, regardless of their size and hydrophilicity/hydrophobicity, were able to permeate mucus and penetrate underlying tissues.

2.5.3. TiO_2 in interaction with the intestinal epithelium

Epithelium is in charge of nutrients and water absorption while restricting the access for potentially noxious substances to the internal organs. Thus, it constitutes a selective—and dynamic—barrier, mediating transport of compounds through the transcellular pathway (i.e., across the cells) and/or the paracellular pathway (i.e., between the cells). It is polarized into an apical and basolateral surface with the apical surface covered with microvilli to increase

the absorptive surface area. There are at least three pathways enabling uptake/translocation of TiO_2 nanoparticles (**Figure 4**): first, they can disrupt the cell junctions (paracellular route), second they can be internalized by the cells (transcellular route, e.g., endocytosis) and finally they can exert a toxic effect on the cells or alter their function, resulting in cell death [53]. In addition, many studies underlined the involvement of the M-cell-rich layer of Peyer's patches which are epithelial cells specialized for the transcytosis of macromolecules and particles [40, 53–56] (**Figure 4**). However, this mechanism of translocation is still under debate since contradictory results were obtained for *in vitro* cells [36, 40, 53, 55–57].

In vitro studies, mainly on Caco-2 cells, converge on the possible TiO_2-mediated disruption of the epithelial barrier. Indeed, subtle or more substantial alterations were depicted, including cytotoxicity [58], alteration of the brush-border microvilli [26, 53], upregulation of nutrient transporters and efflux pumps [59], production of reactive oxygen species [59, 60], misbalance of redox repair systems [59], increase in epithelial permeability [60] and uptake/translocation of TiO_2 nanoparticles [53, 55, 60], at a different extent according to the type of TiO_2 nanoparticles (size and crystal phase) and experimental conditions used.

In line with the findings of Faust et al. [26], recent piece of evidence suggests some adverse effects of oral exposure to E171 on the intestinal mucosa barrier with a putative additional impact on intestinal diseases and colorectal cancer [61–63]. Proquin et al. [63] showed *in vitro* that E171-induced ROS formation and DNA damage through its micro-sized and/or nano-sized fractions in Caco-2 and HCT116 cells. In rodents, Bettini et al. [61] found TiO_2 particles present in Peyer's patches along the small intestine as well as in the colonic mucosa of rats orally given E171 at human relevant levels. No significant change in epithelial paracellular permeability was observed.

2.5.4. Biodistribution of TiO_2

When TiO_2 particles overcome the mucus/microbiota/epithelium-protective triad, they may enter systemic circulation [64, 65] but in an extremely limited amount [36] and infiltrate organs like liver and kidney which are the organs for exogenous chemicals metabolism and for the excretion of metabolic wastes, respectively. But they were also found in lung, spleen and brain [66, 67] and presented a poor clearance [67]. With a half-life of 12.7 days [66], TiO_2 particles may be thus regularly renewed in the organism, suggesting a bioaccumulation [23] but there is an absence of toxicological effects in the conditions of the study [66]. In the terminal ileum of children suspected of having inflammatory bowel disease, the amount of pigment in Peyer's patches became denser with increasing age [68].

3. Conclusion

With the aim to ensure a healthy food, the knowledge about TiO_2 as food additive increased in the last 5 years. Among the large set of TiO_2 samples, E171 food-grade materials have different physicochemical properties from the reference material P25. Indeed, it is characterized by a low specific surface area (around 10 m^2/g), a pure anatase crystalline phase (sometimes traces of rutile), a low

isoelectric point (around 4.1 in ultrapure water) mainly related to the phosphate found at its surface, a mean size of around 140 nm with a distribution spanning from 30 to 300 nm and a fraction of nanoparticles comprised between 17 and 36%. Due to the lack of data on E171, the risk assessment of oral exposure to TiO_2 has been mainly performed with TiO_2 nanomaterials like P25 which possess a different surface chemistry. As TiO_2 has a low absorption rate, it is mostly excreted in the faeces, suggesting that it does not present any toxicity concern. Nevertheless, there is an increasing awareness of proved or suspected deleterious effects of TiO_2 during its transit in the digestive tract, by compromising intestinal homeostasis before absorption in the upper compartments and/or throughout the entire intestine by the non-absorbed fraction. Albeit increasingly recognized as key players in gut health, mucus and microbiota have often been neglected in food nanotoxicology and should now be more deeply investigated. The link with some intestinal diseases needs to be confirmed as well. For all further studies, the use of food-grade forms of TiO_2 is more relevant than that of the nanomaterial P25.

Author details

Marie-Hélène Ropers[1]*, Hélène Terrisse[2], Muriel Mercier-Bonin[3] and Bernard Humbert[2]

*Address all correspondence to: marie-helene.ropers@inra.fr

1 INRA, Biopolymers Interactions and Assemblies, Nantes, France

2 Institute of Materials Jean ROUXEL (IMN), Université de Nantes, CNRS, Nantes, France

3 INRA, Toxalim (Research Centre in Food Toxicology), University of Toulouse, INRA, ENVT, INP-Purpan, UPS, Toulouse, France

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