日本で食品添加物として使用される二酸化チタンの粒子径について測定し、JECFAに情報提供した資料

参考資料３
食品添加物部会
令和５年７月19日

Reports on physicochemical properties of titanium dioxide used as a food additive in Japan, including particle size and distribution, specific surface area, and elemental analysis.

Summary

As part of the safety evaluation of titanium dioxide used as a food additive, physicochemical properties such as particle size and distribution, specific surface area, and elemental analysis were measured. The samples examined in this study were five types of titanium dioxide (samples A, B, C, D, and E) distributed and commercially available as food additives in Japan. Particle size was measured by TEM. The results showed that the ratios of particles less than 100 nm in diameter was 30-50% for samples A and C and less than 10% for samples B, D, and E. The particle size distribution of titanium dioxide in the medium was measured by DLS. To mimic the behavior in the gastrointestinal tract during oral intake, disintegration tests were conducted by dispersing the samples in first, and second fluid, which correspond to the gastric juice and intestinal fluids in the Japanese Pharmacopoeia. The results showed that in almost all samples, the particle size distribution ranged from 1 μm to maximum measurable diameter 6 μm, suggesting the formation of coarse secondary particles in both media. No particles smaller than 100 nm were detected in either sample. The specific surface area of each sample, estimated by BET theory, was found to be the largest for sample A, followed by sample C, and similarly small for the remaining samples B, D, and E. Qualitative analysis by EDX and ICP-AES revealed that sample A contained trace amounts of impurities, while the other four samples were found to be nearly pure titanium dioxide. The dissolved components (Sb, As, Cd, Pb: measured by ICP-MS, Hg: measured by CV-AAS) were below the limit of quantification.

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1. Purpose of the study

As part of the safety evaluation of titanium dioxide used as a food additive, physicochemical properties such as particle size, pore distribution, specific surface area, and elemental analysis were measured. The samples examined in this study were five types of titanium dioxide that are distributed and available as food additives in Japan. The samples were described as Sample A, B, C, D and E in this document. This study was conducted by the Ministry of Health, Labour and Welfare of Japan (MHLW) in 2022.

2. Identification

Chemical name	Titanium dioxide
CAS number	13463-67-7
Chemical formula	TiO_2
Molecular weight	79.87
INS number	171

3. Analysis Items

3-1. Particle size, particle size distribution, and number of particles less than 100 nm in percentage

3-1-1. Transmission electron microscope (TEM)

The long and short diameters of more than 300 primary particles were measured, and then the number and percentage of particles smaller than 100 nm, as well as the average, minimum, and maximum particle diameters were evaluated.

3-1-2. Dynamic light scattering (DLS)

As dispersion medium, first fluid for disintegration test (JP1, pH1.2) and second fluid for disintegration test (JP2, pH6.8) were used in this study. The fluids are prescribed in the Japanese Pharmacopoeia (JP) and mimic gastric juice and intestinal tract, respectively.

3-2. Pore distribution and specific surface area measurement

Pore distribution and specific surface area measurements by nitrogen adsorption BET multipoint method.

3-3. Component analysis

3-3-1. Qualitative analysis by X-ray fluorescence method (EDX)

- Target elements: Na to U
- Reportable limit: 0.1%

3-3-2. Quantitative analysis by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES)

- Target elements: Fe, Si, P, Al, Cr, Zr, Ca, Mg, Ti
- Reporting limit: 0.01% , 0.05% for Si and P

3-4. Analysis of leached components

3-4-1. Quantitative analysis by Inductively coupled plasma mass spectrometer (ICP-MS)

- Target elements: Sb, As, Cd, Pb
- Reportable limit: 1 mg/Kg

3-4-2. Quantitative analysis by reductive evaporation-atomic absorption method

- Target element : Hg
- Reportable limit : 1 mg/Kg

4. Analysis Method

4-1. Particle size, particle size distribution, and number of particles less than 100 nm in percentage

4-1-1. Transmission electron microscope (TEM)

- Equipment: High-resolution transmission electron microscope HF2000 (Hitachi High-Tech)
- Accelerating voltage: 200 kV
- Observation mode: TEM bright field image
- Particle analysis: Mitani Corporation, Image analysis software WinROOF2018

The samples were dispersed in 0.1 wt% hydroxyethylcellulose aqueous solution with sonication for approximately 10 minutes, the samples were dropped onto a TEM grid (Cu grid) with a hydrophilic carbon support film, dried, and then subjected to TEM observation.

4-1-2 Dynamic Light Scattering (DLS)

- Equipment: Particle size distribution analyzer LB-500 (HORIBA, Ltd.)

- Dispersion medium: First fluild for disintegration test and second fluild for disintegration test
- Sample refractive index: 2.53
- Refractive index of medium: 1.33
- Dispersion concentration: 0.2 mg/mL
- Dispersion treatment: Treated in ultrasonic cleaner for 5 min.

4-2. Pore distribution and specific surface area measurement

- Equipment : Micro-Meritics ASAP-2020 pore distribution analyzer
- Pretreatment : Vacuum, 300°C x 3 hr

The specific surface area was obtained from the slope and intercept of the BET plot, the amount of monolayer adsorption from the BET equation, and the cross-sectional area occupied by a single gas molecule multiplied by the amount of monolayer adsorption.

Pore distribution was obtained using a pore distribution curve based on BJH analysis.

4-3. Component analysis

4-3-1. Qualitative analysis

- Equipment: JEOL Ltd. energy dispersive X-ray fluorescence analyzer JSX-3100RII
- EDX measurement conditions

  Tube target element : Rh

  Excitation voltage (kV) : 50

  Measurement time (sec) : 300

  Tube current (μA) : Automatic

  Collimator (mm) : φ7

  Atmosphere : Vacuum

Estimated abundance ratios were calculated by the Fundamental Parameter (FP) method.

4-3-2. Quantitative analysis

Analysis method: ICP emission spectrometry (Fe, Si, P, Al, Cr, Zr, Ca, Mg, Ti)

Equipment: ICPS8100 (Shimadzu Corporation)

4-4. Dissolved component analysis

Elution conditions: 10 g sample was boiled in 50 mL of 0.5 N hydrochloric acid for 15 min.

4-4-1. ICP mass spectrometry (Sb, As, Cd, Pb)
Equipment: Agilent 8800 (Agilent Technologies)

4-4-2. Reduction vaporization-atomic absorption method
Equipment: Mercury analyzer: HG-400 (Hiranuma Sangyo)

5 Results

5-1. Particle size, particle size distribution, and number of particles less than 100 nm percent of the total number of particles

5-1-1. Particle diameter

The results of particle diameter measured by TEM are shown in Table 1. The ratios of particle diameter smaller than 100 nm were 30 to 50% in Samples A and C, while the ratios were less than 10% in Samples B, D, and E. Note that Sample C consisted mainly of irregularly shaped particles with spherical particles adhering to them.

Table1. Particle diameter measurement by TEM

Measurement parameters	Sample	Total number of particles measured	< 100 nm		Particle diameter		
			Number (N)	Ratio (%)	Average (nm)	Min. (nm)	Max. (nm)
Ferret diameter: Horizontal	A	417	192	46.0	117	15	343
	B	371	26	7.0	186	45	425
	C	370	120	32.4	137	30	388
	D	336	20	6.0	197	67	396
	E	345	32	9.3	196	15	537
Ferret diameter: Vertical	A	417	185	44.4	116	7	358
	B	371	33	8.9	187	30	396
	C	370	117	31.6	135	30	381
	D	336	20	6.0	196	60	396
	E	345	22	6.4	195	52	470
Circle equivalent diameter	A	417	198	47.5	111	19	283
	B	371	33	8.9	179	40	396
	C	370	121	32.7	129	32	295
	D	336	19	5.7	188	64	360
	E	345	29	8.4	186	43	428
Absolute maximum length	A	417	109	26.1	136	25	392
	B	371	14	3.8	217	55	479
	C	370	73	19.7	161	42	453
	D	336	8	2.4	228	79	426
	E	345	7	2.0	229	62	544
Diagonal width	A	417	204	48.9	111	7	328
	B	371	36	9.7	173	31	393

	C	370	126	34.1	125	30	281
	D	336	26	7.7	179	59	347
	E	345	34	9.9	178	15	443

5-1-2. Particle size distribution

The results of particle size distribution measured by DLS are shown in Table 2. The results show that the particle distribution ranged from 1 μm to maximum measurable diameter 6 μm for almost all samples dissolved in both the JP1 and JP2. No particles smaller than 100 nm were detected in either sample. Therefore, it can be assumed that coarse secondary particles were formed in both mediums, suggesting accurate particle size could not be obtained from each sample under the conditions of the device used in this experiment. In the comparison of two type of medium, JP1 shown a larger average particle size and frequently detected mode values above 5 μm, indicating a more tendency for agglomeration.

Table 2. Particle size distribution measurement by DLS

Solution	Sample	Measurement number	Particle diameter (μm) Median	Mean	Mode	< 100 nm particle†
First Fluid for disintegration test, pH1.2 The Japanese Pharmacopoeia (JP1)	A	n1	1.8	2.3	1.2	n.d.
		n2	1.5	2.0	1.1	n.d.
		n3	1.5	2.6	5.4	n.d.
		Average	1.6	2.3	2.5	—
	B	n1	2.6	2.9	2.1	n.d.
		n2	1.5	2.2	1.1	n.d.
		n3	1.8	2.6	5.3	n.d.
		Average	2.0	2.6	2.8	—
	C	n1	1.7	2.1	1.2	n.d.
		n2	1.5	2.6	5.4	n.d.
		n3	1.5	2.2	1.1	n.d.
		Average	1.6	2.3	2.6	—
	D	n1	2.0	2.9	5.4	n.d.
		n2	1.9	2.1	2.4	n.d.
		n3	1.6	2.3	1.1	n.d.
		Average	1.8	2.4	3.0	—
	E	n1	2.0	2.6	5.2	n.d.
		n2	2.7	3.0	5.2	n.d.
		n3	1.8	2.3	1.1	n.d.
		Average	2.2	2.6	3.8	—
Second Fluid for disintegration test, pH 6.8 The Japanese Pharmacopoeia	A	n1	1.3	1.7	0.94	n.d.
		n2	1.1	1.5	0.72	n.d.
		n3	1.1	1.8	0.82	n.d.
		Average	1.2	1.7	0.83	—
	B	n1	1.3	1.9	0.94	n.d.
		n2	1.5	1.7	1.2	n.d.

(JP2)		n3	1.5	2.2	0.95	n.d.
		Average	1.4	1.9	1.0	—
	C	n1	1.2	1.7	0.82	n.d.
		n2	1.2	1.9	0.82	n.d.
		n3	1.1	1.7	0.82	n.d.
		Average	1.1	1.8	0.82	—
	D	n1	1.8	2.1	1.2	n.d.
		n2	1.5	2.1	1.1	n.d.
		n3	1.7	2.1	1.2	n.d.
		Average	1.7	2.1	1.2	—
	E	n1	1.5	1.8	0.94	n.d.
		n2	1.5	1.8	1.1	n.d.
		n3	1.5	1.9	1.1	n.d.
		Average	1.5	1.9	1.0	—

† n.d. = not detected.

5–2. Specific surface area and pore distribution

Based on multi-point nitrogen adsorption measurements, specific surface area and pore distribution were estimated by the Brunauer–Emmett–Teller (BET) theory and the Barrett–Joyner–Halenda (BJH) analysis, respectively, and the results are shown in Table 3. No clear pores were observed in the mesopore region for these samples. Comparing the specific surface area values of each sample, Sample A showed the largest, followed by Sample C, and the remaining samples, B, D, and E, showed equally low. In other words, Sample A has the smallest particle diameter, followed by Sample C, and the remaining three samples are comparable. This trend agrees rather well with the TEM observations.

Table 3. Specific surface area and pore distribution estimation by BET and BJH

Sample		Specific surface area (m^2/g)	Mesopore region (1–100 nm)	
	Measurement		Pore volume (cm^3/g)	Pore diameter (nm)
A	n1	10.4	-	-
	n2	10.1	-	-
	n3	10.3	-	-
	Average	10.3		
B	n1	7.8	-	-
	n2	7.4	-	-
	n3	7.2	-	-
	Average	7.5		
C	n1	9.7	-	-
	n2	9.1	-	-
	n3	9.4	-	-
	Average	9.4		
D	n1	7.1	-	-
	n2	7.3	-	-
	n3	7.3	-	-

	Average	7.2		
E	n1	7.9	-	-
	n2	7.4	-	-
	n3	7.6	-	-
	Average	7.6		

5–3. Chemical composition

The results of qualitative analysis by EDX and ICP-AES are shown in Table 4 and Table 5, respectively. Sample A contained trace amounts of impurities, while the other four samples were found to be almost pure titanium dioxide.

Table 4. Qualitative analysis by EDX

Sample	Estimated rate of existence (%) †‡					
	Ti	K	Zr	Nb	P	Ge
A	99.6	0.2	< 0.1	< 0.1	0.2	< 0.1
B	> 99.9	n.d.	n.d.	n.d.	n.d.	< 0.1
C	> 99.9	n.d.	n.d.	n.d.	n.d.	n.d.
D	> 99.9	n.d.	n.d.	n.d.	n.d.	< 0.1
E	> 99.9	n.d.	n.d.	n.d.	n.d.	n.d.

† Elements detected by qualitative analysis for elements from Na to U are listed and calculated the total to be 100%.

‡ n.d. = not detected.

Table 5. Quantitative analysis by ICP-AES

Sample	Chemical composition (wt.%)								
	Fe	Si	P	Al	Cr	Zr	Ca	Mg	Ti
A	< 0.01	< 0.05	0.12	< 0.01	< 0.01	0.01	< 0.01	< 0.01	59.7
B	< 0.01	< 0.05	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	60.3
C	0.02	< 0.05	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	60.3
D	0.08	< 0.05	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	60.3
E	0.01	< 0.05	< 0.05	<0.01	< 0.01	< 0.01	< 0.01	< 0.01	60.4

5–4. Dissolved component analysis

The results of the dissolved component analysis are shown in Table 6. Each sample was

boiled in 50 ml of 0.5 N hydrochloric acid for 15 minutes, and then the elements eluted in the solution were measured using ICP mass spectrometry (Sb, As, Cd, and Pb) and cold vapor atomic absorption spectrophotometry (Hg). Elements generally considered hazardous were found to be below the limit of quantification.

Table 6. Dissolved component analysis

Sample	Dissolved component (mg/kg)				
	Sb	As	Cd	Pb	Hg
A	< 1	< 1	< 1	< 1	< 1
B	< 1	< 1	< 1	< 1	< 1
C	< 1	< 1	< 1	< 1	< 1
D	< 1	< 1	< 1	< 1	< 1
E	< 1	< 1	< 1	< 1	< 1

Abbreviations

BET	Brunauer, Emmett and Teller
CV-AAS	Cold Vapor Atomic Absorption Spectrometry
DLS	Dynamic light scattering
EDX	Energy dispersive X-ray spectrometry
ICP-AES	Inductively Coupled Plasma Atomic Emission Spectroscopy
ICP-MS	Inductively coupled plasma mass spectrometer
INS	The International Numbering System for Food Additives
JP	The Japanese Pharmacopoeia
TEM	Transmission electron microscopy