

MIGRATION OF NANOPARTICLES FROM FOOD CONTACT MATERIALS

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Doctoral Dissertation

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MIGRACIJA NANODELCEV IZ MATERIALOV V
STIKU Z ŽIVILI

Doktorska disertacija

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Abstract

The materials in contact with foodstuffs are various materials which come into contact with foodstuffs during production, processing, preparation and storage of foodstuffs. Materials in contact with foodstuffs must be safe. Safety of certain food contact material depends on the kind and quantity of constituents, which are released from it into foodstuffs. Migrated constituents should not endanger human health, bring about an unacceptable change in the composition of the food, or bring about the deterioration in the organoleptic characteristics of the food. New food contact materials containing nanoparticles have been developed recently. These novel materials are mostly in the research and development stage, but some of them already reached the market or may reach the market soon. The main risk of consumer exposure to nanoparticles present in novel food contact materials is through potential migration of nanoparticles into food. For now, very little information is available on the actual presence and use of novel food contact materials, and the release of nanoparticles from these materials into food.

In this project commercially available non-stick food contact coatings on pans were selected for characterization and study of possible nanoparticles release. Results of release testing were used for the assessment of a possible health risk through exposure to released nano TiO_2 particles. Furthermore, a screening method for detection of nanoparticles present in food contact materials was developed and used for detection of silver nanoparticles released from food contact paper.

Characterisation of non-stick food contact coatings was performed by FTIR, electron and optical microscopy, EDXS and XRD. Characterisation revealed that coatings contained submicron- and nanosized rutile TiO_2 particles and quartz SiO_2 embedded in a silicone polymer matrix. In order to estimate the possible migration of TiO_2 nanoparticles into food, migration tests into simulants (deionised water, 3 % acetic acid and 5 g /l citric acid) were performed (2 h at 100 °C). Besides, thermal and mechanical degradation of the matrix was studied on the selected coating. Simulants, which have been in contact with coatings, were analyzed by ICP-MS after ultrafiltration and by microwave-assisted digestion. The highest concentration of titanium-containing particles was determined in 3% acetic acid (up to 861 $\mu\text{g/l}$ or 147 $\mu\text{g/ dm}^2$). Titanium was present in simulants in ionic form as well. The presence of TiO_2 nanoparticles in 3 % acetic acid was confirmed by SEM-EDXS analysis. Thermal stability study (TG/DSC MS analysis) did not show degradation of the matrix under foreseeable conditions of use, but mechanical degradation studies showed the possible release of titanium-containing nanoparticles (microgram quantities by tribological testing). The matrix degradation results were confirmed by observations of the morphology of the same type of coatings actually used for food preparation. Dissolution from the surface and mechanical abrasion as a mechanism of matrix degradation can both contribute to nanoparticles release from this type of non-stick food contact coatings. The release of nanoparticles from food contact materials due to mechanical degradation has not been described in the literature so far.

Results of migration testing and calculation of possible release from actually used coating were considered together with different food consumption data in the assessment of exposure to TiO_2 nanoparticles. Estimated exposures were compared with DNEL values for neurobehavioral effects and endocrine malfunction in order to assess possible health risks through exposure to released nano TiO_2 particles. Based on the available information, health risk due to exposure to nano TiO_2 particles released from examined quasi-ceramic coating cannot be excluded. However, to confirm the health risk, assessment should be refined with more precise exposure and hazard data.

Additionally, a method for detection of presence and release of nanoparticles from food contact materials, based on the reaction rate of redox reaction between sodium borohydride and methylene blue dye, for which nanoparticles serve as catalysts, was developed and used for detecting the release of silver nanoparticles from food contact paper with printed radio-frequency identification antenna. Knowing that the rate of discolouration depends not only on the concentration of nanoparticles but also on temperature and various properties of nanoparticles (e.g. size and shape), the method is suitable for screening purposes. If catalytic activity in the extract of food contact material is detected, more sophisticated methods of material characterisation should be performed including determination of concentration and physicochemical properties of released nanoparticles.

Research on the migration of nanoparticles from food contact materials and assessment of possible health risk due to exposure to released nanoparticles is still in its infancy. Future research should include release testing into real foods, determination of persistence of released particles in gastrointestinal fluids during digestion and generation of toxicity data on nanoparticles with various physicochemical characteristics.

Povzetek

Materiali v stiku z živili so različni materiali, ki prihajajo v stik z živili med proizvodnjo, predelavo, pripravo in shranjevanjem živil. Materiali v stiku z živili morajo biti varni. Varnost materialov v stiku z živili je odvisna od vrste in količine sestavin, ki se iz njih sproščajo v živila. Snovi ne smejo prehajati v živila v takih količinah, ki bi lahko ogrožale zdravje ljudi ali povzročale nesprejemljive spremembe v sestavi živil oziroma poslabšanje njihovih organoleptičnih lastnosti. V zadnjem času so bili razviti novi materiali za stik z živili, ki vsebujejo nanodelce. Ti novi materiali so večinoma še v fazi razvoja in raziskav, vendar pa so nekateri že na trgu ali bodo na trgu kmalu. Tveganje za zdravje zaradi izpostavljenosti potrošnikov nanodelcem iz novih materialov je prisotno zaradi možnosti migracije nanodelcev v živila. Za zdaj je na voljo zelo malo informacij o dejanski uporabi novih materialov za stik z živili in sproščanju nanodelcev iz teh materialov v hrano.

Za naše delo smo izbrali komercialno dostopne neoprijemajoče se premaze za ponve in jih karakterizirali ter preučevali možno sproščanje nanodelcev iz njih. Rezultate sproščanja smo uporabili za oceno morebitnega tveganja za zdravje zaradi izpostavljenosti sproščenim nanodelcem TiO_2 . Poleg tega smo razvili presejalno metodo za ugotavljanje prisotnosti in sproščanja nanodelcev iz materialov v stiku z živili in jo uporabili za določitev sproščanja srebrovih nanodelcev iz papirja v stiku z živili.

Karakterizacijo premazov smo izvedli z uporabo FTIR, elektronske in optične mikroskopije, EDXS in XRD. Karakterizacija je pokazala, da premazi v preiskanih ponvah vsebujejo delce TiO_2 (rutila) v podmikronski in nano velikosti ter SiO_2 delce, vključene v silikonski polimerni matrici. Za oceno možne migracije TiO_2 nanodelcev v živila smo opravili migracijske preskuse (2 uri pri 100 °C) iz premazov v različne simulante: deionizirano vodo, 3 % očetno kislino in 5 g/l citronsko kislino. Poleg tega smo pri izbranem premazu preučevali termično in mehansko razgradnjo matrice. Simulante, ki so bili v stiku s premazi, smo analizirali z ICP-MS po opravljeni ultrafiltraciji in mikrovalovnem razklopu. Koncentracija titana v delcih, ki so migrirali v simulante, je bila največja v 3 % očetni kislini (do 861 mg/l oziroma 147 mg/dm³). Titan je bil prisoten v simulantih tako v obliki delcev kot v ionski obliki. Prisotnost TiO_2 nanodelcev v 3 % očetni kislini smo potrdili z energijsko spektroskopijo rentgenskih žarkov v vrstičnem elektronskem mikroskopu (SEM EDXS). Preučevanje termične stabilnosti z : termogravimetrično, diferencialno vrstično kalorimetrijo in masno spektroskopijo (TG / DSC MS) ni pokazalo razgradnje matrice pri predvidljivih pogojih uporabe, vendar pa so mehanski testi razgradnje pokazali sproščanje nanodelcev, ki vsebujejo titan (mikrogramske količine pri tribološkem testu). Rezultate razgradnje matrice smo potrdili z opazovanjem morfologije premazov enakega tipa, ki so bili v dejanski uporabi za pripravo živil. Ugotovili smo, da sta raztapljanje s površine premaza ter mehanska abrazija možna mehanizma razgradnje matrice, ki lahko prispevata k sproščanju nanodelcev iz tovrstnih premazov v stiku z živili. Prvi smo opisali sproščanje nanodelcev iz materialov v stiku z živili zaradi mehanske razgradnje matrice.

Rezultate migracijskih preskusov in izračun sproščenih delcev iz materiala, ki je bil v dejanskem stiku z živili, smo uporabili skupaj z različnimi podatki o porabi živil za oceno izpostavljenosti TiO_2 nanodelcem. Ocenjeno izpostavljenost smo primerjali z vrednostmi DNEL za nevrološke vedenjske motnje in endokrine motnje, da bi ocenili morebitna tveganja za zdravje zaradi izpostavljenosti sproščenim nanodelcem TiO_2 . Na podlagi razpoložljivih podatkov smo ugotovili, da tveganja za zdravje zaradi izpostavljenosti nanodelcem TiO_2 , ki so se sprostili iz izbranega kvazi-keramičnega premaza, ni mogoče izključiti. Za potrditev dejanskega tveganja za zdravje pa bo potrebno oceno rafinirati in pridobiti bolj natančne podatke o nevarnosti in izpostavljenosti.

Razvili smo tudi metodo za zaznavanje prisotnosti in sproščanja nanodelcev iz materialov za stik z živili na osnovi določitve hitrosti redoks reakcije med natrijevim borohidridom in barvilom metilen modrim. Pri tej reakciji nanodelci služijo kot katalizator. Metodo smo uporabili za detekcijo sproščanja srebrovih nanodelcev iz papirja z natisnjeno RFID anteno. Ker pa hitrost reakcije ni odvisna samo od koncentracije nanodelcev, ampak tudi od temperature in drugih lastnosti nanodelcev (npr. velikosti in oblike), jo lahko uporabimo kot presejalno metodo. Če v ekstraktu materiala zaznamo katalitično aktivnost, je potrebno za karakterizacijo materiala in določitev koncentracije ter fizikalno-kemijskih lastnosti sproščenih nanodelcev uporabiti bolj sofisticirane metode.

Raziskave migracij nanodelcev iz materialov v stiku z živili in ocena možnega tveganja zaradi izpostavljenosti nanodelcem, ki so migrirali iz materialov v stiku z živili, so še vedno v povojih. Prihodnje raziskave bi morale vključevati določitev sproščanja nanodelcev v živila, določitev obstojnosti nanodelcev v prebavnih tekočinah med prebavo in pridobitev dodatnih informacij o toksičnosti nanodelcev različnih fizikalno-kemijskih lastnosti.

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Abbreviations

ADME	...	Absorption, distribution, metabolism and excretion
Ag	...	Silver
AgCl	...	Silver chloride
Ag ₂ CO ₃	...	Silver carbonate
AgNO ₃	...	Silver nitrate
Ag ₂ O	...	Silver (I) oxide
Al ₂ O ₃	...	Aluminium oxide
Ag ₂ S	...	Silver sulphide
ATR	...	Attenuated total reflectance
CA	...	Contact angle
bw	...	Body weight
CEF panel	...	Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CEN	...	European Committee for Standardization
CO	...	Carbon monoxide
CO ₂	...	Carbon dioxide
CTAB	...	Cetyl trimethylammonium bromide
D _{eff}	...	Effective hydrodynamic particle diameter
DLS	...	Dynamic light scattering
DRIFT	...	Diffuse Reflectance Infrared Fourier Transform
DSC/ TG MS	...	Differential scanning calorimetry/ thermogravimetry coupled with mass spectrometry
DNA	...	Deoxyribonucleic acid
DNEL	...	Derived No-Effect Level
DTU	...	Technical University of Denmark
ECHA	...	European Chemicals Agency
EDXS	...	Energy Dispersive X-Ray Spectroscopy
EDQM	...	European Directorate for the Quality of Medicines
EE	...	Estimated exposure
EFSA	...	European Food Safety Agency
EM	...	Electron Microscope
ENM	...	Engineered nanomaterial
ENO	...	Engineered nanoobject
EVOH	...	Ethylene vinyl alcohol
FAO	...	Food and Agriculture Organisation of United Nations
FCM	...	Food contact material
FE SEM	...	Field Emission Scanning Electron Microscope
FIB SEM	...	Focussed Ion Beam Scanning Electron Microscope

FoodEx	...	EFSA food classification system
FTIR	...	Fourier Transform Infrared Spectroscopy
GI	...	Gastrointestinal
H ₃ BO ₃	...	Boric acid
HCl	...	Hydrochloric acid
HF	...	Hydrofluoric acid
HEPES	...	(4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HNO ₃	...	Nitric acid
IARC	...	International Agency for Research on Cancer
ICP-MS	...	Inductively coupled plasma mass spectrometry
IEP	...	Isoelectric point
LOAEL	...	Lowest Observed Adverse Effect Level
LOQ	...	Limit of quantification
MB	...	Methylene blue
MS	...	Mass spectrometry
NaOH	...	Sodium hydroxide
Ni	...	Nickel
NIAS	...	Nonintentionally added substances
NOAEL	...	No Observed Adverse Effect Level
NP	...	Nanoparticle
PA	...	Polyamide
PALS	...	Phase Analysis Light Scattering
PDI	...	Polydispersity index
PE	...	Polyethylene
PEI	...	Polyethyleneimine
PET	...	Polyethylene terephthalate
PFA	...	Polyfluoroalkoxy
PVC	...	Polyvinyl chloride
PTFE	...	Polytetrafluoroethylene
RCR	...	Risk characterisation ratio
REACH	...	European Union regulation concerning the Registration, Evaluation, Authorisation and restriction of Chemicals
RFID	...	Radio-frequency identification
ROS	...	Reactive oxygen species
SEM	...	Scanning Electron Microscope
SCCS	...	Scientific Committee on Consumer Safety
SCENIHR	...	Scientific Committee on Emerging and Newly Identified Health Risks
SiO ₂	...	Silicium dioxide, Silica
sp ICP MS	...	Single Particle Inductively Coupled Plasma Mass Spectrometry
TEM	...	Transmission Electron Microscope
TDI	...	Tolerable daily intake
TG	...	Thermogravimetric analysis

Ti	...	Titanium
TiN	...	Titanium nitride
TiO ₂	...	Titanium dioxide, Titania
XRD	...	X-Ray Diffraction
AF ⁴	...	Asymmetric Flow Field Flow Fractionation
UV	...	Ultra violet light
Vis	...	Visible part of light spectrum
WHO	...	World Health Organisation
Y	...	Yttrium
Zn	...	Zinc
ZnO	...	Zinc oxide
ZP	...	Zeta potential

Chapter 1

Introduction

1.1. Food Contact Materials

In the food supply and delivery chain, foods are exposed to different food contact materials (FCMs). A variety of materials and their combinations can be used as food contact materials. At the production stage, such materials are metals and alloys (e.g. tools, handling equipment and machinery), plastics (e.g. storage vessels, packaging, tubes), rubbers (e.g. tubes, gaskets), wood (utensils), ion-exchange resins (water preparation). At the food processing and handling stage food contact materials are metals and alloys and also active materials (used for utensils and cookware), glass, ceramics and silicones (kitchenware, cookware, bakeware), textiles (e.g. bags, filters), plastics (utensils, dishes), varnishes and coatings (on metallic cookware and plastics) and wood (spatulas, boards). For serving of food, ceramics and glass, as well as plastics and metals are used. Finally, at the packaging stage all of the above-mentioned materials and some of the other materials or combinations of the materials, like active materials (which deliberately release substances or remove undesirable substances) and intelligent materials (which monitor information about the quality of packaged food), adhesives, cork, rubbers, paper and board, printing inks, regenerated cellulose and waxes, can be used. A symbol that may accompany materials and articles intended to come into contact with foodstuffs (European Commission, 2004) is presented in Figure 1.1.



Figure 1.1: A symbol for materials and articles intended to come into contact with foodstuffs.

1.2. Nanotechnology-Derived Food Contact Materials

In the last decade, novel food contact materials, containing part of the material in the nanoscale dimension, were designed. Such materials are mostly in the research and development stage, but some of them already reached the market or may reach the market soon (RIKILT & JRC, 2014, Vance et al., 2015). Inventories of different nanotechnology-based consumer products (including food contact materials) currently placed on the market exist (e.g. Woodrow Wilson International Centre for Scholars, 2017, DTU Environment, 2017). Nanotechnology-derived food contact materials have certain improved properties compared to conventional materials. They have better mechanical strength, thermal resistance, flexibility, barrier properties, ultraviolet light and flame resistance, antimicrobial and other functional properties, thermal and light stability, mechanical properties etc. According to the Commission's definition of nanomaterial, not all materials containing nanoparticles are nanomaterials. Commission Recommendation on the definition of nanomaterial published recently defines that: "nanomaterial means material containing particles, in an unbound state or as an aggregate or as agglomerate and where, for 50% or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm. In specific cases and where warranted by concerns for the environment, health, safety or competitiveness the number size distribution threshold of 50% may be replaced by a threshold between 1 and 50 %." Fullerenes, graphene flakes, and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials" (European Commission, 2011). Taking into account this definition, some of the food contact materials containing nanoparticles can also be called nanomaterials. Irrespective of the Commission definition, and generally speaking, nanomaterials are all materials having some amount of matter with one or more external dimensions in the nanoscale or which are nanostructured. To such materials engineered nanoobjects(ENOs) are added. Nanoparticles are materials with an external dimension in nanoscale, nanorods or nanotubes have two dimensions in the nanoscale, and nanolayers, coatings or sheets have one dimension in nanoscale. In the context of the thesis, food contact materials containing some amount of the material in the nanoscale dimension are called nanotechnology-derived food contact materials, and for simplicity, ENOs are called nanoparticles.

Nanoparticles can be used in the production of active and intelligent materials and articles, food contact plastic (synthetic polymers and biopolymers), paper and board, printing inks, adhesives and probably other types of food contact materials.

Active materials and articles are materials used for controlled release or absorption of substances. Active materials and articles may contain nanoparticles of silver, gold, zinc oxide, silica, TiO₂, iron and iron oxides, copper and copper oxide, magnesium oxide, alumina nanotubes, carbon nanotubes, carbon black nanoparticles (FAO/WHO, 2009, Silvestre, Duraccio, & Cimmino, 2011, Hatzigrigoriou & Papaspyrides, 2011, Llorens, Lloret, Picouet, Trbojevich, & Fernandez, 2012, Bumbudsanpharoke & Ko, 2015, Smolkova, El Yamani, Collins, Gutleb, & Dusinska, 2015,). These materials have an antimicrobial function, inhibit the growth of microorganisms, and as a result, foodstuffs remain fresh much longer. Other active materials can absorb oxygen and thus keep food fresh (may contain silica nanoparticles or TiO₂ nanoparticles, or can absorb and decompose ethylene (Ag based nanoparticles (Silvestre, Duraccio, & Cimmino, 2011, Hatzigrigoriou & Papaspyrides, 2011). Materials can also release aromas (Woodrow Wilson International Centre for Scholars, 2017). Figure 1.2 shows different active materials containing nanoparticles.

Nanotechnology is also used for manufacturing of intelligent materials and articles. Intelligent materials and articles provide the user with information on the conditions of

the food using nanosensors and nanolabels. Intelligent materials may contain nanosensors, nanocapsules and smart labels (European Commission, 2004, European Commission, 2009a, Han, Yu, Li, & Wang, 2011). For example, intelligent packaging can contain oxygen detecting ink with light-sensitive (TiO₂ nanoparticles) which only detect oxygen when they are “switched on” with UV light], or nanocrystalline SnO₂ as oxygen indicator, or organically modified nanosilica matrix in which 1-hydroxy pyrene-3,6,8-tri sulfonate is immobilized and can be used as CO₂ indicator, or RFID (Radio-frequency identification) tags (for detecting foodborne pathogens or sense the temperature or moisture of a product) that may contain Ag nanoparticles (Silvestre, Duraccio, & Cimmino, 2011, Hatzigrigoriou & Papaspyrides, 2011), magnetic iron oxide nanoparticles for detection of E.Coli (Duncan, 2011).

Nanotechnology is used for production of novel plastic materials (without active or intelligent function), where the presence of nanoparticles improves the properties of synthetic polymers (e.g. polyolefins, polyamide, ethylene-vinyl acetate copolymer, polystyrene, epoxy resins, polyurethane, polyethylene terephthalate, polyvinyl chloride, polystyrene), or biopolymers (e.g. polylactic acid, chitosan, starch, agar.

- *Improved properties for example are:*

- mechanical properties, by addition of nanoclays (Chaudhry et al., 2008, Mihindukulasuriya & Lim, 2014), nanocellulose and carbon nanotubes (Food Standards Agency, 2008, Azeredo, 2009), TiN nanoparticles (FAO/WHO, 2009), silica (Wu, Zhang, Rong, & Friedrich, 2005)
- gas barrier properties, by addition of nanoclays (Blasco & Pico, 2011, Chaudhry et al., 2008, Schmidt et al., 2011, Duncan, 2011, Echegoyen, Rodríguez, & Nerín, 2016), SiO₂ nanoparticles (Tiede et al., 2008), nanofibres of cellulose (Rezaei, Nasirpour, & Fathi, 2015),
- temperature/moisture stability, by addition of nanoclays (Chaudhry et al., 2008, Hatzigrigoriou & Papaspyrides, 2011),
- resistance to fire, by addition of nanoclays, ZnO (Echegoyen, Rodríguez, & Nerín, 2016),
- UV resistance, by addition of TiO₂ and ZnO nanoparticles (Blasco & Pico, 2011, (Martirosyan & Schneider, 2014),
- optical and rheological properties: nanoclays (Hatzigrigoriou & Papaspyrides, 2011),
- nanofibres of cellulose (Rezaei, Nasirpour, & Fathi, 2015),
- biodegradability: nano Zn light catalyst (Blasco & Pico, 2011).

Some polymer materials containing nanoparticles are shown in Figure 1.3.

Food contact paper may also contain silver, gold, TiO₂ and SiO₂ nanoparticles which improve microbiological and chemical characteristics of the food during storage (Peter et al., 2016).

Adhesives made by use of starch nanospheres require less water, time and energy to dry (Blasco & Pico, 2011).

Non-stick quasi-ceramic nanocoatings on metallic cookware are already commercially available. Such coatings prevent sticking and burning of foodstuffs, are supposed to replace the fluorinated polymers (e.g. Teflon®) and may contain silica and TiO₂ (FAO/WHO, 2009). There are also commercially available non-stick nanocoatings for

glass bakeware (no specified data on nanoparticles, Woodrow Wilson International Centre for Scholars, 2017). Nanocoatings on plastics can reduce leftover traces in packaging (nanoscale lipid structures) (FAO/WHO, 2009). Nanocoating on paper makes paper waterproof, antimicrobial and magnetic (manganese ferrite and silver nanoparticles). Articles containing nanotechnology-derived food contact coatings are presented in Figure 1.4.



Figure 1.2: Commercially available active food contact materials containing nanoparticles: antibacterial kitchenware (a), antibacterial tableware (b), nano-silver cutting board (c), tea pot releasing tea flavour (d), refrigerator with deodorant and antimicrobial properties (e), plastic storage bags (f), oxygen scavenging bottle (g) (from the website Woodrow Wilson International Centre for Scholars, 2017), silicone seal which contains antibacterial nanosilver particles (h), baby bottles containing silver nanoparticles (i) (from the paper Bumbudsanpharoke & Ko, 2015).



Figure 1.3: Polymer and paper food contact materials containing nanoparticles. Beer bottle with clay nanoparticles improving barrier properties (a), nanoplastic wrap with ZnO nanoparticles (with some improved properties e.g. UV resistance, reflecting IR, better temperature tolerance, fire proof (b) (from the website Woodrow Wilson International Centre for Scholars, 2017), nanocomposite material with improved mechanical and rheological properties and reduced sensitivity to moisture (starch polymer with added nanoclays)(c) and PA bottles with added nanoclays for better barrier properties (d) (from the paper Bumbudsanpharoke & Ko, 2015).

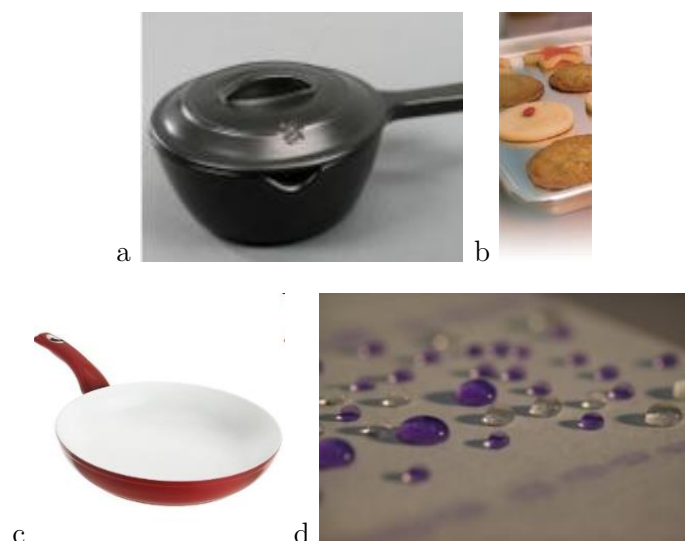


Figure 1.4: Nanocoatings: ceramic nanocoatings (a), non-stick self-assembling nanofilms for glass bakeware (b) quasi-ceramic coating (c) and nanocoating on paper (d) (from the website Woodrow Wilson International Centre for Scholars, 2017).

According to the EFSA inventory (RIKILT & JRC, 2014), the most used nanoparticles in the agricultural, feed and food sector are silver nanoparticles (Figure 1.5). Silver

nanoparticles are also claimed to be used in many other consumer products, not connected to food and feed (Vance et al. 2015). Silver nanoparticles may be intentionally added to food contact materials (as mentioned before) or can be present unintentionally in recycled paper and board as residues originating from, e.g. waste paper containing printed electronics, especially radio-frequency identification (RFID) tags.

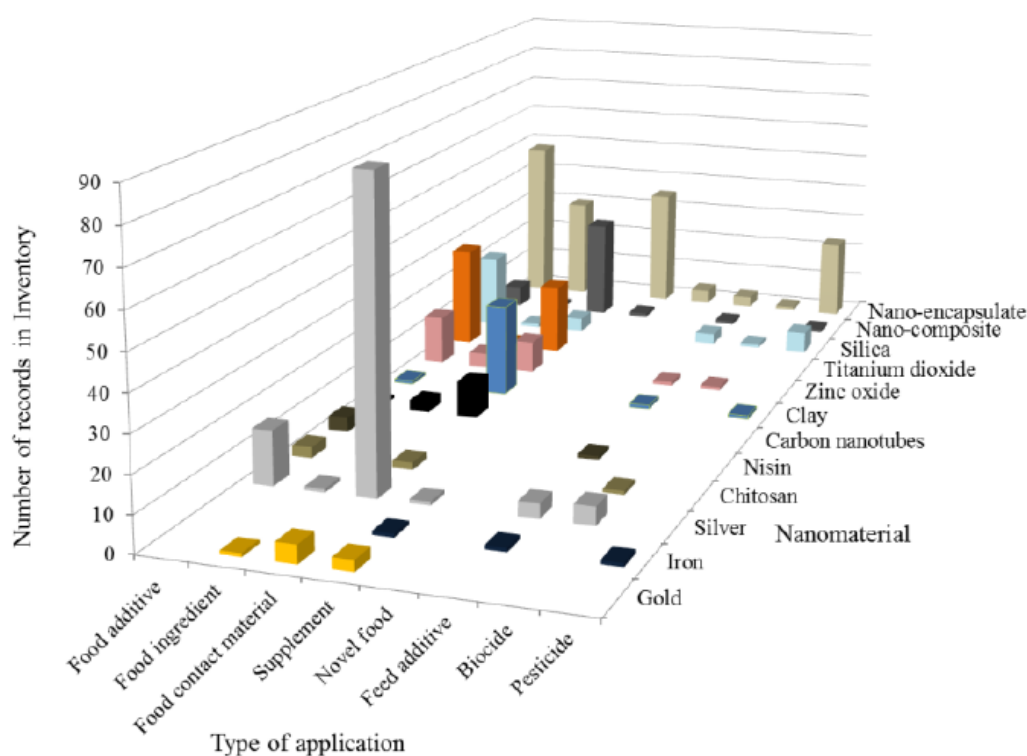


Figure 1.5: Use of different nanomaterials for different food and feed applications. The image is taken from the EFSA Inventory of Nanotechnology applications in the agricultural, feed and food sector (RIKILT & JRC, 2014). It is based on 633 inputs.

1.2.1. European legislation concerning nanotechnology-derived food contact materials

The general requirement of legislation concerning food contact materials (containing nanoparticles or not) is that all food contact materials should be safe as stated in Regulation 1935/2004 (European Commission, 2004).

Nanoform of a substance used in plastics (legislation on plastics is the most elaborate legislation) can only be authorized if included in the specification in the application submitted to European Food Safety Administration (EFSA). Actually, there are two substances used in plastics for some time now, for which the nanoform was included when authorized: silicon dioxide and carbon black (Commission Regulation, 2011).

Silicon dioxide includes synthetic amorphous silicon dioxide primary particles of 1 – 100 nm which are aggregated to a size of 0.1 – 1 μm which may form agglomerates within the size distribution of 0.3 μm to the mm size. There is no restriction regarding its maximum use level.

Carbon black includes primary particles of 10 – 300 nm which are aggregated to a size of 100 – 1200 nm which may form agglomerates within the size distribution of 300 nm – 1 mm. Maximum use level of carbon black in the polymer is 2.5 % w/w.

Regarding new applications for the use of nanoparticles in plastics, not many nanosized substances intended for use in food contact materials have been evaluated by EFSA as safe. These substances are SiO_x coating on polyethylene terephthalate (PET), TiN nanoparticles used as additive in PET, special polymeric additives in nanoform for rigid polyvinyl chloride (PVC), kaolin, which is used in combination with polyacrylic acid, sodium salt and incorporated in ethylene-vinyl alcohol (EVOH) copolymer, zinc oxide, uncoated and coated with [3-(methacrylate)propyl] tri methoxy silane) in all polyolefins, montmorillonite clay modified by dimethyl dialkyl(C16-C18)ammonium chloride (EFSA, 2007, EFSA, 2008, EFSA, 2014a, EFSA, 2014b, EFSA 2015a, EFSA 2015b). The use of these substances was evaluated as safe as long as there is no or very low mass transfer into foods (this applies to all the above mentioned substances) or if they migrate in the form of ions and not as nanoparticles (this applies to nano ZnO used in food contact plastics).

There are nanosized surface antimicrobials incorporated onto or into materials and articles with the function of releasing the substance to the surface only (surface antimicrobials) and intention to maintain hygienic properties or used as a processing antimicrobial, but these applications are not considered as active materials or articles. These food contact materials are considered as "treated articles" and covered by the biocide regulation (European Commission, 2009a).

1.3. Migration from Food Contact Materials

Food contact materials are inseparably connected with foodstuffs. Unfortunately, no food contact material is completely inert, and mass transfer from food contact materials into food occurs. Such mass transfer is generally called migration. There are different factors affecting migration:

- the nature of the food contact material,
- the nature and concentration of the substances present in the food contact material,
- the nature of the foodstuff,
- the extent and the type of contact between the food contact material and the foodstuff,
- the temperature of the contact and the duration of the contact.

Safety of certain food contact material depends on the kind and quantity of constituents, which are released from it into foodstuffs. Migrated constituents should not endanger human health, bring about an unacceptable change in the composition of the food, or bring about the deterioration in the organoleptic characteristics of the food (European Commission, 2004).

In the past, people did not know much about migration. Poisoning caused by food contact materials, according to some theories, occurred already in ancient Rome (lead poisoning). Cases of lead poisoning caused by pottery glazes and tin poisoning with the canned food are known in modern times (Katan, 1996). Nowadays acute health problems rarely originate from food contact materials. Exposure to substances migrated from food contact materials should be monitored and risk of the exposure to migrated substances should be assessed. Special attention should be paid to long-term exposures to low doses

of chemicals (or nanoparticles) in food contact materials. Public and decision-makers should be informed about possible threats and how to avoid those (Bačnik et al., 2016).

1.4. Migration Testing of Plastic Materials and Articles

Determination of substances migrated from plastics into foodstuffs is a very complex task, especially determination of organic substances. It is also time-consuming, as to get realistic data concentration should be determined at the end of the shelf life of the foodstuff. Because of that, migration testing of plastic materials not yet in contact with foodstuffs is mostly performed using solutions simulating real foodstuffs which are called simulants. The material is put in contact with the simulant for a certain time, temperature and surface to volume ratio in order to mimic real migration into foodstuffs. Most extreme conditions of time and temperature foreseeable in actual use are used. After exposure to food contact material (under the most extreme conditions of time and temperature foreseeable in actual use), simulants are analyzed by using the appropriate analytical technique (e.g. gas chromatography or liquid chromatography with appropriate detectors, ICP MS etc.) and concentration of migrated substances are determined. Amount of a substance migrated in food/simulant is called specific migration. Overall migration, on the other hand, is determined as the amount of all nonvolatile substances released from a material or article into food simulants. Overall migration into aqueous simulants is determined simply by evaporating the simulant, whereas the determination of overall migration into fatty food simulants is more complicated and involves gas chromatography phase.

For testing of plastic materials and articles not yet in contact with food, simulants listed in Table 1.1 are used (European Commission, 2016).

Table 1.1: List of food simulants used for testing of plastic food contact materials according to Regulation 10/2011/EC (amendment, August 2016).

Simulant	Abbreviation
Ethanol 10 % (v/v)	Food simulant A
Acetic acid 3 % (w/v)	Food simulant B
Ethanol 20 % (v/v)	Food simulant C
Ethanol 50 % (v/v)	Food simulant D1
Any vegetable oil containing less than 1 % unsaponifiable matter	Food simulant D2
poly(2,6-diphenyl-p-phenylene oxide), particle size 60-80 mesh, pore size 200 nm	Food simulant E

Simulants A, B and C (and deionised water) are used for foods that have a hydrophilic character and are able to extract hydrophilic substances.

Food simulant B is used for those foods, which have a pH below 4.5. Food simulant C is used for alcoholic foods with an alcohol content of up to 20 % and those foods, which contain a relevant amount of organic ingredients that render the food more lipophilic. Food simulants D1 and D2 are assigned for foods that have a lipophilic character and are able to extract lipophilic substances. Food simulant D1 is used for alcoholic foods with an alcohol content of above 20 % and for oil in water emulsions (such as milk and milk products). Food simulant D2 is used for foods, which contain free fats at the surface. Food simulant E is used for testing specific migration into dry foods and for determination of overall migration at high temperatures.

Table 1.2: Food simulants for fruits and vegetables as specified in Regulation 10/2011/EC (amendment in August 2016). For food categories where the cross is followed by (*), the testing in food simulant B can be omitted if the food has a pH of more than 4.5.

Description of food	Food simulants					
	A	B	C	D1	D2	E
Fruit, fresh or chilled:						
A. Unpeeled and uncut						X/10
B. Peeled and/or cut	X	X(*)				
Vegetables , fresh or chilled:						
A. Unpeeled and uncut						X/10
B. Peeled and/or cut	X	X(*)				

For testing, if specific migration limit of a certain substance for the contact with known food is exceeded (specific migration limit is a maximum permitted amount of a substance in food), simulants are chosen from the table published in the above-mentioned Regulation 10/2011 (amended in 2016). Part of that table (describing fruits and vegetables and correspondent simulants) is presented in Table 1.2.

When testing specific migration, the sample is placed in contact with the food simulant in a manner representing the worst of the foreseeable conditions of use as regards the contact time in Table 1.3 and as regards the contact temperature in Table 1.4.

Table 1.3: Contact times for specific migration testing as specified in Regulation 10/2011/EC.

Contact time in worst foreseeable use	Test time
$t \leq 5 \text{ min}$	5 min
$5 \text{ min} < t \leq 0.5 \text{ hour}$	0.5 hour
$0.5 \text{ hours} < t \leq 1 \text{ hour}$	1 hour
$1 \text{ hour} < t \leq 2 \text{ hours}$	2 hours
$2 \text{ hours} < t \leq 6 \text{ hours}$	6 hours
$6 \text{ hours} < t \leq 24 \text{ hours}$	24 hours
$1 \text{ day} < t \leq 3 \text{ days}$	3 days
$3 \text{ days} < t \leq 30 \text{ days}$	10 days
Above 30 days	See specific conditions

Table 1.4: Contact temperatures for specific migration testing as specified in Regulation.

Contact temperature in worst foreseeable use	Test temperature
$T \leq 5 \text{ }^{\circ}\text{C}$	5 $^{\circ}\text{C}$
$5 \text{ }^{\circ}\text{C} < T \leq 20 \text{ }^{\circ}\text{C}$	20 $^{\circ}\text{C}$
$20 \text{ }^{\circ}\text{C} < T \leq 40 \text{ }^{\circ}\text{C}$	40 $^{\circ}\text{C}$
$40 \text{ }^{\circ}\text{C} < T \leq 70 \text{ }^{\circ}\text{C}$	70 $^{\circ}\text{C}$
$70 \text{ }^{\circ}\text{C} < T \leq 100 \text{ }^{\circ}\text{C}$	100 $^{\circ}\text{C}$ or reflux temperature
$100 \text{ }^{\circ}\text{C} < T \leq 121 \text{ }^{\circ}\text{C}$	121 $^{\circ}\text{C}$ (*)
$121 \text{ }^{\circ}\text{C} < T \leq 130 \text{ }^{\circ}\text{C}$	130 $^{\circ}\text{C}$ (*)
$130 \text{ }^{\circ}\text{C} < T \leq 150 \text{ }^{\circ}\text{C}$	150 $^{\circ}\text{C}$ (*)
$150 \text{ }^{\circ}\text{C} < T < 175 \text{ }^{\circ}\text{C}$	175 $^{\circ}\text{C}$ (*)
$T > 175 \text{ }^{\circ}\text{C}$	Adjust the temperature to the real temperature at the interface with the food (*)

* This temperature shall be used only for food simulants D2 and E. For application heated under pressure migration testing under pressure at the relevant temperature may be performed. For simulants A, B, C or D1 the test may be replaced by a test at 100 $^{\circ}\text{C}$ or at reflux temperature for the duration of four times the time selected according to the conditions in the table for contact time.

Specific conditions are used for contact times above 30 days (long term) at room temperature and below. For such use materials and articles are tested in accelerated test conditions, at an elevated temperature for a maximum of 10 days at 60 $^{\circ}\text{C}$. For specific food contact situations, lower temperatures are also used. For example, testing of materials for 10 days at 20 $^{\circ}\text{C}$ cover all storage times at frozen conditions. This test includes the freezing and defrosting processes if labelling or other instructions ensure that 20 $^{\circ}\text{C}$ is not exceeded and the total time above 15 $^{\circ}\text{C}$ does not exceed 1 day in total during the foreseeable intended use of the material or article. Testing for 10 days at 40 $^{\circ}\text{C}$ covers all storage times at refrigerated and frozen conditions including hot-fill conditions and/or heating up to $70 \text{ }^{\circ}\text{C} \leq T \leq 100 \text{ }^{\circ}\text{C}$ for maximum $t = 120/2^{((T-70)/10)}$ minutes. Testing for 10 days at 50 $^{\circ}\text{C}$ covers all storage times of up to 6 months at room temperature, including hot-fill conditions and/or heating up to $70 \text{ }^{\circ}\text{C} \leq T \leq 100 \text{ }^{\circ}\text{C}$ for

maximum $t = 120/2^{((T-70)/10)}$ minutes. Testing for 10 days at 60 °C covers storage above 6 months at room temperature and below, including hot-fill conditions and/or heating up to 70 °C $\leq T \leq 100$ °C for maximum $t = 120/2^{((T-70)/10)}$ minutes.

At first sight, these tables do not seem difficult for use (at least for plastic packaging). However, comparisons of test conditions (and consequently test results) selected for kitchen utensils in different laboratories showed that the selection of test conditions was not always unambiguously clear. To avoid such situations network of European Reference Laboratory and National Reference Laboratories for food contact materials developed a guidance document on the selection of testing conditions (Simoneau, 2009). The updated document is in preparation.

1.4.1. Migration testing of ceramics

Simulant 4 % acetic acid is put into contact with ceramics at a temperature of 22 ± 2 °C for the duration of 24 ± 0.5 hours (European Commission, 1984, European Commission, 2005), after that, the concentration of lead and cadmium is determined by a suitable instrumental method with defined performance criteria. The described test conditions derive from the old legislation. New migration limits, as well as test conditions, are under discussion at the moment.

1.4.2. Migration testing of other food contact materials

For testing of other conventional food contact materials (coatings, paper and board, rubber, glass, cork, metals and alloys etc.) there are no harmonized rules in the European Union. Laboratories use national legislation rules, if available. Council of Europe developed Resolutions and Technical documents which describe requirements and testing for many nonharmonised materials (Council of Europe, 2017). Council of Europe documents elaborated under Council of Europe European Directorate for the Quality of Medicines EDQM are not legally binding. Regardless of that, they are used by industry and many public laboratories. Document describing the specific release limits for different metals released from food contact materials made of metals and alloys and testing of food contact materials made from metals and alloys is most recent (Council of Europe, 2013). New proposed worst-case simulant for contact of metallic materials with acidic foodstuffs is citric acid (5 g/l). For contact with aqueous foodstuffs (with pH > 4.5) the proposed simulant is artificial tap water. Updated technical documents for other nonharmonised materials are in preparation.

Migration testing of coatings is usually performed in the same way as plastics. Testing of food contact materials is complex and procedures are designed for conventional food contact materials.

1.4.3. Characterisation and migration testing of nanotechnology-derived food contact materials

Materials which contain nanoparticles and released nanoparticles should be adequately characterized to obtain important information about their properties. Characterization of nanoparticles in food contact materials and in complex matrices such as food (or in simpler matrices such as food simulants) requires the use of sophisticated techniques and

multiple methodologies. Important information about nanoparticles released from nanotechnology-derived food contact materials is not only determination of chemical composition and their quantity, but also by other parameters such as size, shape, surface coatings (relevant physicochemical properties). Electron microscopy (SEM, TEM) equipped with energy dispersive X-ray spectroscopy (EDXS) unit is essential for general detection of nanoparticles and characterization of nanotechnology-derived food contact materials. Electron microscopy delivers information about size distribution and shape of nanoparticles. Determination of total element concentration is performed by use of other techniques, e.g., ICP-MS. Single particle ICP-MS gives information about mass and number concentration of nanoparticles, the ratio between ionic and nanoform and size distribution of nanoparticles. AF4-ICP-MS enables determination of mass concentration and size distribution of nanoparticles. XRD provides information about the crystal structure; electrokinetic potential measurements are used for determination of surface charge of the particles. Chromatography and other separation methods such as centrifugation and electrophoresis, surface characterization methods and their combinations are often needed (Tiede et al., 2008, Blasco & Pico, 2011, EFSA, 2011, Calzolari, Gilliland, & Rossi, 2012, Mattarozzi et al., 2017). There is a lack of validated methods and of suitable reference materials to assure the accuracy of the results with respect to determined sizes and concentrations.

The release of nanoparticles from nanotechnology-derived food contact materials into foods poses challenges and is not yet studied enough (EFSA, 2009, Liu, Yu, Yin, & Chao, 2012). The release is mostly studied into food simulants. There is inconsistency in the literature whether the release or migration of nanoparticles is possible or not and what are the mechanisms of release. Most of the articles report on migration of silver nanoparticles (e.g. Song, Li, Lin, Wu, & Chen, 2011, Huang et al., 2011, Echegoyen & Nerin, 2013, von Goetz et. al. 2013, Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2013, Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2014, Addo Ntim, Thomas, Begley, & Noonan, 2015, Artiaga, Ramos, Ramos, Cámara, & Gómez-Gómez, 2015, Hannon et al., 2016, Ramos, Gómez-Gómez, Cámara, & Ramos, 2016, Mackevica, Olsson, & Hansen, 2016, Gallochio et al., 2016). Some papers describe nanoclays migration (e.g. Schmidt et al., 2011, Farhoodi, Mousavi, Sotudeh-Gharebagh, Emam-Djomeh, & Oromiehie, 2014, Echegoyen, Rodríguez, & Nerín, 2016), combination of nanosilver and nano TiO₂ (Metak, Nabhani, & Connolly, 2015), migration of carbon black (Bott, Stormer, & Franz, 2014a) and also migration of titanium from nanoTiO₂ polyethylene composite packaging (Lin et al., 2014). Measured concentrations of migrated nanoparticles (if any) are usually very low. Different sophisticated analytical techniques should be applied to detect them in food or simulants with adequate selectivity and sensitivity to quantify them accurately and precisely.

To the best of our knowledge, there are only two papers that refer to the migration of nanoparticles from food contact material into food. One describes the migration of silver nanoparticles from commercially available nanosilver plastic containers and nanosilver coated cling films into different foods (showing the highest migration into orange juice) as reported by Metak, Nabhani, & Connolly in 2015. The other describes the migration of silver nanoparticles from PVC nanocomposite into chicken breasts (Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2013). In the papers complexities associated with detection, characterisation and quantification of migrated particles into real food are emphasized. There are also papers which describe the stability of nanoparticles in food after spiking the food with nanoparticles. Two papers describe the determination of silver nanoparticles by sp ICP MS in spiked chicken meat after enzyme degradation of meat (Loeschner et al., 2013, Peters et al., 2014). The third paper describes the stability of silver nanoparticles in apple juice (Witzler, Kullmer, Hirtz, & Gunther, 2016).

For the moment migration testing is not specially adapted to specific properties of the nanoparticles.

1.5. Potential Health Risks of Nanotechnology-Derived Food Contact Materials

In general, all materials in contact with foodstuffs should be safe including nanotechnology-derived food contact materials. In order to assess possible risk due to potential migration of nanoparticles from food contact materials, a comparison of human exposure to migrated nanoparticles (estimated on the basis of the results of migration studies and food consumption data) to predicted safe levels of exposure, obtained from the results of toxicological studies conducted in animals (hazard characterisation) is performed. The risk assessment paradigm (hazard identification and hazard characterisation followed by exposure assessment and risk characterisation) is used. Hazard identification involves the identification of biological, chemical, and physical agents capable of causing adverse health effects (EFSA, 2017). In the case of nanotechnology-derived food contact materials such agents may be nanoparticles. Hazard characterization, the second step in risk assessment, involves defining the nature of the adverse health effects associated with biological, chemical and physical agents which may be present in food and should involve an understanding of the doses involved and related responses (EFSA, 2017).

1.5.1. Potential harmful effects of nanoparticles

For understanding interactions of nanoparticles with living organisms, a comparison of their size with the size of food substances and living systems is useful (Figure 1.6). Many food substances are nanosized, e.g. food proteins and polysaccharides. Most engineered nanoparticles are larger than lipids, similar size to many proteins, and smaller than cells.

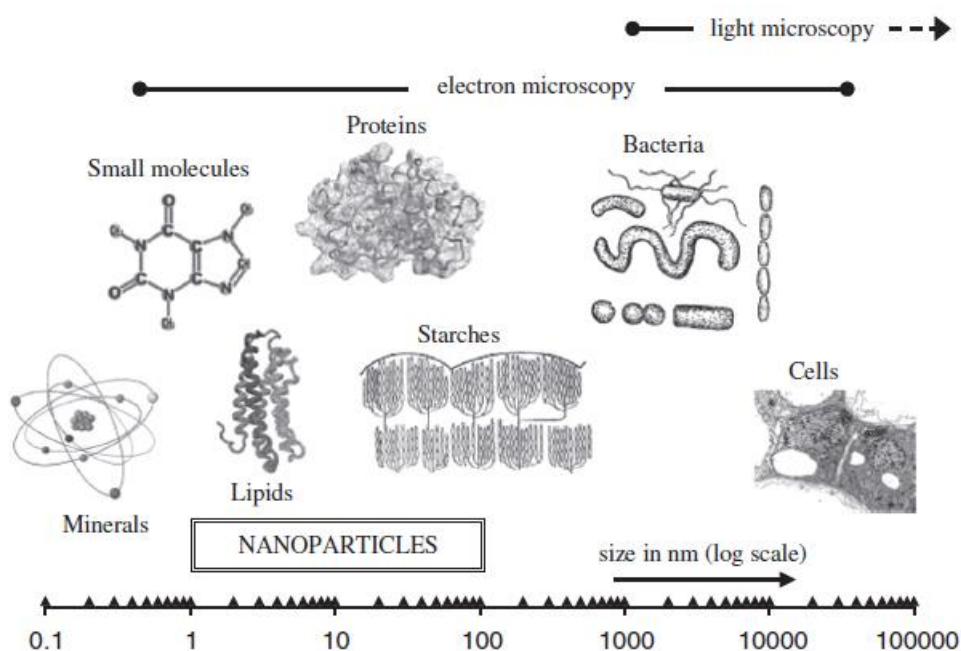


Figure 1.6: Nanoparticles placed in the context of other components in foods (from the book *Nanotechnologies in food* (Chaudhry, Castle, & Watkins, 2010).

If nanoparticles are soluble, it is expected that their toxicity is close to the bulk materials, while insoluble bio-persistent nanoparticles might accumulate in the body (SCENIHR, 2005, EFSA, 2009).

Nanoparticles have unique chemical and physical properties which may play an important role in their potential biological effects. Special properties originate from the large surface area. On the surface of nanoparticles, more atoms are present than in bulk materials, and quantum effects become evident. Their toxicokinetic and toxicodynamic profiles cannot be simply extrapolated from data on their macroscopic counterparts. For that reason thorough characterisation is very important: not only determination of chemical composition and size, but also size distribution, shape, solubility, crystallinity, surface charge, aggregation and agglomeration state, basic morphology, purity, stability, surface area, surface chemistry, surface charge, density, catalytic activity, octanol-water partition coefficient, bioaccumulation potential etc. (Nel, Xia, Madler, & Li, 2006, EFSA, 2009, EFSA, 2011, Tiede et al., 2008, Warheit, Sayes, Reed, & Swain, 2008, Sajid et al., 2015).

For the substances used in food contact materials, the amount of toxicity data needed for safety assessment is related to the expected human exposure level. The higher the exposure, the greater the amount of data required and the so-called “tiered approach” is applied (EFSA, 2016a), as shown in Table 1.5. However, the tiered approach refers to conventional food contact materials. For nanomaterials in contact with food, possible exposure to nanoparticles migrated from food contact materials is specifically considered in EFSA Guidance on nanoscience and nanotechnologies (EFSA, 2011). Due to the limited knowledge on the behaviour and effects of migrated nanoparticles, there is no trigger value under which we could say that there is no health concern. Therefore these novel materials should be considered case-by-case. Information on genotoxicity, absorption, distribution, metabolism and excretion (ADME) and repeated-dose 90 day

oral toxicity study in rodents is required, as well as all other available information on toxicity available (e.g. studies on reproduction and developmental toxicity, studies on long-term toxicity/carcinogenicity, studies on endocrine activity) (EFSA, 2011).

Effects of engineered nanoparticles ingested through gastrointestinal route are not well known yet (EFSA, 2009, Binderup et al., 2013). Studies on nanoparticles show that nanoparticles can enter circulation from the gastrointestinal tract, depending on the physicochemical properties of the nanoparticles, e.g. size and on the physiological state of the organs of entry (Silvestre, Duraccio, & Cimmino, 2011). Generally speaking, there are two major organs for distribution of nanoparticles from circulation: the liver and the spleen (De Jong et al., 2008, SCENIHR, 2014), but all organs may be at risk including the brain and the reproductive system. The smallest gold nanoparticles showed the most widespread distribution in different organs – liver and spleen, as well as blood, lung, kidney, thymus, brain and testis (De Jong et al., 2008). Knowledge of the long-term behaviour of nanoparticles is very limited. The worst-case assumption is that nanoparticles may accumulate in some organs with consequences not yet studied enough. Nanoparticles have the ability to cross cellular barriers, which can lead to the production of oxyradicals and consequently, oxidative damage to the cell (Fadeel, Pietroiusti, & Shvedova, 2012). A major consequence of oxidative stress is damage to nucleic acid bases, membrane lipids and proteins, which in turn may contribute to the development of various diseases.

1.5.2. Exposure to nanoparticles from nanotechnology-derived food contact materials and assessment of possible health risk through exposure to released particles

At the moment, estimation of exposure to nanoparticles originating from food contact materials is difficult to perform because there is still a gap in knowledge about nanoparticles present in nanotechnology-derived food contact materials and released from such materials into food (Chaudhry et al., 2008, Stamm, Gibson, & Anklam, 2012, Noonan, Whelton, Carlander, & Duncan, 2014, Bumbudsanpharoke & Ko, 2015, Jokar, Pedersen, & Loeschner, 2017), although the number of scientific papers about migration of nanoparticles from food contact materials is increasing.

If nanoparticles are present in food due to migration or release, in order to assess a possible health risk, exposure assessment should be performed as presented in the decision tree (Figure 1.7) from the EFSA Guidance document (EFSA, 2011).

Table 1.5: A tiered approach to toxicity testing of substances released from (conventional) food contact materials according to EFSA document Recent developments in the risk assessment of chemicals in food and their potential impact on the safety assessment of substances used in food contact materials (EFSA, 2016a).

Tier number and specifications	Toxicity data required	Additional considerations
Tier 1: Human exposure up to 1.5 µg/kg bw per day or if the substance is classified as Cramer class I(a) and exposure is less than 30 µg/kg bw per day	-3 Genotoxicity studies -Available information including appropriate literature search including the existing information from other databases (e.g. ECHA)	In general, no other toxicity studies are required below this threshold. Exceptions are: (1) if there are existing data indicating the potential to affect endocrine or neural systems; (2) for substances with a high potential to accumulate in humans; (3) for nanomaterials, even if the non-nanoform material has been evaluated and approved for food contact materials.
Tier 2: Human exposure from 1.5 to 80 µg/kg bw per day	As in Tier 1 plus: Extended 90-day oral toxicity study in rodents	A study on ADME should be used to assess the potential for accumulation in humans of substances for which such a potential could be anticipated. If there are existing data indicating endocrine activity suggesting potential effects from prenatal exposure, a 90-day study with a prenatal treatment period or an extended one-generation reproduction toxicity study (EOGRTS) should be considered.
Tier 3: Human exposure higher than 80 µg/kg bw per day	As in Tier 1 and 2 plus (b): -Study on ADME -Studies on reproduction and developmental toxicity - Studies on long-term toxicity/carcinogenicity	If there are existing data indicating endocrine activity suggesting potential effects from prenatal exposure, a chronic study with a prenatal treatment period or an extended one-generation reproduction toxicity study should be considered.

(a): Substances of simple chemical structure with known metabolic pathways and innocuous end products which suggest a low order of oral toxicity according to Cramer classification scheme

(b): The extended 90-day oral toxicity study required in tier 2 might not be necessary as it is covered by the long-term testing.

1.5.3. Assessment of possible health risk through exposure to released particles

The main risk of consumer exposure to nanotechnology-derived food contact materials is through potential migration of nanoparticles into food. EFSA position regarding risk assessment of released nanoparticles is that risk assessment has to be performed on a case-by-case basis (EFSA, 2009). EFSA prepared Guidance on the risk assessment of the application of nanoscience and nanotechnologies in the food and feed chain (EFSA, 2011). If there is no exposure to engineered nanomaterials (e.g. there is no migration from food contact materials, or material is transformed to non-nanoform in food/feed matrix before ingestion), relevant EFSA guidance for non-nanoform should be followed. However, the potential risk for human health may arise if migration of nanoparticles occurs. The risk through exposure of nanoparticles released from food contact materials is assessed taking into account the chemical composition and physicochemical properties of nanoparticles, interactions with tissues, and potential exposure levels.

In general, the risk assessment of oral exposure to nanoparticles is difficult task because of a general lack of data and a number of uncertainties (H. Bouwmeester et al., 2009 Bouwmeester, Brandhoff, Marvin, Weigel, & Peters, 2014, Peters et al., 2014).

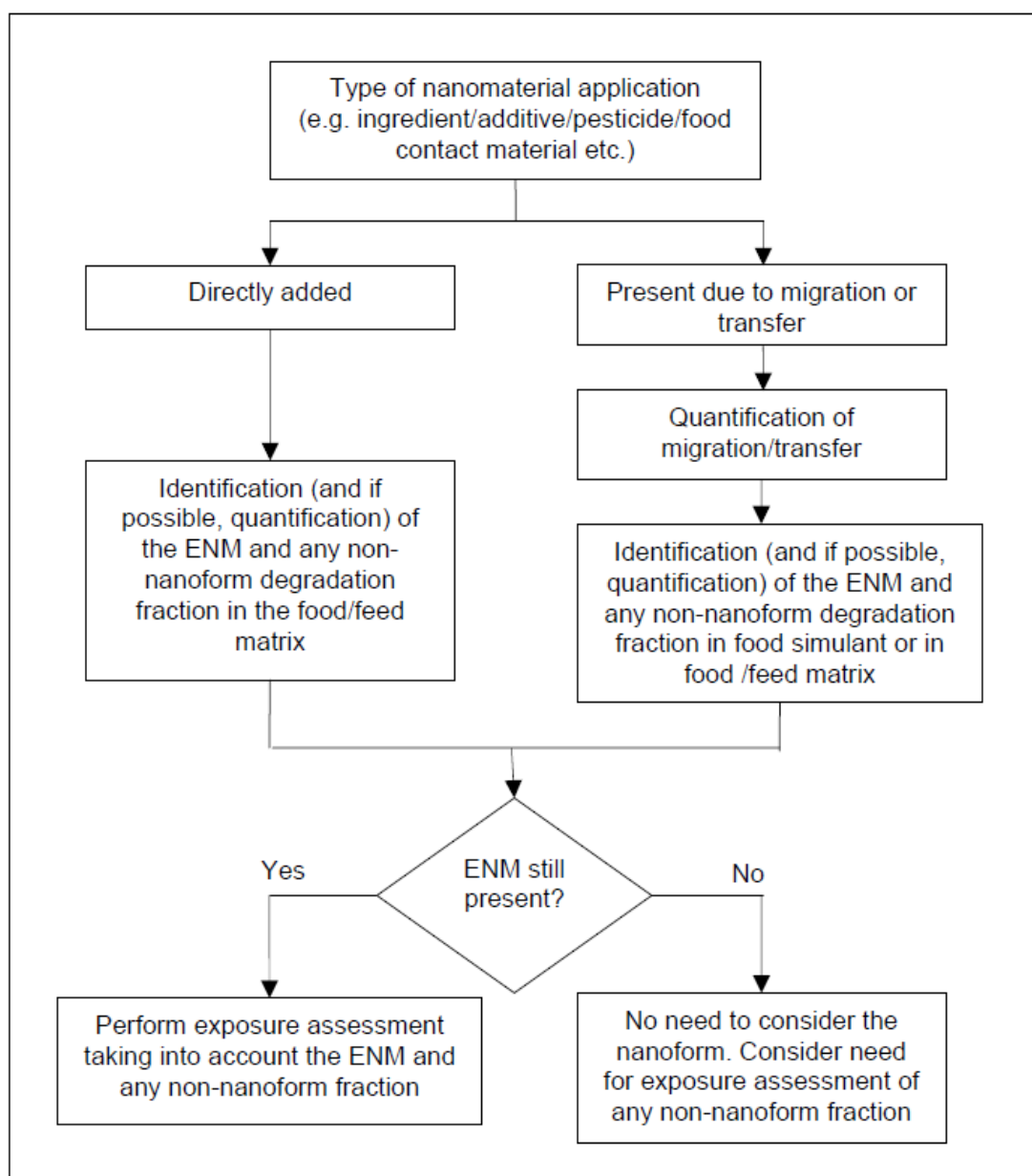


Figure 1.7: Decision tree for performing exposure assessment taken from EFSA document (EFSA, 2011).

Chapter 2

Aims and Hypothesis

Food contact materials are materials which are in contact with food during food production, processing, food storage, preparation and serving. Recently, new food contact materials based on nanotechnology are developed and are already present on the market. These novel materials have improved the mechanical strength, thermal resistance, flexibility, barrier properties, antimicrobial and other functional properties. To achieve these special properties, engineered nano-objects (ENOs) i.e. nanoparticles, nanofibers, nanoplates or other structures are added to polymeric matrices. ENOs have different physicochemical and biological properties in comparison with their bulk counterparts, so possible release into food is not desirable and raised safety concerns. The release of ENOs from the materials into foods is not yet studied enough. There is inconsistency in the literature whether release or migration of ENOs from food contact materials is possible or not, what are the mechanisms of release and what are the possible health risks related to the release. Migration testing is not specially adapted to specific properties of the nanoparticles, and toxicological studies are not available for all ENOs. Characterization of these novel materials and study of ENOs release is complex and requires the use of sophisticated analytical techniques, which are usually not available in official control food contact laboratories.

The aim of the doctoral thesis was to determine if commercially available non-stick food contact materials (pan coatings) contain ENOs, to study if the release of ENOs from such materials into food is possible and to assess if a possible release poses a risk to the consumer's health. The aim was also to develop a simple screening method, which could be used for detection of ENOs present in food contact materials and simulants.

For the study of a possible ENOs release, commercial quasi-ceramic non-stick pan coatings, materials that possibly contain nanoparticles, were selected. For characterisation of such food contact materials, the use of different techniques (Fourier-transform infrared spectroscopy, electron and optical microscopy, XRD analysis) which enable identification of the polymer matrix and the type and morphology of nanoparticles present in food contact material is essential. After characterization, migration testing of characterised food contact material with different simulants was done. Migration of nanoparticles may be caused by various mechanisms:

- (i) desorption, during which the particles adsorbed to the surface of food contact material are released into the food;
- (ii) diffusion, i.e. the movement of particles through the food contact material and, then, their release to food;
- (iii) dissolution when the particles dissolve and ions are released into food;

(iv) matrix degradation, which can be caused by thermal decomposition, mechanical abrasion, UV light or hydrolysis/dissolution of the matrix. Accordingly, additional studies (thermal analysis, tribological and scratch tests) were performed. Obtained results of migration/release were used for screening of exposure. By the use of toxicological data available from the scientific literature, estimated exposure was compared to safe levels of exposure (levels of exposure above which humans should not be exposed), and a possible health risk due to released nanoparticles was characterised.

Our main hypotheses were:

- nanoparticles can be released from quasi-ceramic coatings into the food by different mechanisms,
- a screening method can be used for detection of titanium dioxide and silver nanoparticles present/released from food contact materials.

Chapter 3

Materials and Methods

Three new pans with non-stick coatings (P1-P3) were purchased from the Slovenian market. Three additional pans (U1-U3) were collected after one (U1) or two years (U2 and U3) of typical domestic use on the electric stove (approximately 50-100 hours in total) and analyzed afterwards. Pan U1 was used for one year with careful maintenance (without the use of sharp utensils and dishwasher). Pan U2 was used for two years with careful maintenance. Pan U3 was used for two years with no special care (with use of sharp objects and dishwasher machine). Examinations were performed on rounded samples with a radius of 15 mm, cut off from the pans by punching.

Table 3.1: Samples used in the kitchen and examined in the research.

Sample name	Time in use	Use of sharp objects	Use of dishwasher
U1	1 year	No	No
U2	2 years	No	No
U3	2 years	Yes	Yes

3.1 Chemicals

The main chemicals used during the research work are listed in Table 3.2.

Table 3.2: Chemicals used in the research.

Chemical	Producer
Acetic acid (glacial) 100 % anhydrous, p.a.	Merck KGA, Germany
AgNO ₃	Sigma-Aldrich, USA
Citric acid monohydrate, p.a.	Merck KGA, Germany
CTAB, cetyl trimethylammonium bromide, minimum 99 %	Sigma-Aldrich, USA
Darvan C (ammonium polymethacrylate)	Vanderbilt Minerals, USA
deionised water (ultrapure water, 18.2 MΩ cm)	ELGA Purelab Classic UK and/or Merck Millipore, USA
4 % H ₃ BO ₃	Merck Millipore
HEPES	Acros Organics, USA
30 % HCl	Merck Millipore
40 % HF	Merck Millipore
65 % HNO ₃	Merck Millipore
0.01 M NaOH	Merck Millipore
methylene blue	Fluka Sigma Aldrich, USA
PEI 600 (polyethyleneimine)	Alfa Aesar, Germany
olive oil	Gea, Tovarna olja Gea d.o.o., Slovenia
Sodium borohydride 12 % solution in 40 % aq. Sodium hydroxide solution, nitrogen flushed, 100 ml	Acros Organics, USA
PTFE tape	Facot chemicals, Italy
Ti standard solutions 1000 ± 4 mg L ⁻¹ in 0.1 % nitric acid	Merck Millipore
TiO ₂ P25 nanoparticles	Sigma Aldrich, USA
TiO ₂ rutile nanoparticles	Sigma Aldrich, USA
Trisodium citrate dihydrate	Merck, Germany
Triton X-100 (98–100%)	Merck Millipore

3.2 Methods of Characterisation

3.2.1 Non-stick properties (wetting behaviour)

The sessile drop contact angles formed by deionised water and olive oil on the surfaces were examined by using an optical tensiometer (Theta Lite T101, Attension, Biolin Scientific, Finland). The results are calculated as the mean of five measurements per sample.

3.2.2 Fourier transform infrared spectroscopy

The identification of the coating matrix materials was obtained by Fourier transform infrared spectroscopy (FTIR, Perkin Elmer Spectrum 65, UK) in diffuse reflectance mode (DRIFT). The DRIFT spectra were collected with a resolution 4 cm⁻¹ in the range of 4000-450 cm⁻¹, each generated from 16 scans. The identification of the typical bands was achieved by comparison with reference spectra.

3.2.3 Electron and optical microscopy

Top surfaces and cross-sections of the pan coatings were examined by field emission scanning electron microscopy (FE SEM, JEOL JSM 7600F, Japan) and focused ion beam scanning electron microscopy (FIB SEM, FEI Nanolab Helios 650 dual beam, OR, USA). The chemical composition and element distribution analyses were performed by the use of standardless semi-quantitative energy dispersive X-ray spectroscopy (EDXS). After the qualitative estimation of the amount of added NPs in the non-used pans, further analyses were performed on the selected coatings with apparently the largest number of nanoparticles. The thickness of the coating P2 was observed at the cross-section of the pans by an optical microscope (Zeiss Imager.Z1m, Germany). The appearance of the top surface, the morphology of the scratches and the presence of wear debris were observed by a stereo microscope (Zeiss Discovery V8, Germany) and FE SEM. SEM image of TiO₂ particles in simulant 3 % acetic acid after migration testing was taken by evaporation of the simulant, re-dispersion of the residue in absolute ethanol, and drying of the dispersion directly on a conductive SEM sample holder. Size of the particles embedded in silicone and size distribution were estimated manually from the high resolution SEM images.

3.2.4 X-ray diffractometry

The crystal structure was analyzed by X-ray diffractometry (XRD, Siemens D5000, Germany) using a CuK α radiation (1.5406 Å), with the 2 θ angle scanned from 10° to 70° and a step of 0.04° (holding time 1 s).

3.2.5 Thermal analysis

The thermal degradation of the coating was examined by differential scanning calorimetry/thermogravimetry coupled with mass spectrometry (instrument STA 449 C/6/G Jupiter coupled with QMS 403 C Aëolos, both Netzsch, Germany) during heating

up to 700 °C, with a rate 10 °C /min and argon/20 % oxygen gas atmosphere with a flow rate 10 ml/min.

3.2.6 Scratch and tribological testing

A linear progressive scratch test was performed on the sample P2 using a diamond indenter (Revetest Scratch tester CSM Instruments RST S/N 01-04283, Switzerland) to verify the mechanical resistance of the coating toward scratching with metallic objects (e.g. cutlery). The load was progressively (linearly) increased from 1 N to 30 N, with a rate of 20.7 mm/min and scratch length of 3 mm. The appearance of the wear debris was observed by stereo microscope and FE SEM.

The wear behaviour of the coating P2 was tested on the dedicated tribological tester (UMT-2, CETR, CA, USA) in the form of a pin-on-disk reciprocal sliding configuration to mimic the worst-case long-term conditions. The tribological tests were conducted for one hour at a normal load of 3 N, with a stroke of 5 mm and sliding velocity of 0.03 m/s (3 Hz). Polymeric (PA6) and ceramic (Al_2O_3) pins were used against the pan sample, with contact pressures ranging from 1 MPa to 300 MPa, in order to simulate various scenarios of severity during cutlery contact with the pan surface. Namely, it is estimated that a normal use of wooden or polymer cutlery would result in pressures well below 100 MPa; however, metal contacts with forks or even knives in a pan will easily exceed 300 MPa.

3.2.7 Simulation of dishwashing

Simulation of dishwashing was performed by immersing the punched samples wrapped with PTFE tape (except for the surface of quasi-ceramic coating) in 0.01 M NaOH for 2 hours at 70 °C (vial containing 3 punched samples and 9 ml of NaOH solution). The procedure was repeated 7 times on the same samples.

3.2.8 Migration testing

Samples of intact P2 coatings and P2 coatings after simulation of dishwashing were tested three times successively for 2 hours at 100 °C in an oven with controlled temperature (Binder, WTC Germany) by total immersion into simulants: deionised water (Milli-Q, 18.2 M Ω cm, Merck Millipore, USA), 3 % acetic acid and 5 g/l citric acid (both Merck, Germany, prepared with Milli-Q water). For one migration test, three punched samples were immersed into 9 ml of simulants. Cut edges and surfaces that were not covered by non-stick coatings were protected by wrapping with PTFE tape. The collected migration dispersions were ultrasonicated for 10 minutes in ultrasonic bath prior to further procedures.

3.2.9 Total titanium concentrations determination

3.2.9.1 Filtration and ultrafiltration

The particles present in simulants after migration testing were separated from ions by ultrafiltration. 3 ml of simulants were ultrafiltered using 3kD (ca. 1-2 nm pore size) Amicon Ultra-4 Centrifugal Filter Units (Merck Millipore, USA). Simulants were

centrifuged at 8000 rpm for 30 min to separate particles from the dissolved fraction. Simulants were ultrasonicated in an ultrasonic bath for 10 minutes before ultrafiltration. The debris formed after tribological testing was rinsed with 9 ml of simulant deionised water. Particles larger than 0.2 μm were removed from the dispersion by filtration (cellulose nitrate filter, Sartorius Germany, 0.2 μm).

3.2.9.2 Microwave digestion and ICP-MS measurements

The determination of total titanium concentration (ions and particles) was performed following the two-step microwave-assisted digestion procedure described previously (Vidmar et al. 2016). 4 ml of 65 % HNO_3 , 2 ml of 40 % HF and 1 ml of 30 % HCl (all Merck Millipore, USA) were added to 2 ml of colloidal suspensions in a high-pressure PTFE vessel. The sample was subjected to closed vessel microwave-assisted digestion at the maximal power of 1200 W: ramp to temperature 200 $^{\circ}\text{C}$ in 30 min, at pressure 50 bar, holding at 200 $^{\circ}\text{C}$ for 60 min and cooling for 30 min (microwave oven CEM Mars X, CEM corporation, USA). Then the PTFE vessel was vented, and the vessel cap was removed. 6 ml of 4 % aqueous solution H_3BO_3 was added to complex the residual HF . In the second step of the microwave digestion, the following program was applied: ramp to temperature 200 $^{\circ}\text{C}$ in 15 min at pressure 50 bar, holding at 200 $^{\circ}\text{C}$ for 30 min, and cooling for 30 min. The content was quantitatively transferred to a 100 ml graduated perfluoro alkoxy (PFA) PTFE flask and filled to mark with water. The same procedure was applied to simulants before contact with the sample to determine concentrations of titanium in the blank. The preparation scheme of simulants for ICP-MS measurements is presented in Figure 3.1.

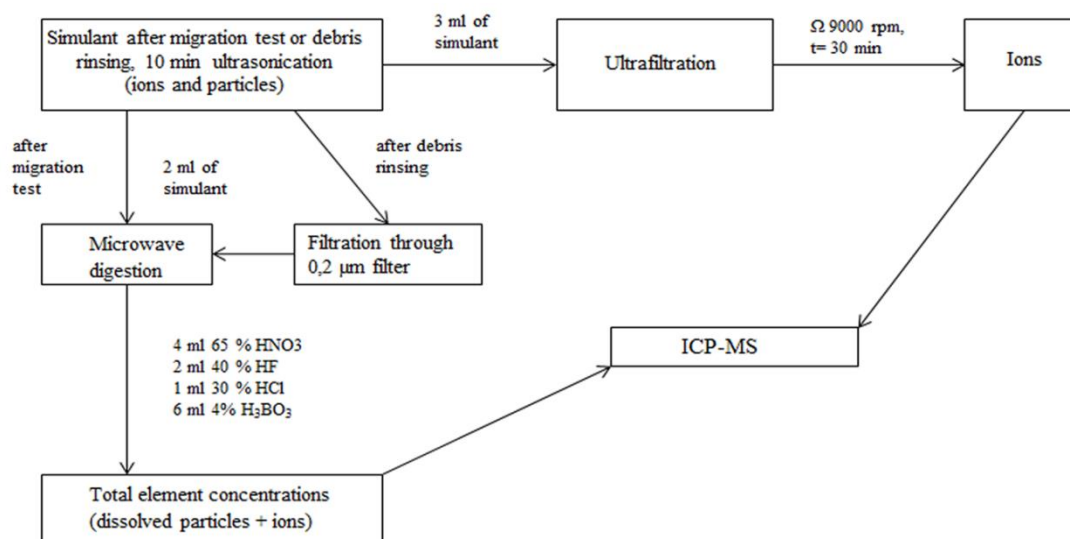


Figure 3.1: Simulants treatment for determination of total and dissolved titanium concentrations.

Concentrations of titanium in ultrafiltrates and digested samples were determined by the use of Agilent 7700 ICP-MS instrument (Agilent Technologies, Tokyo, Japan). ICP-MS operating parameters were optimised for plasma robustness and adequate sensitivity. The parameters of ICP-MS, equipped with a Scott spray chamber, Miramist PTFE nebulizer, and nickel skimmer and sample cones, were set as presented in Table 3.3. To

overcome possible polyatomic interferences from sulphur and nitrogen, ^{48}Ti isotope was monitored in High Energy Collision mode by the use of 10.0 ml/min He gas flow. ^{89}Y isotope was used as internal standard.

Table 3.3: ICP MS instrumental parameters.

Parameter	Type/Value
<i>Sample introduction</i>	
Nebuliser	Miramist
Spray chamber	Scott
Skimmer and sampler cone	Ni
<i>Plasma condition</i>	
Forward power	1550 W
Plasma gas flow	15.9 L min ⁻¹
Carrier gas flow	1.03 L min ⁻¹
He gas flow	10 mL min ⁻¹
Sample depth	8.0 mm
<i>Data acquisition parameters</i>	
Isotopes monitored	^{48}Ti
Isotopes of internal standards	^{89}Y
Integration time	0.3 s

Concentrations of titanium containing particles in simulants were determined by subtracting the ion concentrations, determined after ultrafiltration, from the total titanium concentrations, determined after microwave-assisted digestion.

3.2.10 Analysis of colloidal properties

The electrokinetic potential (zeta potential) of 20 mg/l nanorutile and P25 TiO_2 suspensions with or without addition of dispersants 0.5 % PEI 600, 1 % Darwan C or 1 % Triton X-100 and of scratched coating P2 was calculated by the Phase Analysis Light Scattering system (PALS, ZetaPALS Potential Analyzer, Brookhaven Instruments Ltd), based on the electrophoretic mobility principle. The Smoluchowski model was applied for the ZP calculations. Multi-Angle Particle Sizing option (scattering angle 90 °C, beam wavelength 658 nm, run time 1 min, 90Plus/BIMAS, Brookhaven Instruments Ltd) available on the ZetaPALS instrument was used for the assessment of the polydispersity index (PDI) and the effective hydrodynamic particle diameter (D_{eff}). Suspensions were ultrasonicated for 3 minutes by the use of ultrasonic homogenizer (UP400S, Hielscher Ultrasound technology, Germany, 400 watts, 24kHz), equipped with a 6 mm diameter probe, before zeta potential determination. It was operated at 10 % power output at a frequency 24 kHz and 50 % pulsed operation mode. Coatings were scratched from the pans by scalpel; debris was poured with deionised water and ultrasonicated for 3 minutes. Zeta potential curves of dispersion as a function of pH were determined for dispersions containing 20 mg of debris/l.

3.3 Exposure Assessment

For the screening of exposure to nano TiO₂ released from quasi-ceramic food contact coating, point estimates by considering single values for consumption of food and a fixed value for TiO₂ concentration in food were used. Conservative approach and uncertain inputs were used to ensure “worst-case” estimates of exposure. Assessment of exposure was based on 5 different scenarios. Scenarios from 1 to 3 were based on results of nanoparticles migration study with simulant 3 % acetic acid in combination with different food consumption approaches. A general model which describes exposure to chemicals released from food contact material (the exposure scenarios from 1 to 3, adapted from Pocas & Hogg, 2007) is written as:

$$\text{Estimated exposure} = \text{Concentration} \times \text{Food consumption} \quad (3.1)$$

Estimated exposure is calculated in µg/kg bw/day. Concentration represents the concentration of chemical in food (in µg/g food) and food consumption represents an intake of food which was in contact with food contact material (g food/kg bw/day).

Exposure to nano TiO₂ was assessed by considering the concentration of nano TiO₂ released from the coating into simulants and food consumption data.

Exposure estimate (EE) was calculated using concentration of released TiO₂ according to Equation 3.2:

$$EE = \text{TiO}_2 \text{ release} \times \text{Food consumption} \quad (3.2)$$

EE is estimated exposure in µg nano TiO₂/kgbw/day, calculated from TiO₂ release (in µg nano TiO₂ / g food, and food consumption data (sum of consumption data for vegetables and vegetable products and meat and meat products in g food/kg bw/day).

For the food consumption, anticipated average and high intakes of the food in various population groups (toddlers and adults) were estimated.

Food consumption data for scenario 3 were taken from EFSA Comprehensive European Food Consumption Database (EFSA, 2008), based on FoodEx, hierarchical system with 20 main food categories that are divided into subgroups to 4 levels. For our assessment FoodEx level 1 was used. The statistics for chronic food consumption (as the acute exposure was not considered relevant for the case of food contact materials) was available for the total population (‘all subjects’) and for consumers of respective food categories; we selected the worst-case approach – consumers only.

For scenario 4 body weight of 70 kg was used as the default for the European adult population (aged above 18 years) and 12 kg, as default body weight for toddlers (EFSA, 2012). For estimation of exposure based on Figure 4.14 (scenario 4) and on the results of tribological testing (scenario 5), Equation 3.3 was used:

$$EE = \frac{\text{TiO}_2 \text{ daily release}}{\text{body weight}} \quad (3.3)$$

Daily release (in µg nano TiO₂/day) was divided by default body weights (70 kg for adults or 12 kg for toddlers).

3.3.1 Calculation of released particles from the EM images

Calculation of the particles released from the coating during the use was performed by the assumption that the whole bottom of the pan surface (2.4 dm²), without the rim, is similarly damaged as the area presented in Figure 4.16 (1.58 10⁻⁵ dm²). Mass of released TiO₂ nanoparticles was calculated taking into account that coating material contained both TiO₂ and polymer matrix, and only 13 % of the mass of the coating is related to titanium. The assumption was also made that density of the coating is 1kg/m³ and that only part (approx. ¼) of debris is nanosized (as determined in the tribological test, Chapter 4.2.3.2). Sum of the area of simplified scratches in µm² (as marked in Figure 4.15B) was multiplied by the different depth of scratches (10, 20 and 30 µm) to get a range of volume/mass of debris. The assumption that pan was used two times a week during two years (104 weeks) or 208 times was used for the calculation of daily release according to Equation 3.4:

$$\text{Daily release} = \frac{\text{mass of debris}}{\text{times of use}} \quad (3.4)$$

The daily release is expressed in µg nano TiO₂/day.

3.4 Hazard Characterization

Derived No-Effect Level (DNEL) was calculated using Equation 3.5 (ECHA, 2012) :

$$DNEL = \frac{LOAEL}{AF1 * AF2 * AF3 * AF4} \quad (3.5)$$

DNEL is expressed in µg nano TiO₂/kgbw/day. AF1 is a factor for a dose-response relationship when extrapolating from Lowest Observed Adverse Effect Level (LOAEL) to No Observed Adverse Effect Level (NOAEL), AF2 is a factor for interspecies variation between animals and human, AF3 is a factor for intraspecies variation, AF4 is a factor for extrapolation from subchronic to chronic effects.

3.5 Risk Characterisation

For the risk characterisation, risk characterisation ratio RCR was calculated using Equation 3.6:

$$RCR = \frac{\text{Estimated exposure}}{20 \% DNEL} \quad (3.6)$$

3.6 Catalytic Activity of Nanoparticles

3.6.1 Catalytic activity of TiO₂ nanoparticles

Catalytic activity of TiO₂ nanoparticles in simulant deionised water was investigated by the use of methylene blue, sodium borohydride in neutral medium (buffer (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), at pH 7) as described in the paper written by Corredor et al. in 2015 (Corredor, Borysiak, Wolfer, Westerhoff, & Posner, 2015). Reduction of methylene blue with sodium borohydride in the presence of TiO₂ nanoparticles as catalysts was monitored in 3 ml disposable cuvettes by Cary 50 UV-Vis spectrophotometer (Varian, Australia). 1900 µl of an extract containing 20 mg/l rutile or 200 mg/l or P25 TiO₂ nanoparticles was mixed for two minutes with 500 µl of 0.05M HEPES buffer and 100 µl of 0.26 M sodium tetrahydroborate. To obtain positively charged nano TiO₂ particles, cetyl trimethylammonium bromide (CTAB) reagent was added. After mixing, 100 µl of 0.5 x 10⁻³ M methylene blue (MB) solution was added and discolouration of methylene blue was monitored in 3 ml disposable cuvettes by collecting visible spectra in the wavelength range from 500 to 800 nm by Lambda 950 UV/Vis/NIR spectrophotometer (Perkin Elmer, UK) or Cary 50 UV-Vis spectrophotometer (Varian, Australia).

3.6.2 Catalytic activity of silver nanoparticles

Yellow dispersion of silver nanoparticles, used for calibration curve in screening method, was synthesized from AgNO₃, sodium borohydride and trisodium citrate dihydrate as described in the paper written by Mendis et al. 2016. 6 ml of 0.01 M trisodium citrate was added to a beaker with 4 ml of 0.003 M sodium borohydride and stirred with a magnetic stirrer for 10 minutes. After that, 1 ml of AgNO₃ solutions (with different concentrations: 0.001M, 0.0005 M, 0.00033 M, 0.0002 M and 0.000125 M) were added drop by drop into the solution while stirring, to prepare 15.4 mg Ag nanoparticles/l, 10.3 mg Ag nanoparticles/l, 7.71 mg Ag nanoparticles/l, 5.14 mg Ag nanoparticles/l, 3.09 mg Ag nanoparticles/l and 1.93 mg Ag nanoparticles/l.

The catalytic activity of silver nanoparticles in extracts was investigated by the use of methylene blue, sodium borohydride in basic media (borate buffer, pH 9, Merck, Germany). Reduction of methylene blue with sodium borohydride in the presence of Ag nanoparticles as catalysts was monitored in 3 ml disposable cuvettes by Cary 50 UV-Vis spectrophotometer (Varian, Australia). All solutions were prepared by using deionised water. 1900 µl of extract containing Ag nanoparticles was mixed for two minutes with 500 µl of 0.05 M borate buffer and 100 µl of 0.26 M sodium tetrahydroborate, then 100 µl of 0.25 x 10⁻³ M methylene blue solution was added and discolouration of methylene blue monitored in cuvette by collecting visible spectra in the wavelength range from 500 to 800 nm. Visible spectra for calculation of reaction rate were recorded immediately after the addition of methylene blue (start of reaction) and then in 3 or 5-minute intervals.

Food contact paper containing Ag nanoparticles (printed RFID antenna) was prepared with nanosilver conductive ink (OrgaconTM Nanosilver Screen Printing Ink SI-P2000, Agfa, Mortsel, Belgium), 2 mg per antenna by the use of semi-automatic screen printer. Printing substrate was commercial high-white gloss label paper, with a functional coating on the reverse. 0.4 g of printed paper was cut into small pieces (ca. 5 x 5 mm), and extracted with 5 ml sodium citrate 0.01 M solution, 0.05 M borate buffer and ultrapure water by mixing with a magnetic stirrer for 60 minutes. After extraction,

extraction media was firstly filtered through 1.2 μm filter (Acrodisc 25 mm Syringe Filter with Versapor Membrane, Pall, USA), then through 0.2 μm filter (GHP Acrodisc 25 mm Syringe Filter, Pall, USA). For the reaction, 1900 μl of the filtrate was used.

Average reaction rates over the first 360 s were calculated by the use of Equation 3.7 (adapted from House, 2007):

$$\text{Average reaction rate} = - \frac{[MB \text{ at } t_2] - [MB \text{ at } t_1]}{t_2 - t_1} \quad (3.7)$$

Initial time (0 s) was used for t_1 and 360 s for t_2 . For the calculation of average reaction rate correspondent concentrations of MB were calculated using the calibration curve, prepared with 6 different concentrations of MB at time 0 (10 mg/l, 16 mg/l, 26.6 mg/l, 40 mg/l, 53.5 mg/l and 80 mg/l, or correspondent molar concentrations from 3.12×10^{-5} M/l to 2.5×10^{-4} M/l).

Chapter 4

Results and Discussion

Food contact materials containing nanoparticles available on the Slovenian market were characterized, and possible release of TiO₂ (nano)particles from the selected material was studied by migration testing into food simulants and examination of matrix degradation. After that, assessment of a possible health risk through exposure to released TiO₂ particles was performed. Finally, a screening method for detection of nanoparticles present in/released from food contact materials was developed and used for detection of TiO₂ and silver nanoparticles.

4.1 Characterisation of Food Contact Materials Containing Nanoparticles

For the moment, there are no legal requirements that food contact material containing nanoparticles should be declared as such, in contrast to the requirements on cosmetics (European Commission, 2009b) and foodstuffs (European Commission, 2011). Therefore, in order to find out if the material contains nanoparticles, characterization of the material presumably containing nanoparticles should be performed. Materials containing nanoparticles on the surface may show a non-stick effect, which can be observed by wetting behaviour.

Three new and three used food contact materials – pans (P1-P3 and U1-U3, respectively) with declared non-stick coatings were characterized by wetting behaviour, composition and microstructure.

4.1.1 Wetting behaviour

To confirm the non-stick effect, the wettability of representative coatings (the coating of the new pan P2 and used coating U1) was examined in water and olive oil. The non-stick effect is a result of the composition and/or special structure of the surface. Food particles on such surfaces are easily removed from the surface by water or oil because adhesion between the food particle and the water/oil droplet is higher than between the food particle and the surface of the material.

New and used coatings have the same matrix composition (silicone based polymer) and crystal structure of fillers (as shown by further characterization). However, new and used coatings showed different wetting behaviour, measured by contact angles (CA). The coating P2 was characterized by relatively high contact angles, namely 107° for water and 68° for oil, which is similar to the behaviour of the PTFE properties (Kalin &

Polajnar, 2014). However, after one year of use, water and oil repelling properties of the coating slightly deteriorate: the CA of coating U1 dropped down to 73° for water and to 55° for oil. This could be related to the increase in surface roughness (not examined), which is commonly observed for the materials with various compositions and can be expected to take into account microstructures of the used pan coating surfaces (see Figure 4.1).

4.1.2 Composition and microstructure

Composition and microstructure of the coatings were characterized by the use of Fourier Transform Infrared Spectroscopy, Electron Microscopy equipped with Energy Dispersive X-ray Spectroscopy and X-ray diffractometry (Golja et al., 2017).

FTIR spectra showed that all pan coatings (new and used) contained characteristic bands related to a silicone-based polymer (Smith, 1991, Pirson et al., 1995, Alexandru et al., 2010): the bands at $1300\text{--}1000\text{ cm}^{-1}$ belong to the Si-O-Si group, the bands at about 1260 cm^{-1} and about 1410 to Si-CH₃ group, and the bands at about 2960 cm^{-1} the CH stretching of CH₃ groups. One of the spectra (FTIR spectrum of P2 coating) is presented in Figure 4.2. The broadband centred at $500\text{--}700\text{ cm}^{-1}$, related to the Ti-O bond of TiO₂ (Gao, Masuda, Peng, Yonezawa, & Koumoto, 2003), is also observed in all spectra, as well as the absorption bands corresponding to the O-H group (at $3000\text{--}3700$ and $1620\text{--}1630\text{ cm}^{-1}$). Hydroxyl group absorption band may originate from adsorbed water or polysiloxane (functional groups terminating the chains or unreacted silanols), as well as from silica (Innocenzi, 2003; Tiwari & Hihara 2012). The presence of the Ti-O-Si band was also observed at about 910 cm^{-1} , as a confirmation of the bond between the TiO₂ particles and silicone (Song, Hasegawa, Yang, & Sato, 1988, Tsiourvas, Tsetsekou, Arkas, Diplas, & Mastrogianni, 2011).

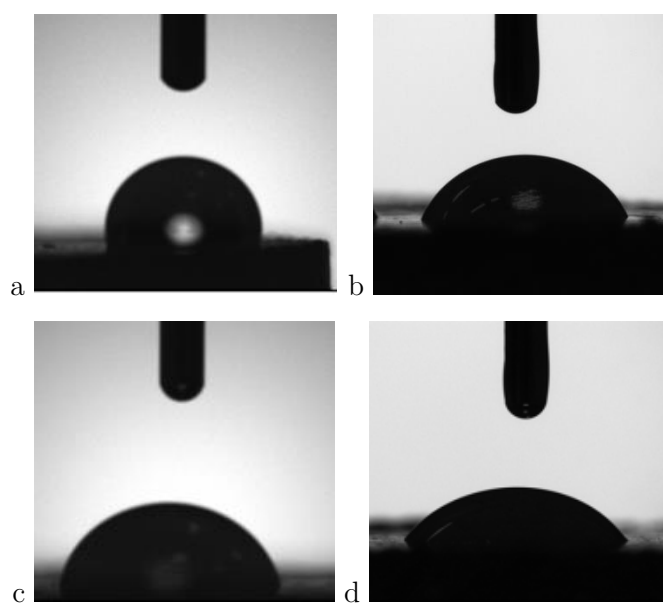


Figure 4.1: Contact angles with deionised water (a) and olive oil (b) of new pan coating P2 and contact angles with deionised water (c) and olive oil (d) of used pan coating U1.

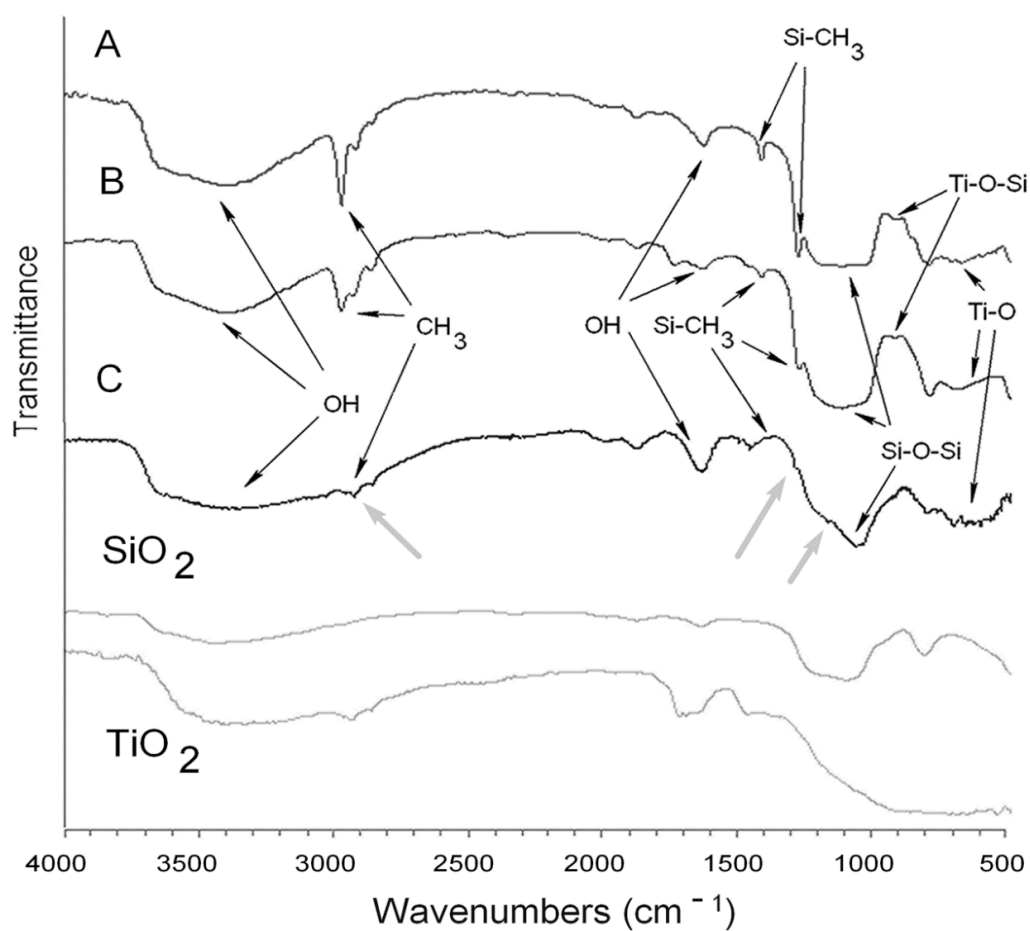


Figure 4.2: FTIR spectra of the samples P2 (A), U1 (B), P2 after heating to 700 °C (C).

Microstructures of the new pan coating surfaces are shown in Figure 4.3. White particles, present at the top coating surface of the samples P1, P2 and P3 (Figure 4.3B, 4.3D and 4.3F), are confirmed by EDXS analysis as Ti containing phase.

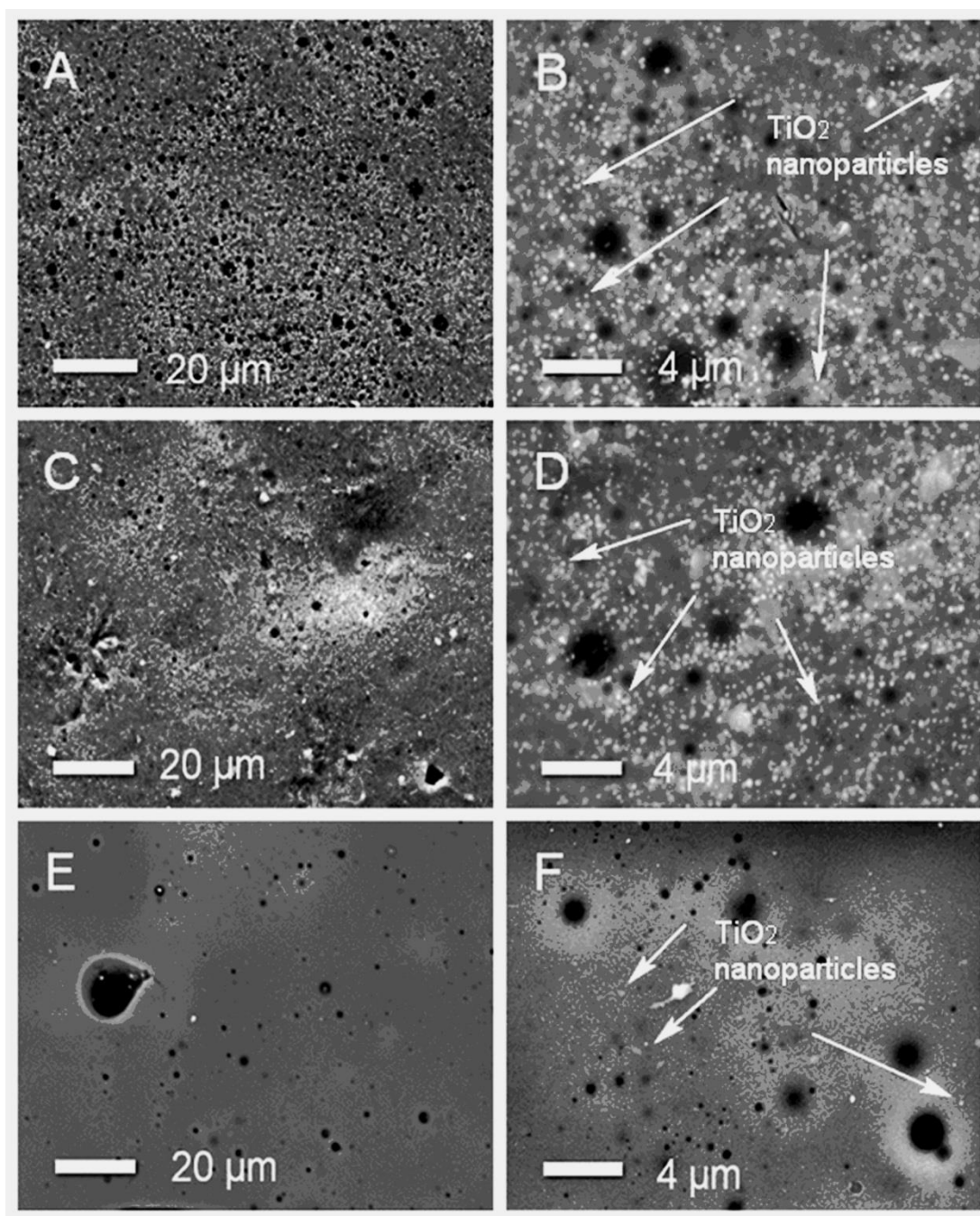


Figure 4.3: Microstructures of the coating surfaces of new pan coatings at two magnifications: P1 (A) and (B), P2 (C) and (D) and P3 (E) and (F) as observed by FE SEM.

The EDXS analysis of the top surface of pan coatings revealed that all the examined samples contained titanium, silicon and aluminium, which indicate the presence of titania (TiO₂) particles embedded in the silicon-rich matrix deposited on an aluminium substrate (Table 4.1). The presence of silica and alumina particles cannot be excluded.

Table 4.1: Estimated concentration of elements (in wt %) using standardless EDXS analysis at the acceleration voltage of 15 kV.

SAMPLE	Ti (wt %)	Si (wt %)	Al (wt %)
New pan P1	22	30	< 1
New pan P2	32	22	< 1
New pan P3	4	44	< 1
Used pan U1	11	38	< 1
Used pan U2	13	36	< 1
Used pan U3	17	31	1

Another feature observed in Figure 4.3 is the presence of spherical voids in all the samples. While the void size in P2 was estimated to be 1 – 4 μm from the SEM images (Figure 4.3D), the observation by low magnification optical microscopy also revealed the presence of cylindrically shaped voids with up to 50 μm in diameter (Figure 4.4), extending through the bottom of the coating. Consequently, the metallic substrate was seen through the hollows. The observed voids may allow a direct contact with the liquid food with the aluminium substrate during cooking and may hence result in contamination of the food by the released aluminium. Aluminium release was actually observed for the sample P2 and 19 other samples with quasi-ceramic coatings into simulants after migration testing (Golja, Zorič, Rotter, Dražić, & Novak, 2016). The release of aluminium from coating P2 into 3 % acetic acid after third migration test (2 hours at 100 °C) was 0.50 mg/l, one of the other coatings (not examined in this work) showed 1.87 mg Al /l of simulant. Specific release of aluminium from the sample P2 was below specific migration limit for aluminium (1 mg/kg), from Regulation on plastics (European Commission, 2011).

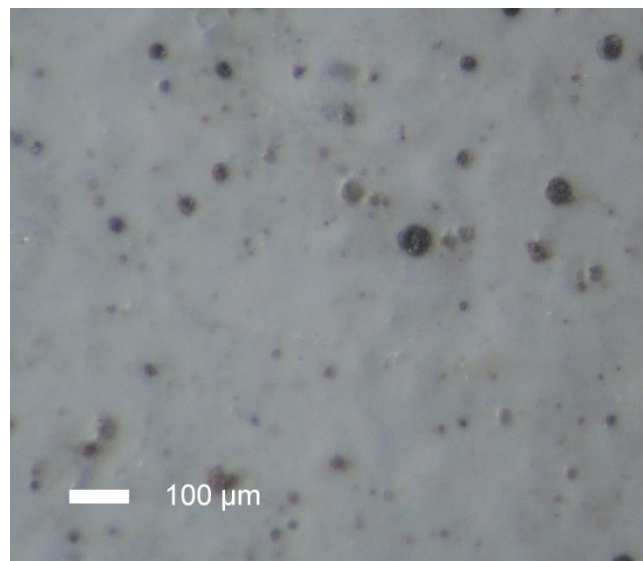


Figure 4.4: Top coating surface of the coating U1 as observed by optical microscopy.

In comparison to the non-used pan coatings, the micrographs of the used pans surfaces (U1-U3) show relatively larger defects (Figures 4.5A-F). At lower magnification (Figures 4.5A, 4.5C and 4.5E), scratches and voids are evident, while at higher magnification, the presence of TiO_2 particles (white spots) and increased roughness of the surface can be seen (Figures 4.5B, 4.5D and 4.5F).

SEM images of the examined pan coatings show that the shape of the TiO_2 is spherical (white inclusions in Figures 4.3B, 4.3D, 4.3F and 4.5B, 4.5D, 4.5F). The size of the particles of coatings was estimated to be in the range from 70 to 250 nm by high magnification FE SEM (see Figure 4.3B and 4.3D, 4.5B, 4.5D and 4.5F). Mean particle diameter in coating P2 was estimated to be 143 nm ($n=318$), with 38 % of the particle size below 100 nm, meaning that at least 38 % are nanoparticles.

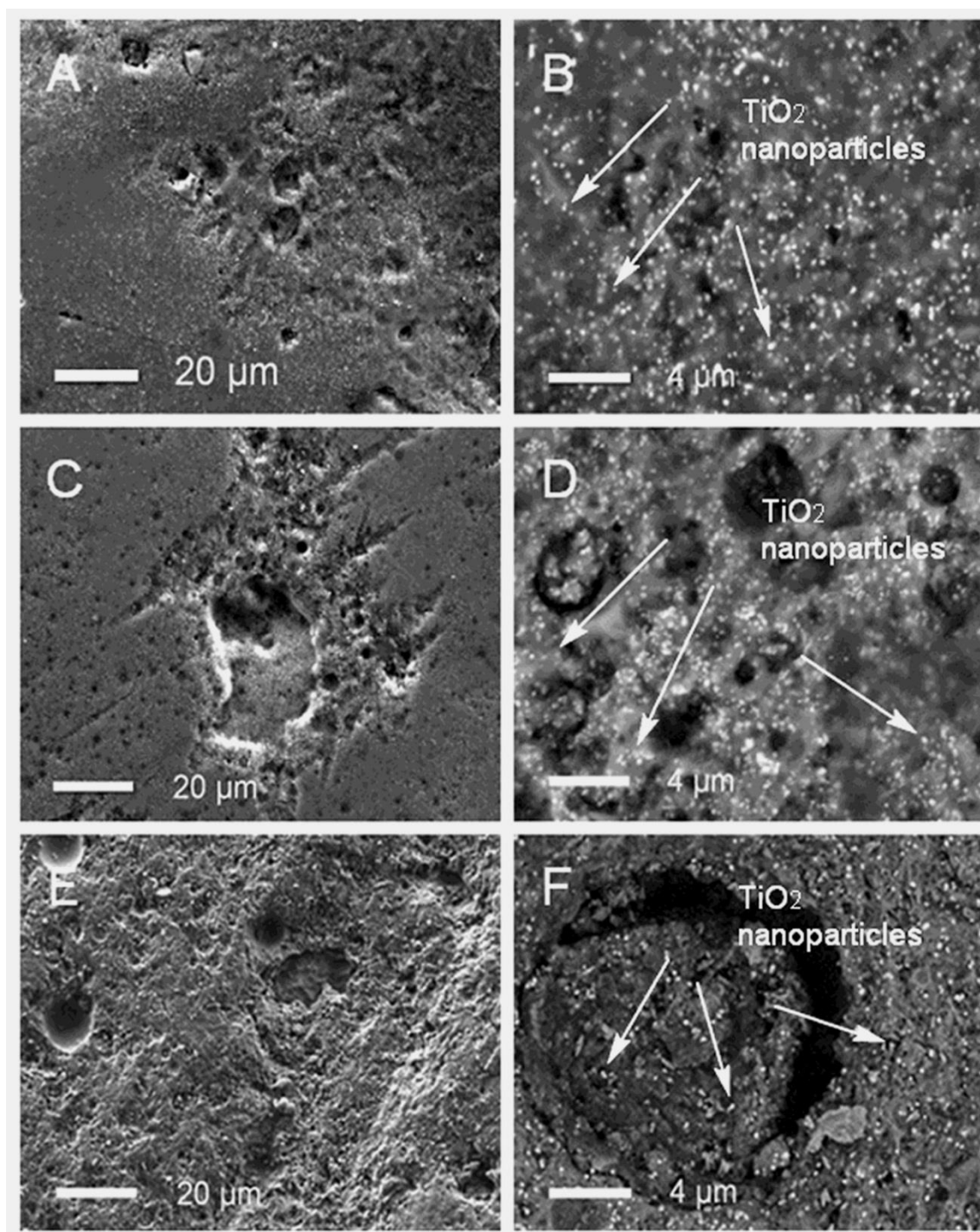


Figure 4.5: FE SEM micrographs of the used pan coatings U1 (1 year of use, careful maintenance) (A) and (B); pan U2 (2 years of use, careful maintenance) (C) and (D) and pan U3 (2 years of use, rough maintenance) (E) and (F).

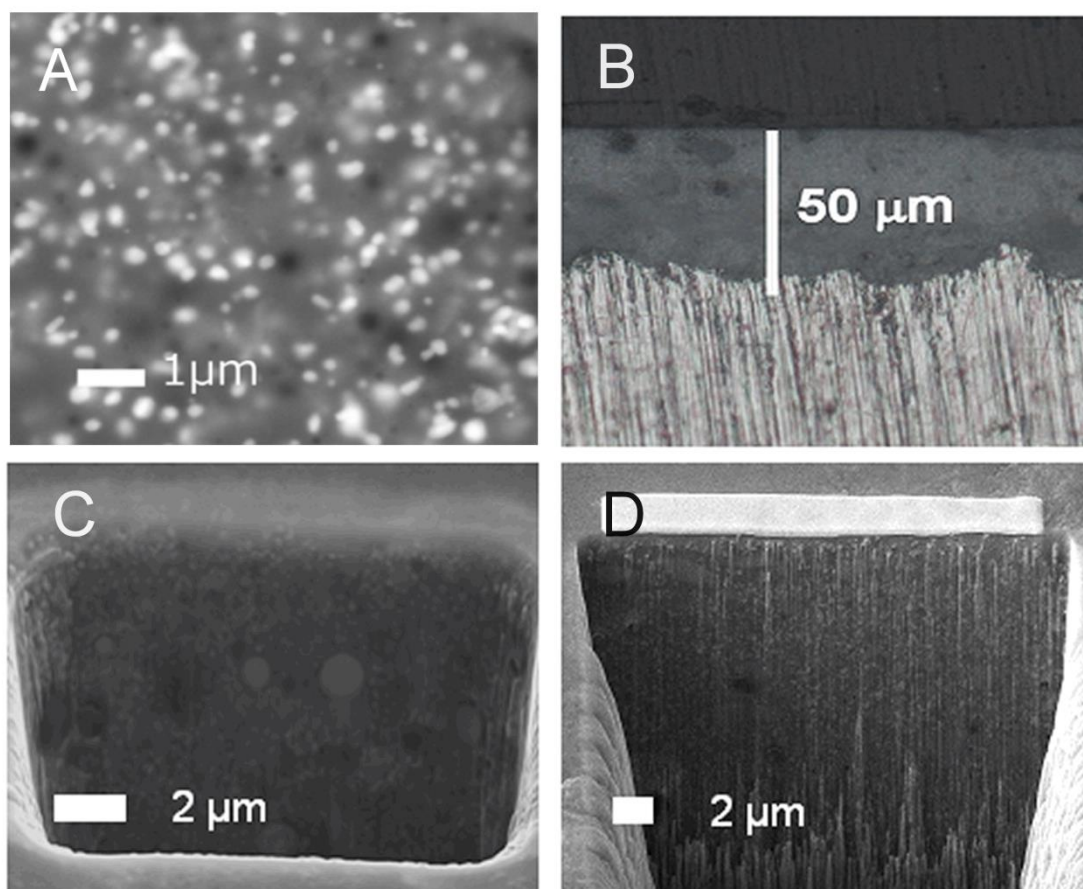


Figure 4.6: SEM image of TiO₂ nanoparticles present in the sample P2 (A). Image (B): Cross section of the coating P2 observed by optical microscope. FIB electron microscopy images of the coatings P2 (C) and U1 (D) shows the presence of particles within the whole thickness. By the use of FIB a cross section through the coating is made and the composition of the coating was observed within the whole thickness.

The new pan coating P2 and used pan coating U1 were selected for further characterization, namely cross section observation and determination of crystal structure. The cross-section observation of samples P2 and U1 by FIB electron microscopy showed the presence of particles within the whole thickness of the coatings (see Figure 4.6 C and 14 D). The coating thickness on the metallic substrate of the coating P2 estimated by an optical microscope was $\sim 50 \mu\text{m}$ (see Figure 4.6B).

The crystal structure of particles present in the new pan coating P2 and used pan coating U1 was determined by XRD measurements. XRD analysis revealed the presence of crystalline TiO₂ in the form of rutile and also SiO₂ in the form of quartz, as well as the presence of a small amount of amorphous phase, indicated by the broad background signal in both coatings (see Figure 4.7). Since aluminium was not detected by the EDXS analysis of the coatings, we conclude that the high observed aluminium peaks in the XRD spectra originate from the substrate pan material. Since rutile is the most thermodynamically stable polymorph of TiO₂ (Hanaor & Sorrell, 2011), no transformation of TiO₂ was expected to be induced during the foreseeable use (maximum

temperature estimated to ~ 350 °C); on the other hand, the crystallisation of the amorphous SiO_2 might appear, but it was not confirmed by the analysis.

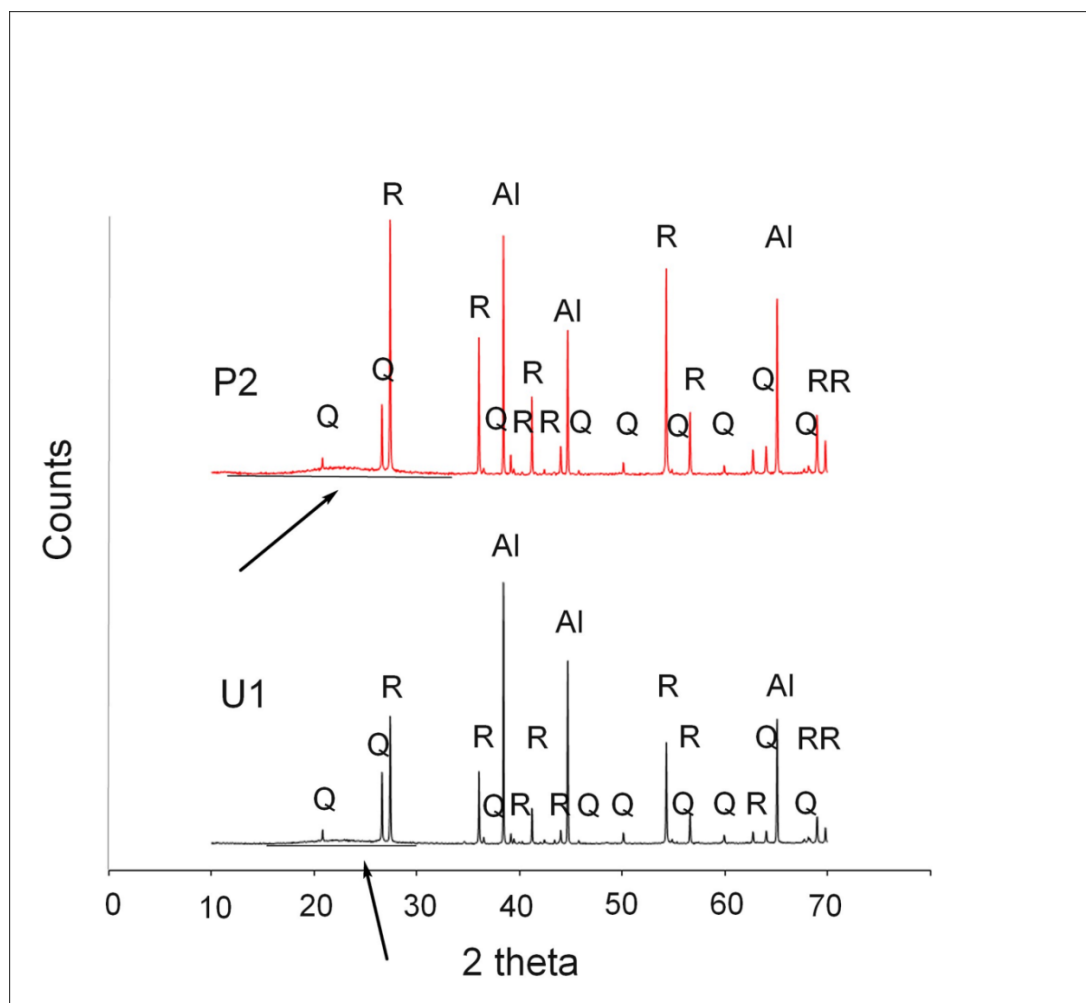


Figure 4.7: X-ray diffractograms of the samples P2 and U1 (Q=quartz, R=rutile, Al=aluminium). Broad background signal suggests the presence of a small amount of the amorphous phase (as shown with arrows).

As mentioned before, determination of different properties such as chemical composition, size, shape, crystal structure, surface charge, solubility, surface coatings on the particles, impurities, etc. of nanoparticles present in novel material and nanoparticles released to food is important as they may influence the toxicity of nanomaterials. Our study determined the chemical composition, size, shape and crystal structure of TiO_2 nanoparticles, the presence of quartz SiO_2 phase and chemical composition of the matrix of the coatings. However, TiO_2 particles were embedded within the polymeric silicone matrix (at least portion of TiO_2 is covalently bonded), so that the characterization of coatings alone could not confirm exposure to nanoparticles. For that reason, the possible release of particles from the material was investigated by migration testing and matrix degradation study.

4.2 Study of (Nano)particles Release from the Coatings

4.2.1 Migration testing

Migration tests were performed with three different simulants: deionised water, 3 % acetic acid and 5g/l citric acid. Deionised water was selected as a simulant in migration tests to represent foods with hydrophilic character and the solutions of acetic acid and citric acid to simulate acidic foods (European Commission, 2011). The citric acid solution is usually used for testing of metals release from metallic food contact materials into acidic foods, according to the Council of Europe Technical Guide (Council of Europe, 2013), and it is known that it is a stabilizing agent for nanoparticles in aqueous suspensions. Test conditions of 2 hours at 100 °C were selected assuming that pans can be used for 0.5 hours at temperature higher than 100 °C; accordingly, a duration of four times the maximum time of use (0.5 hours), or 2 hours was used, as described in the Regulation of plastics (European Commission, 2011).

The concentrations of Ti ions and Ti-containing particles in simulants after migration testing as determined by ICP MS (Golja et al., 2017) are presented in Table 4.2.

Table 4.2: Concentration of Ti in different simulants after migration tests of intact coating P2 (2 hours at 100 °C).

Concentration of Ti in µg /l				
Simulant		deionised water	3 % acetic acid	citric acid 5g/l
Ions	1 st migration	0.058	261	250
	2 nd migration	0.152	272	675
	3 rd migration	0.483	247	3636
Particles	1 st migration	39	405	100
	2 nd migration	< LOQ	791	7
	3 rd migration	< LOQ	861	0*

LOQ = 0.117 µg/l

*titanium was present only in ionic form

It is evident that only a small amount of Ti was released into the deionised water, while many more particles and ions were released into the acidic simulants. More particles were released into the acetic acid solution, while more ions were released into 5 g/l citric acid. The concentrations of released ions in water and citric acid increased with each successive test, while it remained approximately constant in acetic acid solution. On the contrary, the increase in the concentration of Ti-containing particles was only observed in acetic acid solution. TiO₂ particles collected from the acetic acid solution after the third migration test appeared agglomerated in clusters of 800 nm size or larger,

after drying for SEM observation (Figure 4.8). Primary particles smaller than 100 nm are also visible. Released particles have a nearly spherical shape, the same shape as particles embedded in coatings.

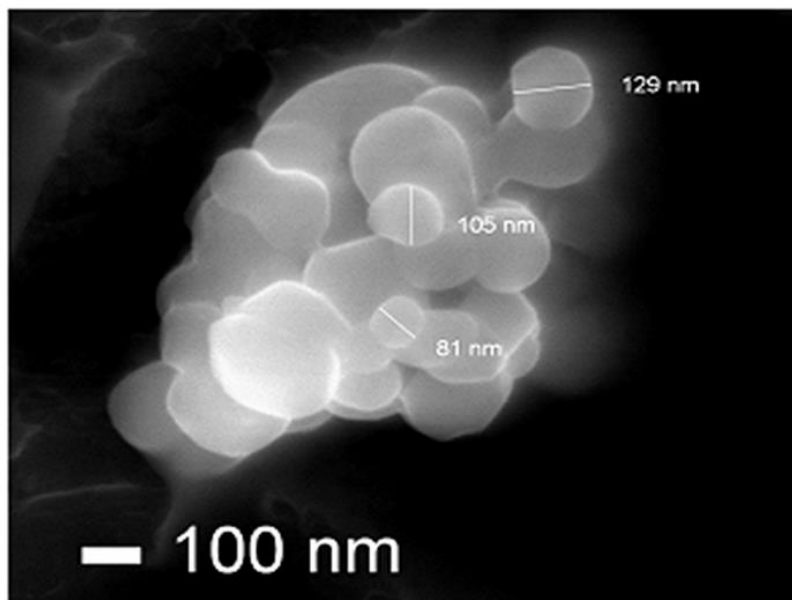


Figure 4.8: SEM image of TiO_2 particles migrated from the sample P2 after testing with simulant 3 % acetic acid (2 hours at 100 °C).

Our results reveal a much higher concentration of particles than that observed by Lin et al. (Lin et al., 2014), who studied the migration of TiO_2 particles from polyethylene film and determined migration of approximately 10 $\mu\text{g/l}$ after 6 hours of contact with 3 % acetic acid. This can be explained by a higher concentration of TiO_2 in our samples.

In general, there are four proposed mechanisms identified to describe the release of nanoparticles from food contact materials into food (Newsome, 2014):

- (i) desorption, during which the particles adsorbed to the surface of food contact material are released into the food;
- (ii) diffusion, i.e. the movement of particles through the food contact material and, then, their release to food;
- (iii) dissolution, when the particles dissolve, and ions are released into food;
- (iv) matrix degradation, which can be caused by thermal decomposition, mechanical abrasion, UV light or hydrolysis/dissolution of the matrix.

In the case of the examined quasi-ceramic food contact coatings, desorption of the Ti containing NPs is not an important mechanism of release, as the particles are not adsorbed on the surface but present within the whole thickness of the coatings (Figure 4.4C and 4.4D). Diffusion is also not a probable mechanism because some titanium is covalently bonded to the silicone matrix (Figure 3.1) and particles are too big for diffusion through the polymer. Due to the limited solubility of TiO_2 within the whole pH region and covalent bond with silicone matrix, the mechanism of dissolution and migration of ions into food was assumed negligible, but this was not confirmed by our results. On the contrary, the dissolution of particles and production of ions is a noteworthy mechanism of release of nanoparticles into food. This is particularly obvious in citric acid solution (Table 4.2), which can be explained by the formation of more

soluble titanium complexes. Partial dissolution of the large particles on the coating surface likely results in the release of smaller TiO₂ particles.

The release of particles was confirmed by the results of migration tests of coating P2 which was before the testing subjected to a simulation of dishwashing. Sodium hydroxide as a simulant for dishwashing detergent removed some of the TiO₂ particles from the surface and for that reason the migration test of the coating after such simulation showed the lower concentration of ions and TiO₂ particles (Table 4.3).

Table 4.3: Concentration of Ti in 3 % acetic acid after migration tests of coating P2 after simulation of dishwashing (7 cycles of contact with 0.01 M NaOH for 2 hours at 70 °C).

Concentration of Ti in µg /l		
Simulant	3 % acetic acid	
Ions	1 st migration	64
	2 nd migration	32
	3 rd migration	34
Particles	1 st migration	11
	2 nd migration	32
	3 rd migration	271

The surface detachment was also the mechanism proposed by Echegoyen and Nerin as an explanation of their observed migration of silver nanoparticles from food container (Echegoyen & Nerin, 2013). Lin who studied the migration of Ti from nano –PE composite packaging into simulants concluded that the migration of nanoparticles might occur via dissolution from the surface and cut edges (Lin et al., 2014). The release caused by cutting edge effect (release of nanoparticles from the cut edges of the samples) was also discussed by Bott (Bott, Stormer, & Franz, 2014b). We believe that in our case the release did not occur from the cut edges as they were covered by PTFE tape.

The release of ions and particles into 5 g/l citric acid and 3 % acetic acid was higher than 10 µg/kg food, so released Ti ions and TiO₂ particles can be considered as NIAS (non-intentionally added substances) according to Regulation 10/2011.

Overall migration was also determined in the third migration test of intact P2 coating with simulant 3 % acetic acid (2 hours at 100 °C). It was 3.9 mg/dm² (23 mg/l), and it is below the overall migration limit of 10 mg/dm² from the Regulation on plastics (European Commission, 2011). The limit was used for comparison, although coatings are excluded from the scope of this Regulation. The value indicates that besides TiO₂, other components of the coatings are migrating into the simulant as well.

Migration test was performed by the use of test conditions for plastic food contact materials and food simulants described in EU Regulation 10/2011 (European Commission, 2011) and Council of Europe Technical Guide (Council of Europe, 2013). However, release testing with simulants used for traditional food contact materials and

the use of the same test conditions as for testing of traditional food contact materials may not be appropriate and may not represent the worst-case approach for testing of novel food contact materials.

In order to confirm that the same approach as for conventional materials is suitable, release into real food should be performed and results compared with release into simulants. However, nanoparticles may not be stable in the simulant of interest and/or after performing particular testing, especially if the time of testing (and time of simulant storage before measurements of concentration) is long and testing is performed at elevated temperatures. Agglomeration, aggregation, sedimentation and dissolution may take place which may lead to inaccurate results. Released particles (depending on their surface charge) may be adsorbed to the surface of the glass container. The classical approach does not take into account the impact of nanoparticles properties like surface charge, surface coatings or possible chemical reactions of nanoparticles with components of food. Contact with real foods (proteins and other components of the foods) may change the release and properties of nanoparticles. The classical approach is based on the diffusion model, and diffusion is only one of the mechanisms for the possible release of nanoparticles into the food. Some other researchers also have doubts about the suitability of “classical” approach for testing of novel food contact materials (Ntim, Thomas, & Noonan, 2016, Jokar, Pedersen, & Loeschner, 2017).

4.2.2 Characterisation of rutile nanoparticles in dispersions

When determining the concentration of nanoparticles in dispersions, to avoid underestimation and obtain accurate results, maintaining stable dispersion, by avoiding agglomeration and settling of nanoparticles throughout the analytical procedure, needs to be ensured. Agglomerates of nanoparticles can be separated by introducing the mechanical force (ultrasonication) or by overcoming weak attractive forces by electrostatic, steric or electrosteric interaction with the addition of dispersing agents that are adsorbed on the particles surface (Mandzy, Grulke, & Druffel, 2005, Letzel et al., 2016).

In order to examine the stability of rutile nanoparticles (crystal modification of TiO_2 nanoparticles present in our coating) in simulants, the zeta potential of commercial powder dispersions was measured with or without the addition of different dispersing agents. Dispersants PEI (cationic surfactant polyethyleneimine), Darvan C (anionic surfactant ammonium polymethacrylate) and Triton X-100 (a non-ionic surfactant containing hydrophilic polyethylene oxide chain and an aromatic hydrocarbon lipophilic or hydrophobic group) were used. The region of low dispersion stability typically appears at zeta potential values from - 30 to +30 mV, whereas at zeta potentials higher than +30 or lower than - 30 mV mean that dispersions will be stable (Thio, Zhou, & Keller, 2011).

As observed in Figure 4.9, applied dispersing agents did not improve the stability of rutile TiO_2 nanoparticles in aqueous suspension in comparison with rutile nanoparticles without dispersants (Vidmar, Milacic, Golja, Novak, & Scancar, 2016). Rutile dispersed in water without the addition of dispersants has the isoelectric point IEP at pH 5.3. Addition of PEI 600 slightly shifted IEP toward a higher pH (5.6), while Darvan C and Triton X-100 shifted IEP toward a lower pH (4.5). The positively charged PEI complex with negatively charged TiO_2 nanoparticles, and shift IEP towards higher pH (Dempsey, Lee, Cowan, & Suh, 2013). Negatively charged Darvan C (ammonium salt of polymethacrylic acid) changes the surface charge of TiO_2 nanoparticles to a more negative value, by increasing the electrostatic repulsion between the particles and shifting IEP towards a lower pH (Singh, Nayak, Samal, Bhattacharjee, & Besra, 2012). The same

happens with Triton X-100. Hydrophobic chains of Triton X-100 absorbed onto nanoparticles surface repulse each other and stabilise the dispersion. Stability of dispersions made with or without dispersants is similar in acidic pHs, while in alkaline pH, the stability of dispersion is higher without dispersants. A possible reason for that is the formation of surfactants aggregates on the nanoparticle surface due to hydrophobic interactions between nonpolar chains of the surfactants. These aggregates destabilize nanoparticles in suspension and decrease the zeta potential. In conclusion, a more efficient dispersion of nanoparticles in Milli-Q water was achieved by the use of ultrasonication, then by the use of dispersants.

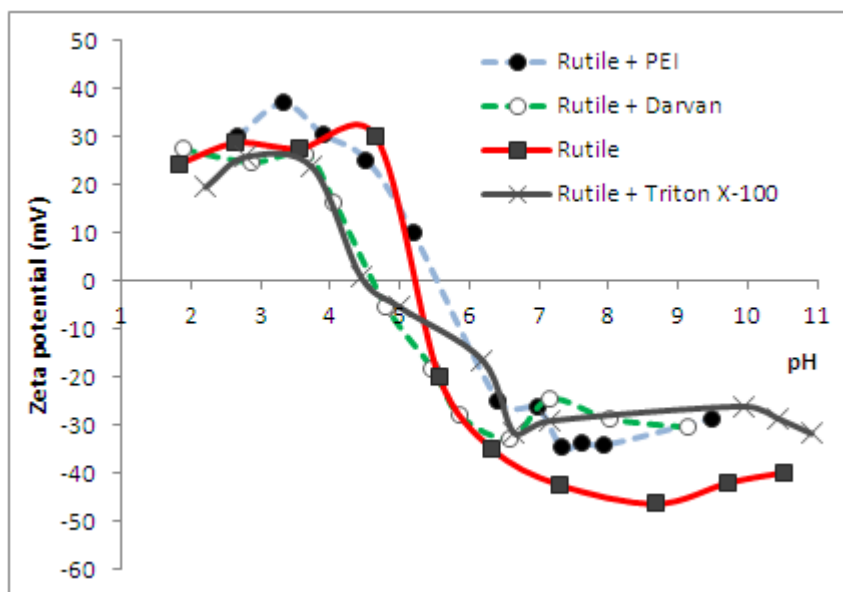


Figure 4.9: Zeta potential of freshly prepared 20 mg/l rutile TiO₂ suspensions in deionised water with the addition of different dispersing agents, as a function of pH.

Determination of dispersion stability of particles scratched from the coating P2 was observed as well. Zeta potential was measured in dispersion with deionised water, after ultrasonication. Zeta potential is preferably determined in deionised water to avoid discrepancies between tests in different solvents and pH/ionic conditions. Zeta potential as a function of pH for the same coating on two different days showed a bit different curves, with isoelectric point IEP between 4.2 and 4.7 (Figure 4.10). Zeta potential is a function of the surface charge of the particles, so it is evident that scratching produces not only particles of different sizes (as described under matrix degradation studies) but also particles with slightly different surface charges. It is evident from Figure 4.10 that particles produced by scratching do not form stable dispersions at low pH. Dispersion shows better stability at higher pH values (which are not characteristic for the foodstuff).

Particles migrated during migration test with simulant 3 % acetic acid (into which most of the TiO₂ particles migrated) were characterized at inherent pH (3.9) by dynamic light scattering (DLS), which calculates the distribution on an intensity basis and polydispersity index (PDI) and effective (hydrodynamic) particle diameter D_{eff} were determined. Measurements were performed one day after migration testing. Observed polydispersity index 0.480 indicated broad, polydisperse particle size distribution. Hydrodynamic particle diameter D_{eff} 536 nm showed agglomeration of the particles.

These findings are in accordance with Figure 4.8, where an agglomeration of particles is well visible.

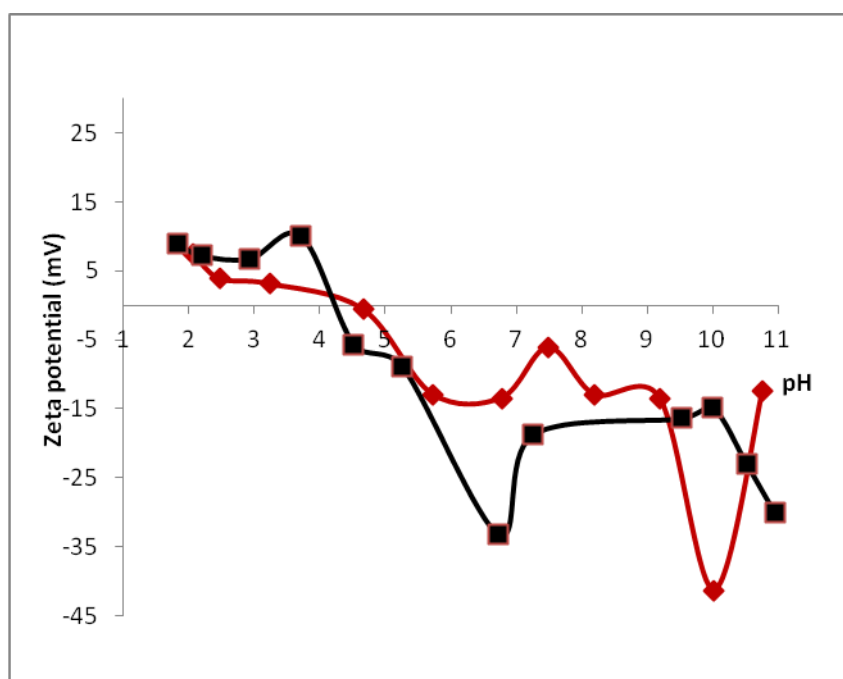


Figure 4.10: Zeta potential curves for the twice scratched coating P2 dispersed by ultrasonication in deionised water.

4.2.3 Matrix degradation studies

The matrix degradation of the non-used pan coating P2 was studied by the examination of thermal stability and wear resistance. The influence of UV light on matrix degradation was neglected as indoor exposure of food contact materials to UV light is not particularly relevant, and TiO_2 in the form of rutile is not as strong a photocatalyst as anatase (van Dyk & Heyns, 1998). Hydrolysis/dissolution of the silicone matrix itself was not studied, as it is not expected to occur at neutral or moderately acidic pH of foods.

4.2.3.1 Thermal degradation

Thermal stability of the pan coating P2 was examined by DSC/TG MS analysis up to 700 °C. Within the temperature range of foreseeable use on the stove (up to 450 °C, as written in instructions of use for one of the new pans), the weight loss was minor, while the total weight loss up to 700 °C was ~8 % (see Figure 4.11).

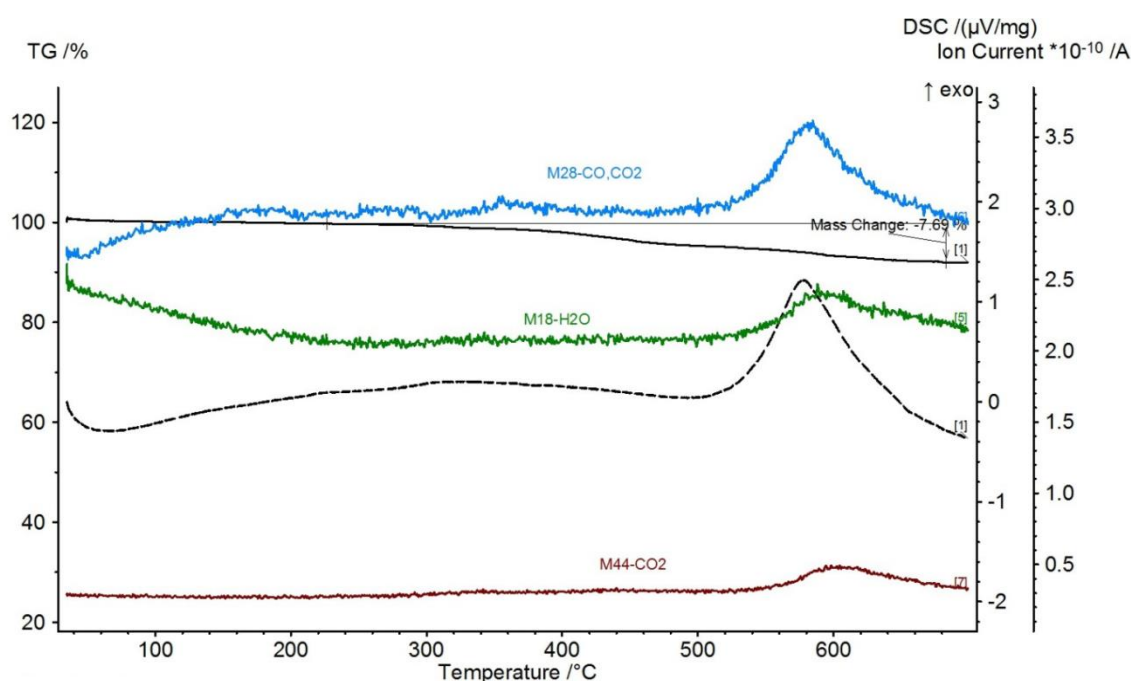


Figure 4.11: The DSC/TG MS curves for the sample P2 within the temperature range of 35 – 700 °C at the heating rate of 10 °C/min. The MS signals recorded during decomposition show MS fragment ion curves for CO/CO₂ ($m/z = 28$) (blue curve), water ($m/z = 18$) (green curve) and CO₂ ($m/z = 44$, brown curve). The black curve shows a change in mass of the sample (7.69 %). Dashed curve (DSC) shows exothermic effect with maximum intensity at 580 °C.

The peak at 600 °C is accompanied by the formation of CO and CO₂. Furthermore, the first exothermic change, indicating decomposition of the material, appears only over 500 °C, which is above the foreseeable temperature during the use.

From the appearance of the coating P2 after the DSC/TG MS analysis (Figure 4.12A), it is obvious that the white TiO_2 particles are not firmly incorporated into the matrix after heating to 700 °C, and fine particles became more exposed (Figure 4.12B).

The mapping of the area by EDXS analysis (Figure 4.12C and 4.12D) confirmed white particles as being Ti-rich and the surrounding material as Si-rich. These results indicate that, under certain conditions, titania (rutile) particles can be easily released from the matrix.

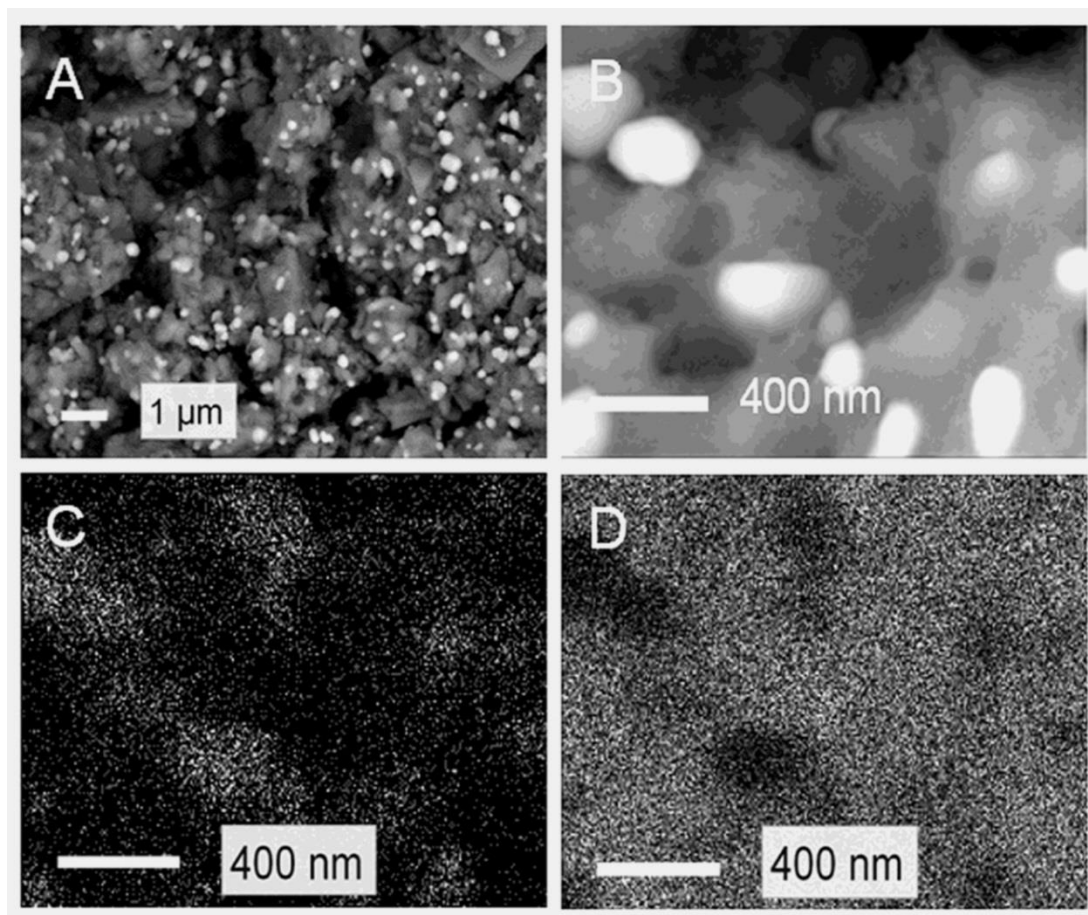


Figure 4.12: Scanning electron micrographs of the sample P2 after heating at 700 °C (DSC/TG MS analysis) (A) and (B); EDXS elemental mapping of titanium and silicon distribution (C) and (D). White particles on image C are titanium rich and white matrix on image (D) are silicon rich.

Furthermore, after the heating to 700 °C, there are almost no characteristic bands for silicone visible in the FTIR spectrum (Figure 4.2C), except for very low intensity bands at 2963, 1413 and 1261 – 1263 cm^{-1} (labelled by grey arrows), confirming that a large amount of organic phase has transformed to CO and CO₂, while the inorganic phase of TiO₂ and SiO₂ is remained.

Thus, the results of the thermal stability analysis suggest that the examined coating is relatively stable under the foreseeable conditions of use (up to 450 °C), and the TiO₂ NPs are not expected to be released by thermal decomposition of the silicone-based matrix (at least in a short-term use). However, under more severe conditions, i.e. by overheating, or by repeated heating during long-term use, particles release due to thermal degradation of the matrix cannot be excluded. This presumption is also supported by the appearance of the coatings after 1 and 2 years of use (Figure 4.5).

4.2.3.2 Mechanical degradation

In order to estimate the probability of nanoparticles release by mechanical wear, two sets of analysis were performed: in the first, a scratch test was performed under increasing load (simulating the worst case of use by, e.g. knives, forks), while in the second, wear test at constant load for a long time was used to simulate a long term use. It should be noted that conditions of scratch and wear tests were selected solely as an approximation of simulation and not as a precise simulation study.

Scratch test was performed with the load of the pin increased up to 30 N. Visible deformations appeared only at loads above 4 N, where scratches followed by chipping of the material at high loads were observed (Figure 4.13A). The particles created by chipping of the coating material were small, below 1 μm , and had irregular shapes (Figure 4.13B). This reveals a change from a predominantly elastic deformation at low loads to a brittle fracture under increased load. The appearance of the cracks after scratching the quasi-ceramic coating with high crosslink density was reported to happen even under less severe conditions (Tiwari and Hihara, 2012), where the load was only 0.5 N.

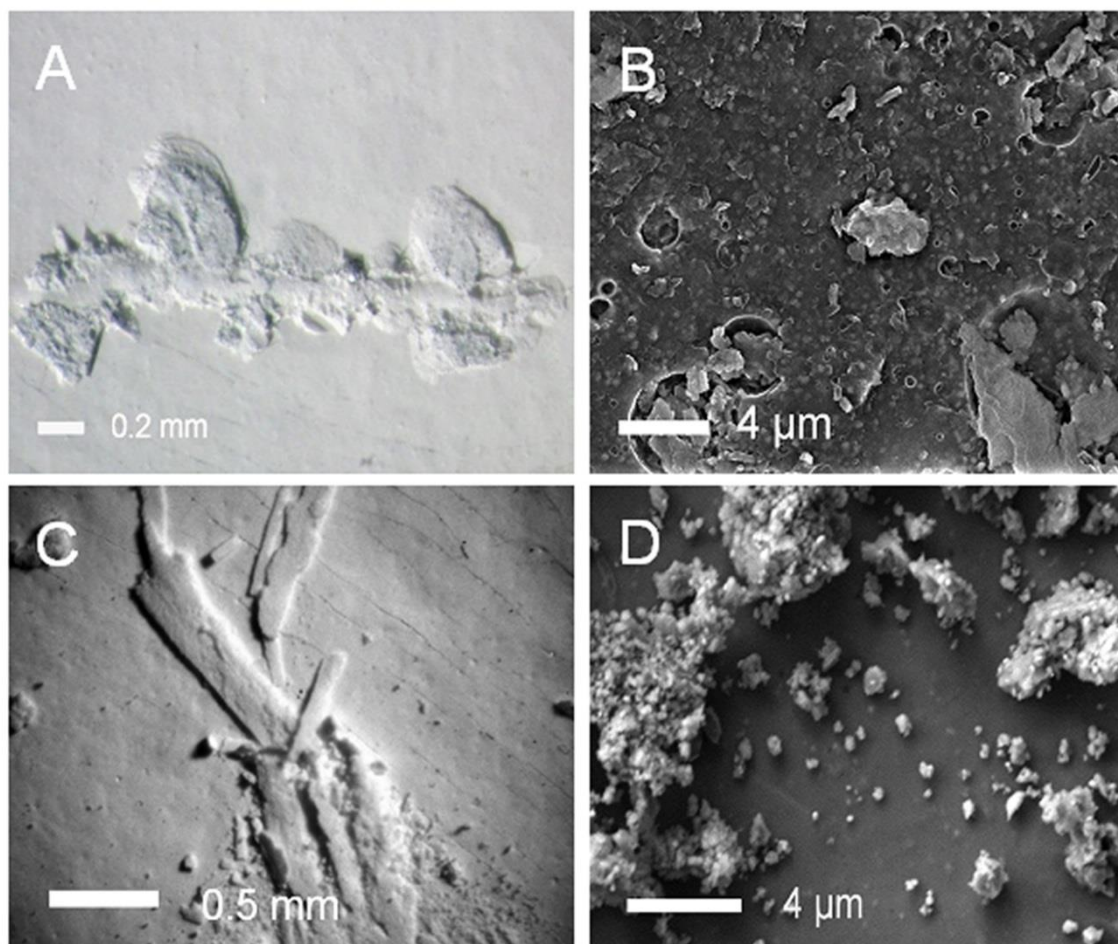


Figure 4.13: Sample P2: low magnification image of the scratch (A) and SEM image of the formed particles (B), debris after wear test: stereomicroscope image (C) and SEM micrograph (D).

The scratches are formed during actual use as well, as shown in Figure 4.14.

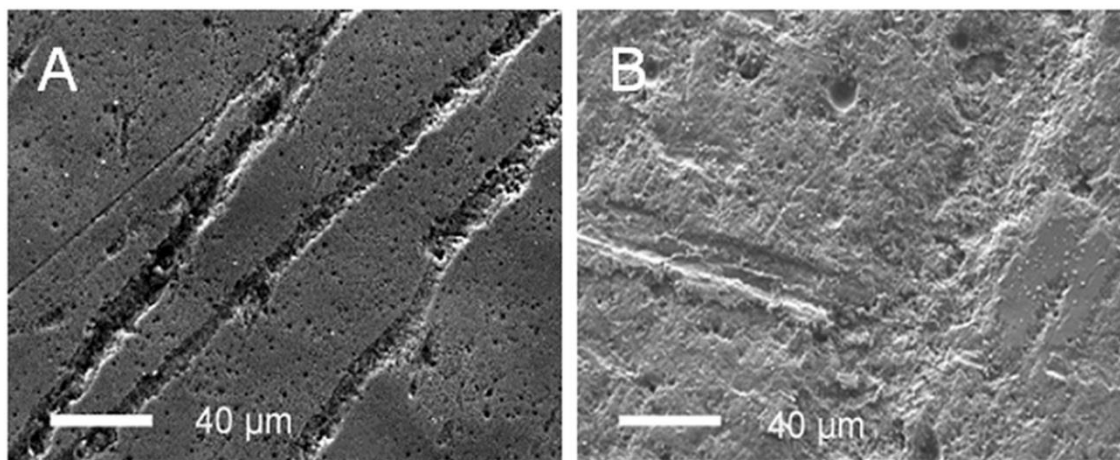


Figure 4.14: Surface scratches on used pan coatings U2 (A) and U3 (B).

In the second set of analysis, the coating was tested under tribological conditions for a longer time (1h). The intention was to simulate the use of tableware under various contact conditions, from mild, i.e. recommended use, to more severe ones, which can take place by use of any hard and sharp object, such as a fork or a knife.

Under mild conditions, simulated by the polymer loaded at pressures below 50 MPa, no visible wear appeared. In contrast, when higher contact pressures were applied by using steel or ceramic counterparts, i.e. above 200 MPa, the brittle nature of the coating became evident, especially above 300 MPa (Figure 4.13C). The produced wear debris was micro- and nanosized and had an equiaxial shape (Figure 4.13D). As expected, the debris composition was in agreement with that one of the coating determined by the EDXS analysis before the wear test.

From the experiments, it is evident that the release could be minor in the short term, while more severe conditions, prolonged time and, in particular, the combination of both, might result in a distinct particles release (Golja et al., 2017). This is supported by the appearance of the coatings after actual use (see Figure 4.5 and Figure 4.14), where the strongly damaged structure of the coating implies easy particle removal and possible release into food.

ICP MS measurements showed that the tribological test, at specific conditions of testing described above, had produced up to 22 µg of titanium in the form of particles; (0.4 to 5.5 µg of titanium in the form of particles smaller than 200 nm) (Golja et al., 2017). Recalculating to TiO₂, it gives 0.7 to 9.2 µg of particles smaller than 200 nm TiO₂ (Figure 4.15).

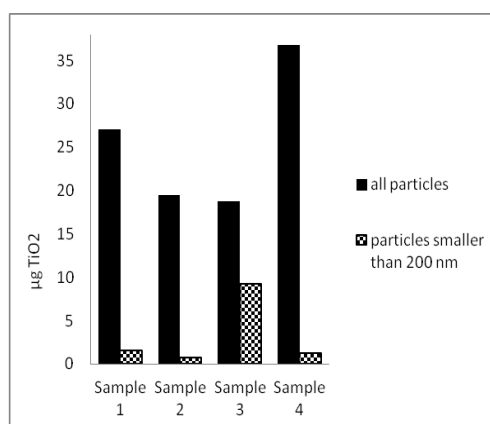


Figure 4.15: Mass of titanium containing particles in debris after tribological testing rinsed with deionised water from the sample P2 and determined by ICP MS.

Under the same wear conditions, the observed mass of the particles in the debris of four different samples of the same coating varied. This is not surprising due to the chipping nature of the coating also evident from Figure 4.13.

Scratch and tribological testing followed by ICP-MS measurement confirmed degradation of the quasi-ceramic coatings caused by mechanical wear during the use (cooking and cleaning) and formation of partially nanoparticulate debris.

4.2.3.3 Calculation of nanoparticles released in actual use (based on EM image of the used pan)

Scratches on the surface are actually formed during the use and coating material (including particles) is released into food. From the image of the coating U2 being in use for two years with careful maintenance and handling, with no use of sharp objects and dishwasher machine (Figure 4.16A), the quantity of nano TiO₂ released from the simplified scratches marked with white lines (Figure 4.16B) was calculated as described in 3.3.1. The mass of released nanoparticles for different depths of the scratches recalculated as the daily release of nano TiO₂ particles is presented in Table 4.4. This calculation is probably an underestimation of nanoparticles release because actual release occurs not only as a scratch (in depth) but also from the surface and is not visible.

Table 4.4: Calculated quantities of released nano TiO₂ for different depth of scratches.

Scratch depth in µm	Release of nano TiO ₂ from the pan during the 2-year use in mg	Daily release of nano TiO ₂ in µg
10	1.7	8
15	2.5	12
20	3.3	16

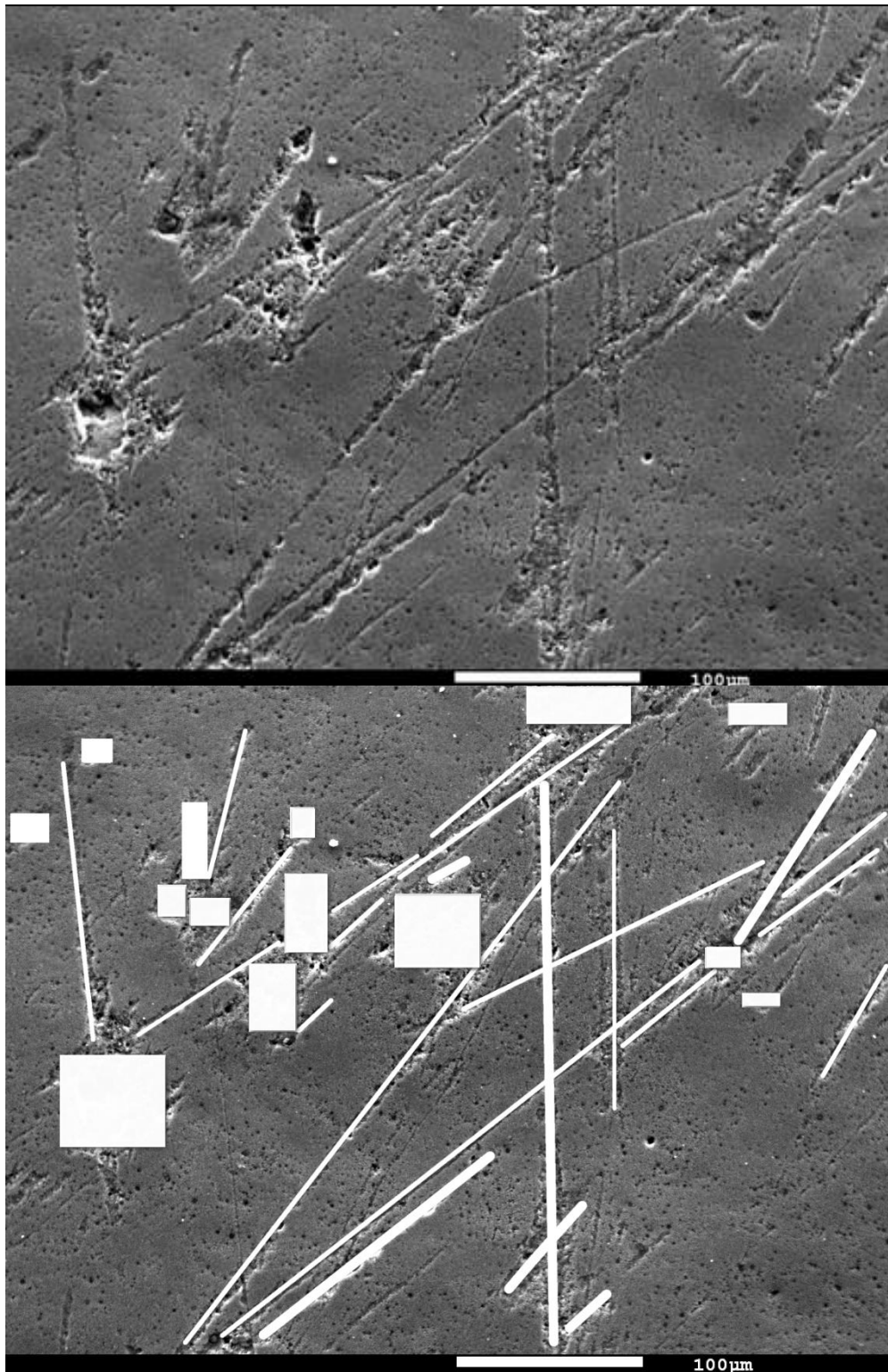


Figure 4.16: FE SEM micrograph of the pan coating in use for 2 years U2 (top) with well visible scratches. The area of the scratches is simplified for easier calculation and marked white (bottom).

4.3 Assessment of Possible Health Risk Through Exposure to Released Nano TiO₂ Particles

To assess a possible health risk, the risk assessment paradigm (hazard identification and hazard characterisation followed by exposure assessment and risk characterisation) was used.

4.3.1 Hazard identification and characterisation

TiO₂ nanoparticles are identified as agents which are potentially capable of causing adverse health effects. Our experiments showed that exposure to TiO₂ nanoparticles released from the quasi-ceramic coating into foods is possible. In order to assess whether or not such release into food poses a risk to consumers, the data on the toxicity of nano TiO₂, as evaluated in toxicity studies, were reviewed. After that, possible hazard was characterised by calculation of Derived No-effect Levels.

Bulk TiO₂ has been considered for many years as an inert material, nontoxic to humans and the environment. For the bulk TiO₂ NOAEL value of 2250 mg/kg bw/day is established based on chronic (721 days) oral study on rats (EFSA, 2017).

A literature search was performed for data on toxic effects of nano TiO₂. Important sources were the review of Iavicoli (Iavicoli, Leso, & Bergamaschi, 2012), Heringa (Heringa et al., 2016) and data from the SCCS opinion on the safe use of nano TiO₂ as a UV filter (SCCS, 2014).

Many studies showed that nano TiO₂ could be absorbed by the gastrointestinal tract, it can bioaccumulate and cause changes in various organs of animals (Jani, McCarthy, & Florence, 1994, Bockmann, Lahl, Eckhert, & Unterhalt, 2000, Cui et al., 2010, Duan et al., 2010, Koeneman et al., 2010, Shi, Magaye, Castranova, & Zhao, 2013, Wang et al., 2013, Tassinari et al., 2014, Sang et al., 2014, Jovanović, 2015, Bettini et al., 2017). Some other studies (Cho et al., 2013, Jones et al., 2015, MacNicoll et al., 2015) show limited absorption in the gastrointestinal tract. Geraets et al., (2014) described limited absorption in the gastrointestinal tract, but they concluded that limited uptake and slow tissue elimination, in the long run, might result in potential tissue accumulation. There are contradicting results regarding genotoxicity of nano TiO₂ as well. In the study published in 2011 (Sycheva et al., 2011), mice treated by oral gavage for 7 days with TiO₂ NPs (33 nm, anatase) presented genetic damage in the cells of bone marrow and liver. However, used doses were high: 40, 200 and 1000 mg/kg bw day. Uboldi study (Uboldi et al., 2016) showed by cell transformation assay and the micronucleus test (*in vitro* tests) that rutile TiO₂ nanoparticles appeared to be slightly genotoxic, whereas anatase TiO₂ NPs did not induce any significant neoplastic or genotoxic effect. Woodruff (Woodruff et al., 2012) evaluated 10 nm uncoated sphere TiO₂ anatase particles using the Ames test (reverse mutation assay) and single-cell gel electrophoresis Comet assay. Their results showed no genotoxicity. Also, Chen (Chen et al., 2014) evaluated the genotoxicity of TiO₂ nanoparticles (anatase, 75 nm) in vivo and in vitro. Intragastric testing of rats (up to 200 mg/kg body weight for 30 days) did not cause damage to chromosomes in rat bone marrow cells. In vitro study on Chinese hamster lung fibroblasts (V 79 cells) showed significant DNA damage at concentration 100 µg/l after 24 h treatment using comet assay. Bettini study (Bettini et al., 2017) showed the absence of genotoxicity for food grade TiO₂ (300-400 nm, 36 % of particles were < 100 nm, mainly anatase) *in vivo* at low doses (rats were dosed daily by intragastric gavage for 7 days). Petković et al. (Petkovic et al., 2011) reported that in HepG2 cells genotoxic potential of TiO₂

nanoparticles varies with particle size and crystalline structure. Both nanorutile- and anatase-induced intracellular ROS formation, DNA strand breaks and oxidative damage; nanoanatase was a stronger inducer than rutile. ROS formation was demonstrated by Jin et al. as well (Jin et al., 2011) only for nano-anatase.

Regarding carcinogenicity of nanosized TiO_2 it was known that inhaled or intratracheally administered nano, and fine-sized TiO_2 has been observed to induce lung cancer in rats, and the International Agency for Research on Cancer (IARC) has classified TiO_2 as possibly carcinogenic to humans (class 2B), (WHO, 2010), whereas possible carcinogenicity of nano TiO_2 by other routes of exposure (oral, dermal) still needs to be evaluated (WHO, 2009). National Cancer Institute reported in 1979 on a 2-year oral carcinogenicity study (NCI, 1979). They concluded that no adverse effects or carcinogenicity were found up to a dose of 2500 mg/kg bw/d of pigment TiO_2 . The annexes of the report, however, do show some dose-related non-neoplastic effects that indicate a provisional LOAEL of 1250 mg/kg bw/d. It is important to note that TiO_2 material used for the study was not characterized well and there is no information on the presence of nanoparticles in the tested substance. Recent Bettini study (Bettini et al., 2017) showed that commercial food additive E171 containing nano TiO_2 triggers the preneoplastic stages of carcinogenesis in the large intestine in rats orally exposed to E171 additive for 100 days (in 40 % of the animals). Rats were dosed daily through drinking water.

Guo et al. (Guo, Martucci, Moreno-Olivas, Tako, & Mahler, 2017) showed that titanium dioxide nanoparticle (30 nm) ingestion alters nutrient absorption in an in vitro model of the small intestine.

Group of Tassinari (Tassinari et al., 2014) observed that endocrine-active tissues are the target of TiO_2 particles (anatase, < 25 nm, 5 days study on rats) at oral dose levels relevant to the human dietary intake. Above described Bettini study also showed that food-grade TiO_2 particles cause imbalances in intestinal and systemic immune responses and induce low-grade mucosal inflammation (Bettini et al., 2017).

Sayes et al. correlated the nanoscale titania structure with toxicity by studying cytotoxicity and inflammatory response with human dermal fibroblasts and human lung epithelial cells (Sayes et al., 2006). They concluded that diverging toxicological properties may originate from a different crystal structure and that anatase is 100 times more toxic than rutile in the nanoparticulate form.

Titania nanoparticles have also been linked to Crohn's disease from gastrointestinal intake (Lomer, Thompson, & Powell, 2002).

Different toxicity studies reported on effects on liver (Wang et al., 2013, Shukla, Kumar, Vallabani, Pandey, & Dhawan, 2014), heart (NCI, 1979, Wang et al., 2013), sperm and testosterone levels (Jia, Sun, Yan, Zhou, & Wang, 2014). Heringa study (Heringa et al., 2016) of nano TiO_2 particles via oral exposure with the inclusion of toxicokinetic information showed potential risk for liver ovaries and testes. They stated that higher absorption might occur at lower doses.

In a meta-analysis of experimental studies regarding toxicity *in vitro* and short time, animal studies of exposure to nano TiO_2 (Chang, Zhang, Tang, & Wang, 2013), an association between kidney toxicity and nano TiO_2 was also identified. The crystallinity of the particles in this paper was not stated.

Scientific Committee on Consumer Safety (SCCS) performed a review of toxicological data concerning TiO_2 as described in their Opinion on Titanium Dioxide (nanoform) COLIPA n° 75 (SCCS, 2014). In the Opinion, subchronic (60 days) oral study in mice for anatase with particle size 5 nm was considered. On the basis of neurobehavioral effects

observed in the study, SCCS concluded that Lowest Observed Adverse Effect Level (LOAEL) for nanoanatase TiO₂ is 5 mg TiO₂/kg bw/day.

From SCCS LOAEL value, the level of exposure above which humans should not be exposed, Derived No-Effect Level (DNEL), was estimated taking into account relevant safety factors (adapted from REACH guidance (ECHA, 2012) using Equation 3.5.

Factor 3 was used for safety factor AF1, as it is recommended in the majority of cases (Roy & Skoglund, 2014, ECHA, 2012), default factor 17.5 was used for AF2 (interspecies variation between mice and humans (ECHA, 2012, Heringa et al., 2016), default factor 10 was used for AF3 (ECHA, 2012) and default factor 2 was used for AF4 (ECHA, 2012, Heringa et al., 2016).

By applying these factors and under the assumption that LOAEL is applicable, we calculated Derived No-Effect Level (1) by using Equation 3.5:

$$DNEL(1) = \frac{5 \frac{mg}{kg\ bw} / day}{3 * 17.5 * 10 * 2}$$

$$DNEL(1) = 0.0047\ mg/kg\ bw/day\ or\ 4.7\ \mu g/kg\ bw/day$$

This value is lower than DNEL calculated in Danish report regarding consumer risk assessment for nanoparticle in the Danish market (Christensen et al., 2015). In Danish report, DNEL was calculated in the range 0.01 – 0.02 mg nano TiO₂ /kg bw/day. The probable difference originates from the use of factors – in their calculation, AF4 factor (factor for extrapolation from subchronic to chronic effects) was not taken into account.

In a more recent paper on risk assessment of titanium dioxide nanoparticles via oral exposure (Heringa et al., 2016), available hazard data for *in vivo* oral studies on TiO₂ nanoparticles were compiled and reviewed. Among them, key studies were selected according to determined criteria: repeated exposures, preferably chronic studies, adequate characterization of nano TiO₂ particles, lowest value of the NOAEL or of the LOAEL among similar studies on the same endpoints, including hazard analysis of liver and spleen, coverage of additional endpoints/tissues/organs, coverage of both rutile and anatase TiO₂. No single study fulfilled all of these criteria; therefore they selected four key studies and one major supportive study. No study reported on carcinogenic effect. No selected study was performed with rutile TiO₂ nanoparticles, only with anatase nanoparticles. Among selected studies, the lowest LOAEL value of 1 mg/kg bw/day was reported by Tassinari (Tassinari et al., 2014) for oral 5 days study of nanoanatase. In their study spherical 20-60 nm and irregular 40-60 nm primary size anatase nanoparticles, mostly present as large agglomerates (mean diameter 1.6 µm) were administered intragastrically to rats. Critical effects observed at LOAEL were an endocrine malfunction (histopathological effect in thyroid, adrenal and ovary, thyroid system disruption, change in concentration of testosterone in serum).

By applying LOAEL of 1 mg/kg bw/day from Tassinari study and safety factors, calculated Derived No-Effect Level (2) was lower than by applying LOAEL of 5 mg/kg bw/day SCCS):

$$DNEL (2) = \frac{1 \frac{mg}{kg \text{ bw}} / day}{3 * 10 * 10 * 10}$$

$$DNEL (2) = 0.00033 \text{ mg/kg bw/day or } 0.33 \text{ } \mu\text{g/kg bw/day}$$

In the calculation, safety factor 3 was used for dose-response relationship when extrapolating from LOAEL to NOAEL (AF1), factor 10 for interspecies variation (AF2), factor 10 for intraspecies variation (AF3) and factor 10 for extrapolation from a 5-day (acute) study to lifelong (chronic) exposure (AF4).

DNEL (2) value derived from LOAEL from Tassinari study was lower than DNEL (1) value derived by SCCS. Primary particles were larger and mostly present as large agglomerates. The study considered in SCCS report was performed on mice, and in Tassinari study, toxicological effects on rats were investigated.

It should be noted that primary TiO₂ particles released from our food contact material were different from TiO₂ particles in above-mentioned toxicity studies. Particles released into simulant 3 % acetic acid were larger than particles used in studies (primary particles > 80 nm), had a different crystal structure than most of the above-mentioned studies and may be partially bonded to silicone.

It should also be noted that in contact with food nanoparticles may be coated with food ingredients which may also influence their toxicity.

4.3.2 Screening of exposure to nanoparticles released from the coating

Characterisation of representative quasi-ceramic coating P2 revealed that coating contains rutile TiO₂ nanoparticles in size range from 70 to 200 nm, embedded in a silicone matrix. Although particles are embedded, migration testing and matrix degradation studies showed the release of TiO₂ nanoparticles from the surface of material into simulant 3 % acetic acid and due to mechanical degradation during the use. For that reason, it is likely that consumers are exposed to TiO₂ nanoparticles via the gastrointestinal (GI) tract. Following the decision tree (Figure 1.7) from the EFSA document (EFSA, 2011), it can be concluded that, in order to assess a possible health risk, exposure assessment should be performed.

Results of migration studies showed that the highest release of nanoparticles was observed by migration testing with simulant 3 % acetic acid (Table 4.3): 861 µg titanium /l. For the exposure assessment, this value is recalculated to TiO₂ and 1436 µg TiO₂/l simulant (food) is obtained. For further calculations, 1400 µg TiO₂/l of food was considered. In the field of food contact materials it is generally accepted that 1 l of simulant equals 1 kg of food. So for exposure assessment migration of 1400 µg TiO₂/kg food (1.4 µg TiO₂/ g food) was considered. Not all released particles are nanosized, but for the worst case assumption, it was assumed that all of the particles released are nano-sized. We are aware that it would be more appropriate to determine the release of nanoparticles into real food. Unfortunately, due to the complexity of the food matrixes and difficult determinations of nanoparticles in the food, such determinations were not possible, and testing with simulants usually used for food contact materials was performed. Testing with simulants for conventional migrants in most cases overestimates

the migration into real foods. At the moment, due to the lack of data, it is not possible to conclude if migration of nanoparticles into simulants overestimates or underestimates the migration into foods.

Assessment of exposure was based on 5 different scenarios. Scenarios from 1 to 3 were based on results of nanoparticles migration study with simulant 3 % acetic acid in combination with different food consumption approaches. Scenario 1 considered use of the old food consumption approach, where consumption of 1 kg food per day by a person with the weight of 60 kg is assumed. This default value was used by the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF) based on the Guidelines of the Scientific Committee on Food for the presentation of an application for safety assessment of a substance to be used in food contact materials prior to its authorisation (EFSA, 2012).

Scenario 2 took into account food consumption data listed in the new EFSA document (EFSA, 2016a).

Summary statistics on food consumption data from EFSA Comprehensive European Food Consumption database were used as a quick screening tool to assess chronic exposure to released nano TiO₂ particles in scenario 3.

Scenarios 4 and 5 were not based on migration testing results, but on the calculation of released particles from the EM image of the coating which was in actual use for two years (U2) and in the determination of particles release by tribological testing respectively. The calculated daily release was divided with adults/toddlers body weights, as described in 3.3.1.

4.3.2.1 Exposure scenario 1 (conservative food consumption approach)

The old, conservative scenario considers consumption of 1 kg of foodstuffs in contact with the material(s) containing a certain substance per day and takes into account 60 kg as the weight of the person consuming food. This scenario does not distinguish between different types of foods and different population, e.g. the low body weight of infants and young children. According to this scenario consumption of food which has been in contact with certain food contact material is 17g/kg bw/day. Estimated exposure EE in µg nano TiO₂/kg bw/day, calculated from TiO₂ release (1.4 µg nano TiO₂ /g foodstuffs) using Equation 3.2 was 24 µg nano TiO₂ /kg bw/day.

4.3.2.2 Exposure scenario 2 (new EFSA approach for food contact materials, food categories 3 and 4)

In the recent EFSA document (EFSA, 2016a), EFSA elaborated four different food categories for which food contact materials are intended to be used and food consumptions to be considered for the estimation of exposure (Figure 4.17).

Category 3 would be appropriate if in the pan special food for infants and toddlers is prepared (food consumption to be considered for the estimation of exposure is 50 g/kg bw /day). Estimated exposure for category 3 with consideration of food consumption of 50 g/kg bw /day and release value 1.4 µg TiO₂ /g foodstuff was 70 µg/kg bw/ day.

Vegetables and meat which can be prepared in pans would be covered by category 4. These are foods other than water, baby bottle contents, milk and milk products and non-

alcoholic drinks or solid foods specifically intended for infants and toddlers. Taken from the table, exposure for category 4 (level of consumption 20 g/kg bw /day) is considered appropriate to cover the consumption by all population groups. Estimated exposure for food category 4 (with consideration of release 1.4 µg TiO₂ /g foodstuff) was 28 µg/kg bw /day.

Category	Food categories for which the FCM containing the substance under evaluation are intended to be used	Population driving the consumption ^(a)	Food consumption to be considered for the estimation of exposure (g/kg bw per day)
1	Water and baby bottle contents such as reconstituted milk formula	Infants ^(b)	150
2	Milk, milk products and other non-alcoholic drinks (e.g. fruit and vegetable juices)	Toddlers ^(c)	80
3	Solid foods specifically intended for infant and toddlers	Toddlers	50
4	Foodstuffs not covered by categories 1, 2 and 3	Toddlers	20

(a): This means that the critical population (infants or toddlers) consuming the foods grouped in a category has the highest consumption of one or more of the foods in that category; this does not mean that the critical population consumes all food types falling into that category.

(b): Infants are young children aged up to 12 months.

(c): Toddlers are young children aged from 12 months up to and including 36 months.

Figure 4.17: Table showing food consumption figures based on the categorization of application(s) of the food contact material(s) containing the substance under evaluation. Table is taken from the EFSA document (EFSAa, 2016).

4.3.2.3 Exposure scenario 3 (EFSA Comprehensive European Food Consumption Database)

For exposure scenario 3 a meal consisting of vegetables and meat cooked in the pan was considered. Many vegetables (e.g. sauer kraut, tomatoes) are acidic foods. Migration testing showed the highest release of nanoparticles into simulant for acidic foods. Consumption of meat was also taken into account because it is often prepared in such pans and during the cooking the sharp utensils can be used which can scratch the coatings and produce nanoparticles release. Chronic food consumption statistics for the consumption of meat and vegetables (in g/kg bw/day) for Slovenia is not available yet. The assumption that Slovenian dietary habits are similar to dietary habits of neighbouring Slovenian countries was made. Therefore consumption data on meat and vegetables for Italy, Austria and Hungary were extracted from EFSA Comprehensive European Food Consumption Database (EFSA, 2016b), as presented in Table 4.5. Chronic food consumption data for the fourth neighbouring country (Croatia) was not available in the database. Hungarian data was not available for toddlers. In addition, German chronic food consumption statistics were extracted as well. Data were extracted for mean and 95th percentile intakes (for consumers only) for Foodex Level 1 (for raw data see Appendix A). Foodex is a food classification system developed by EFSA. Foodex Level 1 includes 20 categories of food.

Direct comparison between food consumptions data for different countries is not appropriate due to possible different methodologies of data acquisition. However, in order to obtain the range of exposures, the highest consumption of vegetables and meat (in

Italy) and the lowest consumption of vegetables and meat (in Austria) were used for further estimation of exposure, by multiplying TiO_2 release obtained by migration testing and food consumption data according to Equation 3.2 (as described in the 3.3).

Results of estimated exposure to TiO_2 nanoparticles released from the pan according to exposure scenario 3 are presented in Table 4.6.

Table 4.5: Consumption data for vegetables and vegetable products including fungi and meat and meat products for Italy, Austria, Hungary and Germany. Data are extracted from EFSA Comprehensive European Food Consumption Database.

ITALY				
Type of food	Adults consumption in g/kg bw/day		Toddlers consumption in g/kg bw/day	
	Mean consumption	95th percentile	Mean consumption	95th percentile
Vegetables and vegetable products (including fungi)	3.454	7.046	5.796	21.625
Meat and meat products	1.661	3.260	3.725	7.517
Sum	5.115	10.306	9.521	29.142
AUSTRIA				
Type of food	Adults consumption in g/kg bw/day		Toddlers consumption in g/kg bw/day	
	Mean consumption	95th percentile	Mean consumption	95th percentile
Vegetables and vegetable products (including fungi)	1.642	4.433	2.486	6.249
Meat and meat products	1.547	3.87	2.978	6.672
Sum	3.189	8.303	5.464	12.921
HUNGARY				
Type of food	Adults consumption in g/kg bw/day		Toddlers consumption in g/kg bw/day	
	Mean consumption	95th percentile	Mean consumption	95th percentile
Vegetables and vegetable products (including fungi)	2.187	4.702	-	-
Meat and meat products	2.599	5.078	-	-
Sum	4.786	9.780		
GERMANY				
Type of food	Adults consumption in g/kg bw/day		Toddlers consumption in g/kg bw/day	
	Mean consumption	95th percentile	Mean consumption	95th percentile
Vegetables and vegetable products (including fungi)	1.704	4.385	4.037	9.46
Meat and meat products	1.514	3.592	2.763	6.794
Sum	3.218	7.977	6.8	16.254

Table 4.6: Range of estimated exposures EE to nano TiO₂ particles released from food contact material P2 into vegetables and meat by the use of EFSA Comprehensive Food Consumption Database (exposure scenario 3).

	EE for mean consumption in µg nano TiO ₂ /kg bw/day	EE for 95th percentile consumption in µg nano TiO ₂ /kg bw/day
Highest estimated exposure for adults (Italian data)	7.2	14
Highest estimated exposure for toddlers (Italian data)	13	41
Lowest estimated exposure for adults (Austrian data)	4.5	12
Lowest estimated exposure for toddlers (Austrian data)	7.6	18

4.3.2.4 Exposure scenario 4 (nanoparticles released in actual use calculated from the image)

Nanoparticles daily release was calculated from the EM image (Figure 4.16) for three different depths of the scratches (described in 4.2.3.3). Taking into account only this, visible degradation, exposure was assessed to be in the range from 0.1 to 0.2 µg /kg bw/ day for adults (70 kg according to new default EFSA value, EFSA, 2012) and from 0.6 to 1.3 µg /kg bw/ day for toddlers (12 kg) as presented in Table 4.7. Exposure was calculated by the use of Equation 3.4 (as described in 3.3.1). Calculated daily release (in µg nano TiO₂/day) was divided by default body weights (70 kg for adults or 12 kg for toddlers).

Table 4.7: Range of estimated exposures EE to nano TiO₂ particles released from food contact material U2 as calculated from Figure 4.16 (scenario 4).

Daily release of nano TiO ₂ in µg	EE for adults in µg nano TiO ₂ /kg bw/day	EE for toddlers in µg nano TiO ₂ /kg bw/day
8	0.1	0.6
12	0.2	1.0
16	0.2	1.3

4.3.2.5 Exposure scenario 5 (nanoparticles released by tribological testing)

Tribological testing performed on the intact quasi-ceramic coating P2 (results presented in 4.2.3.2) caused mechanical degradation of the matrix and particles release up to 9.2 µg

of TiO₂ nanoparticles. This quantity was used for exposure assessment scenario 5. Assuming that it represents daily release, estimated exposure is 0.1 µg nano TiO₂/kg bw/day for adults and 0.8 µg nano TiO₂/kg bw/day and it is in the same order of magnitude as exposure scenario 4.

4.3.2.6 Overview of exposure estimates

Estimated exposures based on different approaches are collected in Table 4.8. As expected, exposure estimates quite differ depending on the used scenario. Scenario 1 is old and does not distinguish between toddlers and adults or different types of food. Scenario 4 takes into account real use but it is probably an underestimation because this scenario takes into account only the visible release. Scenario 5 was just an attempt of simulation (it is actually similar to calculation). Scenario 3 (use of food consumption data from EFSA Comprehensive European Food Consumption Database) gave lower estimated exposures than the use of other food consumption scenarios. In this scenario, only the meal consisting of meat and vegetables cooked in the pan was assumed, while in the worst case, various foods could be prepared in the pan, therefore it is also an underestimate, but probably more realistic than scenarios 1 and 2.

It should be noted that Rompelberg et al. estimated dietary intake of TiO₂ nanoparticles from food, food supplements and toothpaste in the Dutch population (Rompelberg et al., 2016). The estimation was based on Dutch National Food Consumption Survey and on average measured total Ti or TiO₂ particle levels in such products. In the estimation, food contact materials were not taken into account. The estimated mean intake of TiO₂ NPs ranges from 0.55 µg/kg bw/day for 7-69-year-old people to 2.16 µg/kg bw/day for young children. Ninety-fifth percentile (P95) values are 0.74, 1.61 and 4.16 µg/kg bw/day, respectively. Our results showed that exposure to TiO₂ nanoparticles from food contact coating P2 is for scenarios 4 and 5 comparable to estimated dietary intake of TiO₂ nanoparticles from food, food supplements and toothpaste in the Dutch population. For other scenarios, exposure is higher.

According to the tiered approach to toxicity testing from EFSA document, based on exposure levels for conventional materials as presented in Table 1.5 and taking into account our exposure assessment (scenarios from 1 to 3) the estimated exposure range would be from 4 µg nano TiO₂/kgbw/day (Austrian data for mean consumption of adults) to 70 µg nano TiO₂/kgbw/day (special foods for toddlers according to new EFSA document) and it would fall within Tier 2. Exposure estimations based on scenario 4 (calculated from the image) and scenario 5 (based on tribological test) would fall within Tier 1. However, we must not forget that the tiered approach is appropriate for conventional food contact materials. For nanotechnology-derived food contact materials, all available toxicological data should be obtained and taken into account.

Table 4.8: Estimated exposures to TiO₂ nanoparticles from food contact coating P2 based on different scenarios.

Scenario	EE in $\mu\text{g nano TiO}_2/\text{kgbw/day}$	
	EE adults	EE toddlers
1 (old scenario) (17 g food/ kg bw/day)	24	
2 (EFSA new document, special food for toddlers, 50 g food/ kg bw/day and other types of food, 20 g food/ kg bw/day*)		70
	28	
3 (migration test and Italian food consumption data from EFSA Food Consumption database)	7-14	13-41
3 (migration test and Austrian food consumption data from EFSA Food Consumption database)	4-12	8-18
4 (calculation from the image of the pan in actual use)	0.1-0.2	0.6-1.3
5 (tribological test of matrix degradation)	0.1	0.8

*According to the EFSA document (EFSA, 2016a), this value should cover consumption by all population groups although based on food consumption of toddlers.

Alternatively, exposure to nanoparticles released from nanotechnology-derived food contact materials can be expressed in terms of the amount of material used which contains nanoparticles in question (adapted from Pocas & Hogg, 2007), Equation 4.8:

$$\text{Estimated exposure} \left(\frac{\mu\text{g}}{\text{kg bw}} \right) = \frac{\text{Migration} (\mu\text{g}/\text{dm}^2) \times \text{Material usage} (\text{dm}^2/\text{kg bw /day})}{\text{day}} \quad (4.8)$$

However, data on material usage for quasi-ceramic coatings were not available. It should be noted that in our exposure assessment effects of possible changes during storage, effects of stability of the TiO₂ nanoparticles as well as effects of digestion were not considered.

4.3.3 Risk characterisation

After review of hazard characteristics of TiO₂ nanoparticles and assessments of exposure, possible risk was characterised by comparison of exposures estimated in scenarios 3 (migration test and Italian or Austrian food consumption data) and 4 (released nanoparticles calculated from the image) with Derived No-Effect Levels (DNEL) 1 and 2 calculated (in absence of Tolerable Daily Intake Value) from the Lowest Observed Adverse Effect Level (LOAEL). The risk to humans can be considered to be adequately controlled if the estimated levels of exposure do not exceed the appropriate DNEL. Risk characterisation ratio (RCR) was calculated by dividing estimated exposures by two different reference value DNELs. In the field of food contact materials, 20 % of the reference value is often considered because the reference value also covers other exposure sources (e.g. exposure to nano TiO₂ from food additives, cosmetics, textiles) (Equation 3.6).

For calculation of the risk characterisation ratio, estimated exposures from Table 4.8 were taken into account together with DNEL (1) = 4.7 µg/kg bw/day and 20 % DNEL = 0.94 µg/kg bw/day and DNEL (2) = 0.33 µg/kg bw/day and 20 % DNEL = 0.066 µg/kg bw/day. By applying these values, a range of risk characterisation ratios was obtained. Results are presented in Table 4.9.

By the use of DNEL (1) risk characterisation ratios for scenario 3 are higher than 1 for different consumption data, for toddlers and adults, showing that risk may not be adequately controlled. For scenario 4 (released nano TiO₂ particles calculated from the SEM image) risk characterisation ratios are for adults (for both depth of scratches assumed) and for toddlers taking into account the depth of scratches 10 µm lower than 1. Taking into account the depth of scratches 20 µm and 30 µm, for toddlers, RCRs are larger than 1.

By the use of DNEL (2) risk characterisation ratios are much higher than 1 for all exposure scenarios, all consumption levels and both populations.

Exposure assessment followed by risk characterization described above suggests that there may be a risk for neurobehavioral effects and endocrine malfunction due to exposure to nano TiO₂ particles released from quasi-ceramic coatings into acidic foods (foods with pH < 4.5) and due to exposure based on mechanical matrix degradation. If DNEL (2) is considered (endocrine malfunction), even with the assumption that rutile nanoparticles are 100 times less toxic than anatase (Sayes et al., 2006), risk characterisation ratios would be larger than 1 for exposure scenario 3 for both toddlers and adults.

Risk characterisation ratios based on conservative calculations for different scenarios presented in Table 4.9 showed that risk of neurobehavioral effects and endocrine malfunction for adults and for toddlers due to exposure to nano TiO₂ from quasi-ceramic coatings could not be excluded. Therefore, further refinement of exposure assessment should be performed. Such refinement would include improvement of release testing (preferably determination of release into real food) and improvement of food consumption assessment. It would also be very important to determine if released particles remain in nanoform during digestion.

Besides, new toxicological data is needed. Toxicological data are available only for anatase TiO₂ nanoparticles, smaller than particles which were released from quasi-ceramic coatings. It is known that the crystal structure and size may influence the toxicity of the particles. Therefore toxicological data on nano TiO₂ rutile with a larger particle size and preferably toxicological data on particles released by mechanical degradation of the quasi-ceramic coatings would be very useful. Particles partially

bonded to the polymer may have a different toxicity than TiO_2 particles which are not bonded to the polymer.

Table 4.9: Risk characterisation ratios RCR for nano TiO_2 particles released from food contact material P2, for adults and toddlers based on exposure scenarios 3 and 4, for two different Derived No-Effect Levels.

Risk characterisation ratios for the use of DNEL (1) (neurobehavioral effects)				
	Adults mean	Adults 95 percentile	Toddlers mean	Toddlers 95 percentile
Scenario 3				
Italian data	7.4	14.9	13.8	43.6
Austrian data	4.3	12.8	8.5	19.1
	Adults (depth of scratches 10 μm)	Adults (depth of scratches 30 μm)	Toddlers (depth of scratches 10 μm)	Toddlers (depth of scratches 30 μm)
Scenario 4				
calculation from the image	0.11	0.21	0.64	1.38
Risk characterisation ratios for the use of DNEL (2) (endocrine malfunction)				
	Adults mean	Adults 95 percentile	Toddlers mean	Toddlers 95 percentile
Scenario 3				
Italian data	106	212	197	621
Austrian data	61	182	121	273
	Adults (depth of scratches 10 μm)	Adults (depth of scratches 30 μm)	Toddlers (depth of scratches 10 μm)	Toddlers (depth of scratches 30 μm)
Scenario 4				
calculation from the image	1.5	3	9	20

4.3.4 Risk assessment uncertainties

In order to better understand the outcome of the risk assessment, uncertainties associated with different steps of assessment should be considered. Many different uncertainties should be taken into account: uncertainties in the physicochemical characterization of the engineered nanomaterial, uncertainties in the hazard characterization of the engineered nanomaterial and uncertainties in exposure assessment.

Uncertainties regarding physicochemical characterisation and exposure assessment are mostly connected to migration testing. Conventional simulants and time/temperature/surface to volume conditions were used although it is not known if they represent the worst case for the release testing of novel materials. That means that uncertainties are present due to the difference between testing with simulants and testing with real food. TiO₂ nanoparticles are not particularly stable in simulants and may sediment out of dispersion which may contribute to the uncertainty of their determination in simulants. Due to a positive surface charge in the acidic region, released TiO₂ particles may be attracted to the negative surface charge of the glassware which can cause additional uncertainties in the determination of concentration. Measurement uncertainties of ICP MS quantification of release were also present. Possible surface coatings on nanoparticles were not determined but may contribute to overall uncertainty. Worst case assumption that all released particles were nanosized was used although released particles agglomerate in simulant 3 % acetic acid. Mechanical degradation of the matrix may lead to the release of nano TiO₂ particles partially bonded to silicone matrix, but the concentration of such particles was not determined. For particles originating from the matrix degradation study, only results for particles smaller than 200 nm were taken into account, so an overestimation was made. The assumption that 1 l of food is equal to 1 kg of food introduces an additional uncertainty. Release into simulants was increasing in every subsequent test, so actual exposure during many uses may be higher. Assumptions were also made that all released nano TiO₂ particles are ingested and absorbed in the nanoform, although the structure and properties of the nanoparticles were undetermined and therefore difficult to relate to the structure and properties of the nanoparticles used in the toxicity studies.

In the absence of Slovenian food consumption data, the assumption was made that Slovenian dietary habits are similar to dietary habits of neighbouring countries. For the calculation of particles released based on the image, it was assumed that a simplified area of scratches is representative for the whole pan.

The largest uncertainties in the risk assessment, besides uncertainties in consumption data (especially in scenarios which do not take into account differences between populations), are probably connected with hazard characterisation. Toxicity studies have not been conducted on exactly the same nanoform of TiO₂ as was used for toxicity testing. In the absence of studies conducted with rutile nanoparticles, results of studies on nanoanatase were used. Most of the toxicological studies were done on smaller particles. For that reason, hazard assessment and calculated DNEL may differ for the larger rutile particles, nanoform of the TiO₂ present in quasy-ceramic coatings. Some of the toxicity studies on TiO₂ have been performed without relevant particle characterization (size, crystal structure, shape) and with the administration of very high doses of TiO₂ which are not relevant to daily human uptake (Jovanovic, 2015). Furthermore, the toxicity of the released particles partially bonded to silicone matrix is not known. Nanoparticles present in the body are covered with protein corona which influences their toxicity (Lee, Choi, Webster, Kim, & Khang, 2015). Nanoparticles released from the food contact materials are, after contact with food, probably covered with proteins or other components of the food (sugars, fats), and it is very likely that these coronas (coatings) influence their toxicological behaviour in the body. What happens after contact of particles released with components of food was not studied yet. Use of different safety factors for calculation of DNEL is a source of uncertainty as well, and the assumption that only 20 % of the reference value is connected to food contact materials is a source of uncertainty as well.

4.4 Screening Method for Detection of Nanoparticles Present in/Released from Food Contact Materials

Analytical techniques for characterisation of nanoparticles present in food contact materials and released into foods or simulants require sophisticated instrumentation, highly skilled personnel and demanding sample preparation. However, sophisticated techniques are not available in small food contact laboratories. There is a need for a screening method capable of screening their presence in food contact material, before use of other techniques. For that purpose, the catalytic activity of TiO_2 nanoparticles (present in quasi-ceramic coatings) and silver nanoparticles (most often used nanoparticles in food contact materials) was tested.

Catalytic activity is listed among the main parameters for characterization and identification of nanomaterials, in the EFSA document on recent developments in the risk assessment of chemicals in food and their potential impact on the safety assessment of substances used in food contact materials (EFSA, 2016a). Nanoparticles may catalyze a redox or other reactions which may result in a much larger biological response even with small amounts of the catalytically active nanoparticles. Recently, several authors reported on catalytic reactivity of nanoparticles (Mallick, Witcomb, & Scurrall, 2006, Cuenya, 2010, Gupta, Singh, & Sharma, 2011, Marques et al., 2013, Suvith & Philip, 2014, Ganapuram et al., 2015, Corredor et. al., 2015). Corredor et al. reported on the use of catalytic properties of nanoparticles for determination of nanoparticles in environmental and biological samples. The method is based on the reduction of methylene blue with sodium borohydride, which results in a colour change from blue into colourless due to the formation of colourless leucomethylene blue (Figure 4.18). The reduction reaction is thermodynamically and not kinetically favoured. Nanoparticles serve as an electron relay in the redox reaction electron from the electron donor sodium borohydride to the electron acceptor methylene blue (Figure 4.19).

Without the presence of nanoparticles as catalysts, the reaction is very slow. However, if nanoparticles are present, the reaction is much faster. Corredor et al. studied silver, gold, CeO_2 , SiO_2 and VO_2 nanoparticles. TiO_2 nanoparticles were not included in their study.

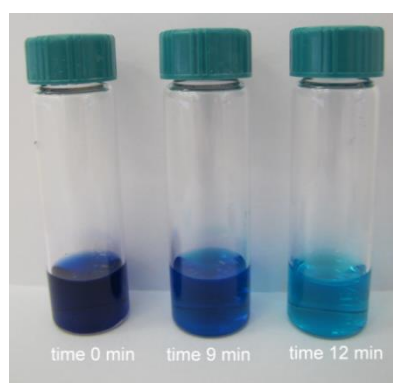


Figure 4.18: Change of colour of methylene blue in the presence of Ag nanoparticles 10.3 mg/l.

4.4.1 Detection of nano TiO₂ particles released into simulant

To find out if the method is applicable for detection of TiO₂ nanoparticles released from quasi-ceramic coatings into food simulants after migration testing, sodium borohydride and methylene blue dye redox pair, the method described by Corredor was applied. Rutile nanoparticles were used in concentration 20 mg/l, but the reaction was very slow and barely detectable after 20 minutes (Figure 4.20). Knowing that surface charge of nanoparticles has a high influence on the catalytic reactivity of nanoparticles and positively charged nanoparticles possess higher reactivity than negatively charged particles (Corredor et al.2015), this nonreactivity can be explained by the negative surface charge of rutile nano TiO₂. The commercial rutile that we used was negatively charged at inherent pH and positively charged only below pH 5.5, as shown in Figure 4.19. Simple pH adjustment and use of rutile dispersions as catalysts in the acidic region were not appropriate because sodium borohydride cannot be used at acidic pH due to hydrolysis. For that reason, in order to find out if nano TiO₂ particles (rutile or anatase, which can also be present in food contact material) can be positively charged at higher pH values, surfactant cetyl trimethylammonium bromide (CTAB) was added to nanoparticles P25 when dispersed in water. P25 is a commercial powder of TiO₂ nanoparticles which contain mixed rutile and anatase phase and its zeta potential at pH 7 is near IEP (Novak et al., 2016). The positive charge was achieved at higher pH values (see Figure 4.21) and the reaction was repeated. The slightly faster reaction was achieved, but only for high concentrations (200 mg/l) of P25 nanoparticles as presented in Figure 4.22.

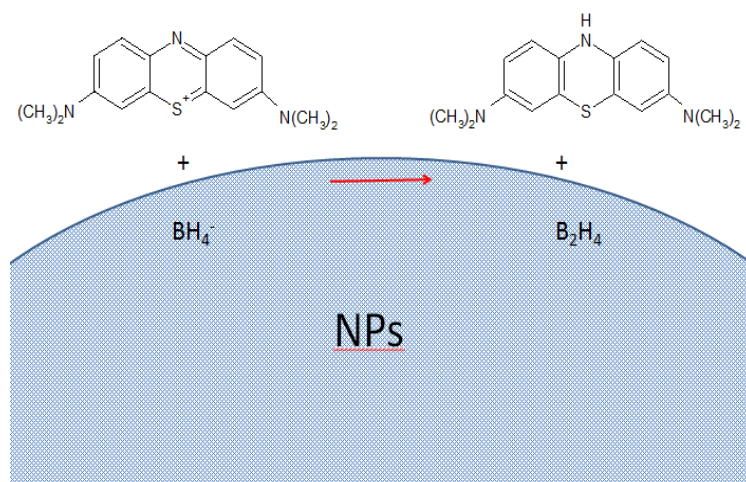


Figure 4.19: Nanoparticles are serving as an electron relay in the redox reaction between sodium borohydride and methylene blue dye.

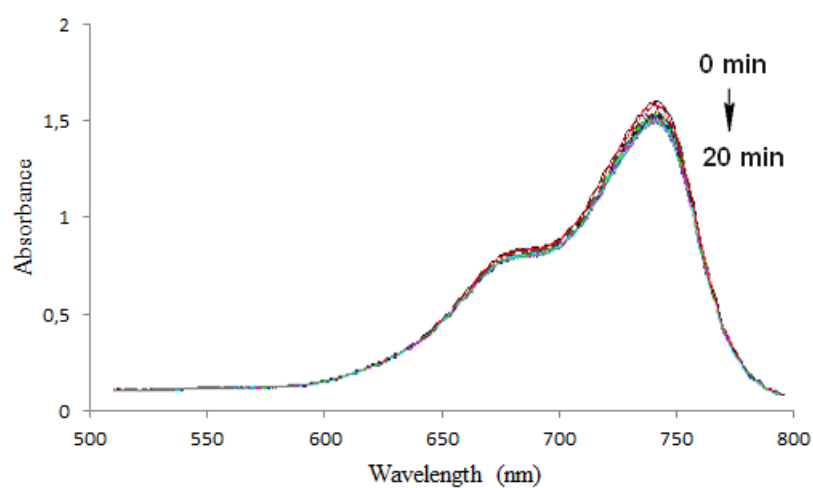


Figure 4.20: Change in the visible spectra of methylene blue solution after the addition of sodium borohydride in the presence of TiO₂ rutile (20 mg/l) nanoparticles during 20 minutes (spectra were collected every 30 seconds).

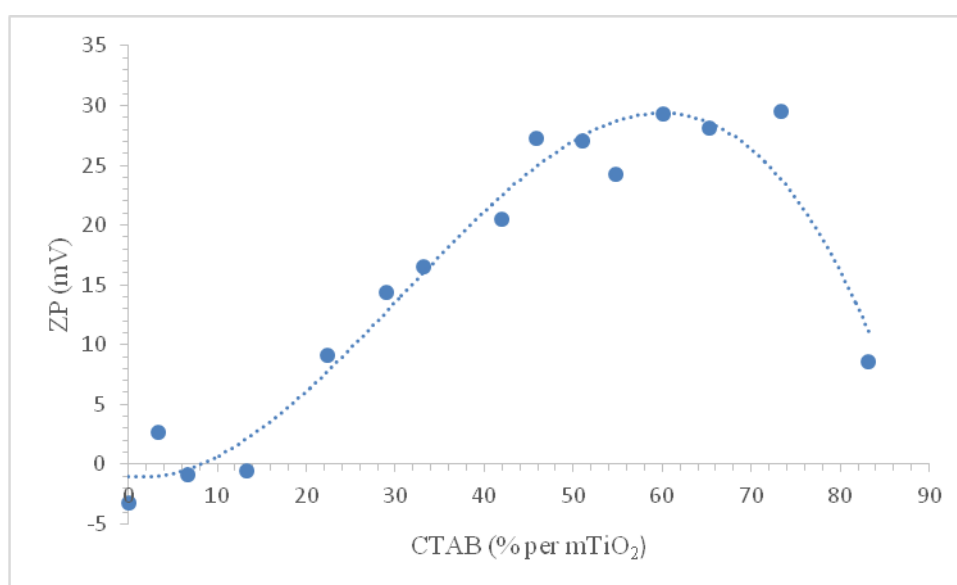


Figure 4.21: Zeta potential of TiO₂ nanoparticles P25 with different additions of CTAB.

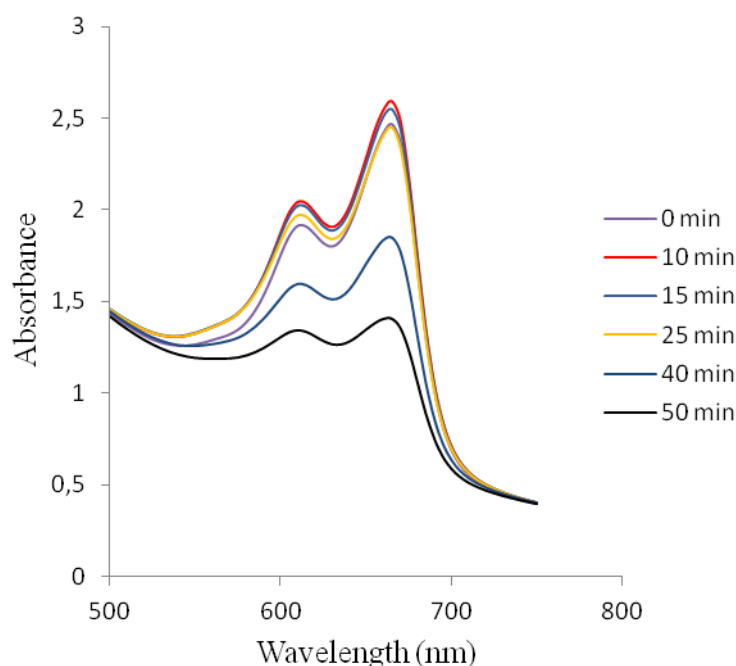


Figure 4.22: Discolouration of methylene blue with sodium borohydride in the presence of 200 mg/l nano TiO₂ P25.

It is possible to use the Corredor method for detection of higher concentration (200 mg/l) of nano TiO₂ particles in simulant deionised water, although the reaction catalyzed by these particles was still quite slow. As seen in Figure 4.22, significant discolouration was observed only after 50 minutes. Furthermore, the tested nanoparticles were a mixture of rutile and anatase (P25), and anatase is expected to be a better catalyst than rutile. In conclusion, a method is not sufficiently sensitive for determination of particles migrating from our food contact material into simulant deionised water. From quasi-ceramic coatings, much lower concentrations of nano TiO₂ (rutile) particles are expected to be released into simulant deionised water (39 µg Ti/l or 65 µg TiO₂/l as determined by ICP MS). Higher concentrations of nano TiO₂ particles were released into simulant 3 % acetic acid, but the method based on the catalytic activity of TiO₂ nanoparticles is not applicable to acidic simulants due to rapid sodium borohydride hydrolysis in acidic media (Rohm & Haas, 2003).

4.4.2 Detection of silver nanoparticles in extracts of food contact paper

Silver nanoparticles are most often nanoparticles used in the application of nanotechnology in the agricultural, feed and food sector including food contact materials (RIKILT & JRC, 2014). RFID antennas (which may contain silver nanoparticles) are printed on paper and are becoming increasingly common because of the possibility of producing RFID tags more economically, ecologically and rapidly. Papers containing RFID antennas may be included in the recycling process and may become part of food

contact papers. From such food contact papers, silver nanoparticles may be released into foods, and for that reason, a screening method would be useful.

Corredor method (Corredor et al., 2015) was modified and used for the detection of silver nanoparticles in simulant deionised water and two extraction solvents. The method was modified by using a different buffer solution. Instead of the HEPES buffer (with pH 7), borate buffer with higher pH 9 was used knowing that reducing agent sodium tetrahydroborate is more stable in alkaline solutions. Silver nanoparticles (yellow dispersion) needed for calibration curve were synthesized *in situ* by reduction of AgNO_3 with sodium tetrahydroborate in the presence of trisodium citrate at room temperature (as described by Mendis et al. 2016). Synthesized particles had a negative charge (zeta potential measured immediately after synthesis was -39.9 eV), due to the adsorbed citrate ions. Effective diameters of the silver nanoparticles were measured at inherent pH (8.9) by dynamic light scattering (DLS) and varied from $25 - 42$ nm. The size of synthesized silver particles was slightly larger than described in the Mendes paper ($10 - 30$ nm). According to TEM images in the Mendes paper, particles synthesized in such a way are spherical in shape with smooth morphology.

Concentrations of methylene blue at different reaction times (needed for calculation of reaction rates) were determined from calibration curve presented in Figure 4.23. A calibration curve was prepared by mixing solution of sodium borohydride and different concentrations of methylene blue without the addition of silver nanoparticles.

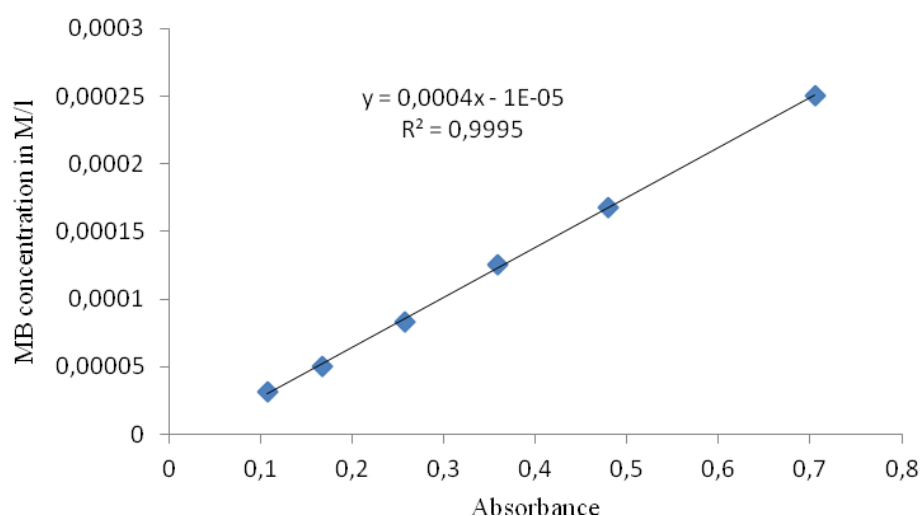


Figure 4.23: Plot showing the concentration of methylene blue vs absorbance at time 0, without the presence of nanoparticles.

Visible spectra were recorded immediately after the addition of methylene blue to a dispersion containing silver nanoparticles and sodium tetrahydroborate in phosphate buffer solution (time 0) and after that in intervals of 3 minutes as shown in Figure 4.24. In comparison to TiO_2 nanoparticles (shown in Figure 4.22), silver nanoparticles caused much faster discolouration of methylene blue for lower concentrations of silver nanoparticles than TiO_2 nanoparticles, because they are better catalysts for this reaction.

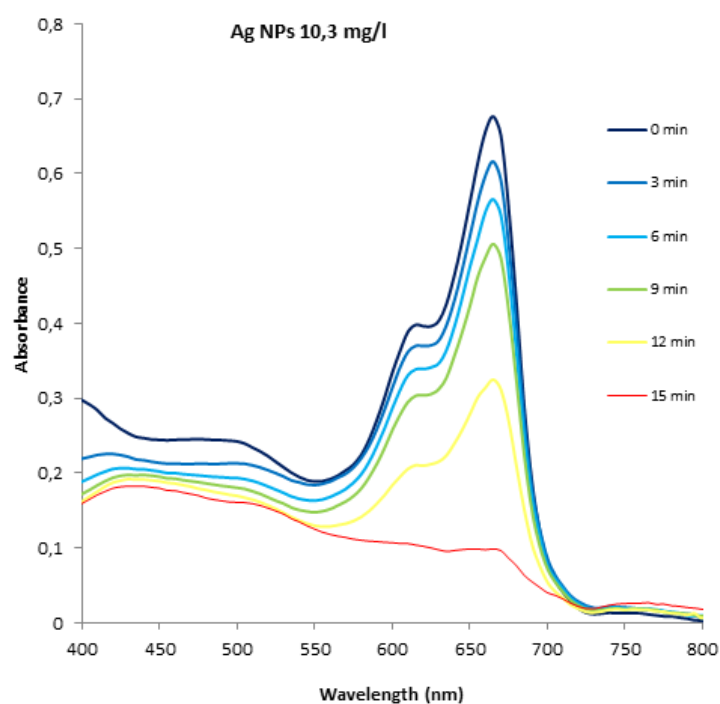


Figure 4.24: Discolouration of methylene blue solution with sodium tetrahydroborate during 15 minutes in the presence of 10.3 mg/l silver nanoparticles.

Higher concentrations of silver nanoparticles cause faster reactions, as expected. It is clearly visible in Figure 4.25.

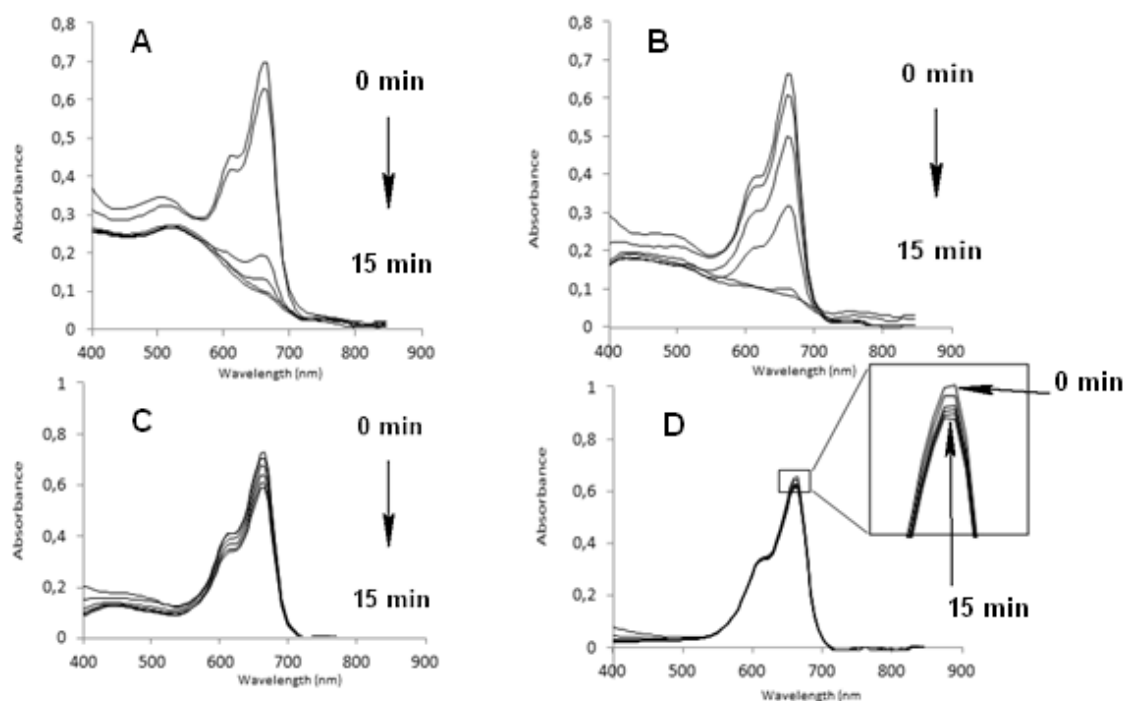


Figure 4.25: Discolouration of methylene blue solution with sodium tetrahydroborate at different concentrations of silver nanoparticles: A=15.4 mg/l, B 10.3 mg/l, C: 7.7 mg/l, D: 1.93 mg/l. Spectra were taken every 3 minutes.

Molar concentrations of methylene blue at different reaction times (as presented in Table 4.10) were used for calculation of average reaction rates over the first 360 seconds for different concentrations of Ag nanoparticles using Equation 3.7.

Table 4.10: Calculated concentrations of methylene blue at time 0 s, 180 s and 360 s in the presence of different concentrations of catalysts silver nanoparticles.

Time in s	Calculated concentrations of MB (in mM/l) 15.4 mg/l Ag NPs	Calculated concentrations of MB (in mM/l) 10.3 mg/l Ag NPs	Calculated concentrations of MB (in mM/l) 7.7 mg/l Ag NPs	Calculated concentrations of MB (in mM/l) 1.93 mg/l Ag NPs
0	0.266	0.255	0.288	0.256
180	0.234	0.228	0.275	0.253
360	0.113	0.208	0.268	0.250

Average reaction rate as a function of the concentration of silver nanoparticles is presented in Figure 4.26. The correlation between average rate and concentration of silver nanoparticles is exponential with acceptable correlation coefficient.

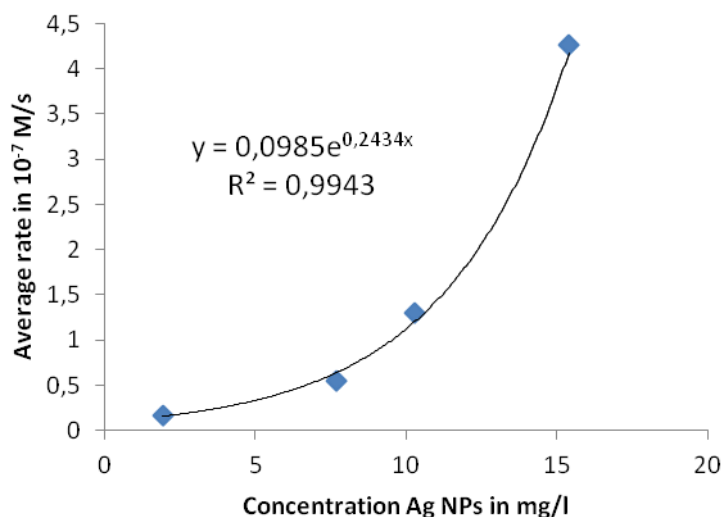


Figure 4.26: Average reaction rate over the first 360 s vs concentration of Ag nanoparticles.

The concentration of Ag nanoparticles present in simulant deionised water was calculated from Figure 4.26 by determination of the reaction rate. Higher reaction rates indicate a higher concentration of Ag nanoparticles in the simulant. From the curve, in Figure 4.26,

it can be assessed that the method can detect the presence of Ag nanoparticles in deionised water at a concentration from 2 mg/l up to 15 mg/l.

The method was applied to a paper containing silver nanoparticles. Silver nanoparticles may be added to food contact materials as antimicrobials (Echegoyen & Nerin, 2013) or can be present unintentionally in recycled paper and board as residues originating from e.g. printed electronics, especially radio-frequency identification (RFID) tags. As a model for food contact paper containing silver nanoparticles, paper with printed RFID antenna was used (Figure 4.27). Cut pieces of printed paper were mixed with a magnetic stirrer with different extraction solvents: deionised water, sodium citrate and borate buffer. Deionised water is a simulant often used for analysis of food contact paper (CEN, 1997a, CEN, 1997b), sodium citrate was expected to stabilise silver nanoparticles in dispersion as a capping agent (Bastús, Merkoçi, Piella, & Puntès, 2014) and borate buffer with pH 9 was expected to have a positive impact on the stability of sodium borohydride.

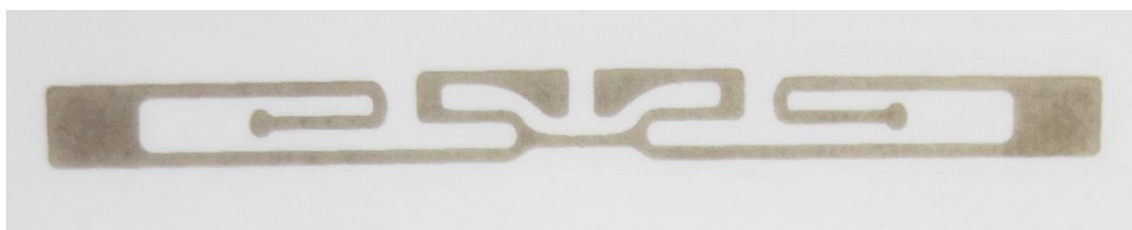


Figure 4.27: RFID antenna printed with a printing ink containing silver nanoparticles. RFID antenna was printed on commercial high-white gloss label paper with nanosilver conductive ink, using semi-automatic screen printer.

After 1 hour of mixing with different extraction solvents, the printed antenna was not completely released from the paper, and silver grey ink was still visible on cut pieces. Irrespective, catalytic activity of Ag nanoparticles released from the paper was detected in all extracts. Concentrations of Ag nanoparticles determined in different extracts are presented in Table 4.11.

Table 4.11: Results of determination of Ag nanoparticles in extracts of paper with RFID antenna for different extraction solvents.

Sample	Concentration of Ag nanoparticles in paper extracts (mg/l extract)
1 (0.01 M sodium citrate)	2.7
2 (0.05 M borate buffer)	8.9
3 (deionised water)	4.3

The highest concentration of Ag nanoparticles was observed for extraction with borate buffer. Higher pH of borate buffer has a positive impact on the stability of sodium borohydride, the reducing agent used in the screening method. At lower pH (extraction with sodium citrate and deionised water) more silver ions may be formed from the

particles. The rate of forming of silver ions from silver nanoparticles depends on the silver particle size, particle functionalization and the local environment (Liu & Hurt, 2010, Liu, Sonshine, Shervani, & Hurt, 2010, Addo Ntim, Thomas, Begley, & Noonan, 2015). Silver nanoparticles are also not persistent in dispersions containing dissolved oxygen. During long extraction, other particles (Ag_2S , AgCl , Ag_2O , Ag_2CO_3), with different catalytical activity may be formed as well. For the further studies, it would be useful to test if shorter extraction with borate buffer would give higher results. Extraction solvent sodium citrate is not only the capping ligand but also the reducing agent. If silver ions are released from nanoparticles present in the material, the citrate may reduce them into nanoparticles especially after the addition of sodium hydride during the screening procedure. However, that was not the case for our tested paper. For further studies, it would be useful to test the use of other extraction solvents.

The method was also applied to two different solutions (containing 170 mg/l AgNO_3 and 0.85 mg/l AgNO_3), containing only silver ions (no nanoparticles). Strong reducing agent sodium borohydride reduced a high concentration of silver ions (170 mg/l AgNO_3 solution) to larger (visible) particles immediately after addition, and visible black suspension occurred. For a smaller concentration of silver ions (0.85 mg/l AgNO_3 solution), concentrations of the same order of magnitude as described for some food contact applications (e.g. in the paper Cushen, Kerry, Morris, Cruz-Romero, & Cummins, 2014), use of the method showed negligible catalytic reaction (reaction rate was $5.2 \cdot 10^{-09}$ M/s). It would be useful to examine further the use of screening procedure for dispersions containing both silver nanoparticles and ions.

The Corredor method that we modified is based on the calculation of a reaction rate. The reaction rate depends not only on the concentration of nanoparticles and temperature but also on the size and shape of nanoparticles as well (Cuenya, 2010, Suvith & Philip 2014). Silver nanoparticles have unprecedented dynamic behaviour in dispersions and on surfaces (Glover, Miller, & Hutchison, 2011). New nanoparticle production may occur through a process involving oxidation and dissolution of silver from the surface of the particle, diffusion of the silver ion across the surface in an adsorbed water layer and formation of new, smaller particles by chemical and/or photo reduction. The silver nanoparticles and silver ions in dispersions are also very sensitive to oxidation and reduction and can be influenced by the presence of other reactive species (Addo Ntim, Thomas, Begley, & Noonan, 2015).

We can conclude that the use of the method for detection of silver nanoparticles in extracts of food contact paper is possible. However, for the samples containing silver nanoparticles for which we do not know the size, shape and other characteristics, the method is suitable only for screening purposes. If catalytic activity in the extract of food contact paper is detected, more sophisticated methods of material characterization and determination of silver nanoparticles concentration and properties should be used.

Chapter 5

Conclusions

The present study reports on the most relevant physicochemical characteristics of commercially available non-stick food contact quasi-ceramic pan coatings and on testing a possible nanoparticles release into food. Both new and used pans were analysed. Results of release testing were then used for the assessment of health risk through exposure to released nano TiO₂ particles. Furthermore, a screening method based on catalytic properties of nanoparticles was used for the detection of nanoparticles present in food contact materials.

The analysis confirmed that all the studied samples contained fine TiO₂ particles embedded in an approximately 50 µm thick silicone polymer matrix. In the coatings, SiO₂ was present as well. SEM, FTIR and XRD analyses revealed that the TiO₂ particles are in the form of rutile and their estimated size is in the range of 70 to 250 nm.

The study considered different mechanisms of nanoparticles release. The migration of TiO₂ particles from the coatings was caused by the dissolution mechanism. The large TiO₂ particles were partially dissolved from the surface of the coatings which resulted in the release of smaller TiO₂ particles. Migration was tested with three simulants: deionised water, 3 % acetic acid and 5g/l citric acid, three times successively for 2 hours at 100 °C. ICP-MS analysis of simulants after migration tests, combined with ultrafiltration, demonstrated that titanium is released from the coatings in the form of both, particles and ions, although TiO₂ particles are embedded in the silicone matrix. The highest release of Ti was observed into acidic simulants; release of particles into 3 % acetic acid and release of ions into 5/l citric acid. The mechanism of particles release due to matrix degradation was studied as well, considering thermal and mechanical degradation. The results of DSC/TG MS analysis suggest that within the temperature range of recommended use on the stove (up to 450 °C), the weight loss was minor, while considerable weight loss can appear above 500 °C. After heating to 700 °C the organic phase largely transforms to CO and CO₂ leaving TiO₂ and SiO₂ as the prevailing phases. Therefore, thermal degradation is also a probable mechanism of release of TiO₂ particles, but only at temperatures above recommended. Mechanical degradation studies (scratch and wear tests), simulating the use of the sharp utensils, showed chipping of the material and formation of partially nanoparticulate debris, so the most probable mechanism of matrix degradation for this type of coatings is mechanical abrasion. The release of nanoparticles from food contact materials due to mechanical degradation has not been described in the literature so far.

Based on the results of migration tests and on calculation of the amount of particles released from the coating, which was in use for two years, taking into account different exposure scenarios and derived no-effect levels, we conclude that a health risk due to

exposure to nano TiO_2 particles released from the food contact coating cannot be excluded. However, to confirm the health risk, assessment should be refined.

To refine the risk assessment and to clarify if exposure to TiO_2 nanoparticles released from quasi-ceramic coatings poses a risk to health, additional research should be done. Release into foods during actual use (which is still very challenging and difficult task) should be performed. After that, adequate test conditions and simulants mimicking the release into foods could be determined with certainty. Nanoparticles may have very different properties, and generalisation of their properties is questionable. For that reason, appropriate test conditions are probably to be determined on the case-by-case basis. To refine exposure assessment for the Slovenian population, specific consumption data for Slovenians should be obtained. Additional toxicological information regarding long-term toxicity of titania nanoparticles (in particular rutile nanoparticles) should be available to identify hazards and to obtain dose-response data needed for more precise characterization of the hazards. Also, the persistence of released particles in gastrointestinal fluid during digestion should be determined. It would be worth performing toxicological testing of particles actually released from quasi-ceramic coatings by matrix degradation (after the tribological test), as particles partially bonded to the polymer are formed by mechanical degradation and such particles may have different toxicological properties than rutile TiO_2 alone.

Our work was focused on the release of TiO_2 nanoparticles, but quasi-ceramic coatings also contained SiO_2 particles, and further work is required to examine the possible release of SiO_2 (nano)particles into food.

For detection of nanoparticles present in nanotechnology-derived food contact materials, sophisticated instrumentation, highly skilled personnel and demanding sample preparation are needed. However, it is possible to detect the presence and release of nanoparticles from food contact materials into the deionised water by the use of the simple method, based on catalytic properties of nanoparticles. The method is based on the redox reaction between electron donor sodium borohydride and electron acceptor methylene blue dye by determination of reaction rate of methylene blue discolouration. For TiO_2 nanoparticles the method is usable only for concentrations much higher than found in migration experiments. However, for silver nanoparticles (which are the most often used nanoparticles in the application of nanotechnology in the agricultural, feed and food sector including food contact materials) the method can be used for detection of silver nanoparticles released from/present in food contact paper. Paper containing RFID antenna was used in the study. Knowing that the rate of discolouration reaction depends not only on the concentration of nanoparticles but also on temperature and various properties of nanoparticles (e.g. size and shape), the method is suitable for screening purposes. If catalytic activity in the extract of food contact material is detected, more sophisticated methods of characterisation should be used including determination of released nanoparticles concentration and their physicochemical properties.

There are still many questions to be answered regarding the migration of nanoparticles from food contact materials. It should be stressed that future work should focus on the determination of migration of nanoparticles from nanotechnology-derived food contact materials into different foodstuffs, determination of persistence of released particles in gastrointestinal fluids during digestion and on generating toxicity data of nanoparticles with various physicochemical characteristics.

Appendix A

Information on food consumption in some European countries was found in the Comprehensive Food Consumption Database. Data from the Database allowed us to estimate the consumers' exposure to possible hazards (in our case nanoparticles) in food, which is a fundamental step in risk assessment. In the Database, special food classification system 'FoodEx' was used for categorization of all foods and beverages provided by 20 Member States and collected from 22 different national dietary surveys.

Summary statistics from the database enabled quick screening for chronic exposure (acute exposure was not considered relevant for the case of food contact materials) to substances that may be found in the food chain and may originate from the food contact materials. In the database, dietary surveys and food consumption data for each country are divided by categories including age; food group (over 1,500) and type of consumption, covering both regular and high consumption and thus allowing calculations to be tailored to each category of consumer.

The statistics for chronic food consumption was available for the total population ('all subjects') and for consumers of respective food categories; we selected the worst-case approach – consumers only.

Foodex L1	Nr Consumers	% Consumers	Mean	P5	Median	P95
Grains and grain-based products	2,313	100.0%	3.682	1.548	3.515	6.387
Vegetables and vegetable products (including fungi)	2,312	100.0%	3.454	1.072	3.084	7.046
Starchy roots and tubers	1,545	66.8%	1.092	0.105	0.944	2.558
Legumes, nuts and oilseeds	964	41.7%	0.455	0.042	0.335	1.264
Fruit and fruit products	2,153	93.1%	3.036	0.285	2.628	7.267
Meat and meat products (including edible offal)	2,293	99.1%	1.661	0.476	1.537	3.260
Fish and other seafood (including amphibians, rept)	1,602	69.3%	0.989	0.079	0.857	2.480
Milk and dairy products	2,298	99.4%	2.801	0.337	2.531	6.258
Eggs and egg products	1,718	74.3%	0.418	0.045	0.314	1.161
Sugar and confectionary	2,114	91.4%	0.329	0.044	0.271	0.796
Animal and vegetable fats and oils	2,313	100.0%	0.586	0.260	0.565	0.986
Fruit and vegetable juices	1,298	56.1%	0.798	0.009	0.100	3.390
Non-alcoholic beverages (excepting milk based beve	2,229	96.4%	2.555	0.500	2.000	6.390
Alcoholic beverages	1,813	78.4%	1.893	0.000	1.270	6.044
Drinking water (water without any additives except	2,275	98.4%	10.197	2.432	8.906	22.564
Herbs, spices and condiments	2,261	97.8%	0.163	0.006	0.080	0.663
Food for infants and small children	9	0.4%	0.362	0.017	0.346	0.945
Products for special nutritional use	107	4.6%	0.304	0.005	0.042	0.867
Composite food (including frozen products)	501	21.7%	0.843	0.189	0.641	2.051

Figure 6.1: Chronic food consumption statistics (consumers only, INRAN SCAI 2005-06) for Italian adults extracted from EFSA Comprehensive European Food Consumption Database in September 2016. Mean, P5, median and P95 are expressed in grams per day per kilogram of body weight (g/kg bw/day).

Foodex L1	Nr Consumers	% Consumers	Mean	P5	Median	P95
Grains and grain-based products	35	97.2%	9.757	2.782	7.699	18.841
Vegetables and vegetable products (including fungi)	34	94.4%	5.796	0.682	4.505	21.625
Starchy roots and tubers	20	55.6%	3.124	0.058	3.663	6.824
Legumes, nuts and oilseeds	13	36.1%	1.530	0.856	1.365	2.321
Fruit and fruit products	31	86.1%	11.368	0.374	11.128	25.758
Meat and meat products (including edible offal)	33	91.7%	3.725	0.581	3.409	7.517
Fish and other seafood (including amphibians, rept)	23	63.9%	3.920	0.910	3.810	6.594
Milk and dairy products	36	100.0%	28.209	4.583	24.639	73.990
Eggs and egg products	22	61.1%	1.057	0.134	1.013	2.400
Sugar and confectionary	22	61.1%	1.291	0.242	0.939	3.564
Animal and vegetable fats and oils	35	97.2%	1.298	0.346	1.204	2.441
Fruit and vegetable juices	20	55.6%	10.243	0.097	11.081	22.110
Non-alcoholic beverages (excepting milk based beve)	4	11.1%	7.544	3.125	3.911	19.231
Alcoholic beverages	10	27.8%	0.002	0.000	0.001	0.007
Drinking water (water without any additives except	35	97.2%	30.804	9.231	28.033	58.500
Herbs, spices and condiments	30	83.3%	0.334	0.014	0.116	0.934
Food for infants and small children	27	75.0%	8.667	0.889	6.639	20.410
Products for special nutritional use	4	11.1%	0.333	0.014	0.250	0.818
Composite food (including frozen products)	6	16.7%	6.661	1.282	4.567	19.912

Figure 6.2: Chronic food consumption statistics (consumers only, INRAN SCAI 2005-06) for Italian toddlers extracted from EFSA Comprehensive European Food Consumption Database in September 2016. Mean, P5, median and P95 are expressed in grams per day per kilogram of body weight (g/kg bw/day).

Foodex L1	Nr Consumers	% Consumers	Mean	P5	Median	P95
Grains and grain-based products	305	99.0%	3.603	0.945	3.392	7.453
Vegetables and vegetable products (including fungi)	246	79.9%	1.642	0.131	1.297	4.433
Starchy roots and tubers	109	35.4%	1.275	0.423	1.073	3.250
Legumes, nuts and oilseeds	71	23.1%	0.494	0.035	0.369	1.159
Fruit and fruit products	263	85.4%	2.780	0.361	2.190	7.139
Meat and meat products (including edible offal)	251	81.5%	1.547	0.191	1.196	3.870
Fish and other seafood (including amphibians, rept)	64	20.8%	1.095	0.142	1.086	2.176
Milk and dairy products	294	95.5%	2.264	0.248	1.761	6.081
Eggs and egg products	33	10.7%	0.425	0.239	0.387	0.877
Sugar and confectionary	223	72.4%	0.344	0.038	0.225	1.079
Animal and vegetable fats and oils	263	85.4%	0.222	0.024	0.190	0.508
Fruit and vegetable juices	156	50.6%	3.198	0.186	2.177	10.179
Non-alcoholic beverages (excepting milk based beve	287	93.2%	7.823	1.504	6.331	18.713
Alcoholic beverages	144	46.8%	4.840	0.604	2.945	15.789
Drinking water (water without any additives except	300	97.4%	17.289	3.943	16.083	36.008
Herbs, spices and condiments	270	87.7%	0.491	0.010	0.290	1.622
Products for special nutritional use	11	3.6%	2.090	0.108	0.404	6.460
Composite food (including frozen products)	281	91.2%	3.483	0.771	2.886	8.348
Snacks, desserts, and other foods	98	31.8%	0.905	0.128	0.686	2.588

Figure 6.3: Chronic food consumption statistics (consumers only, ASNS Survey) for Austrian adults extracted from EFSA Comprehensive European Food Consumption Database in September 2016. Mean, P5, median and P95 are expressed in grams per day per kilogram of body weight (g/kg bw/day).

Foodex L1	Nr Consumers	% Consumers	Mean	P5	Median	P95
Grains and grain-based products	128	100.0%	8.955	4.202	8.602	15.361
Vegetables and vegetable products (including fungi)	126	98.4%	2.486	0.405	2.032	6.249
Starchy roots and tubers	97	75.8%	1.998	0.291	1.653	5.319
Legumes, nuts and oilseeds	41	32.0%	0.496	0.108	0.336	1.111
Fruit and fruit products	111	86.7%	3.684	0.179	3.172	9.449
Meat and meat products (including edible offal)	126	98.4%	2.978	0.725	2.745	6.672
Fish and other seafood (including amphibians, rept)	43	33.6%	1.777	0.443	1.502	3.770
Milk and dairy products	124	96.9%	10.232	1.207	9.124	20.775
Eggs and egg products	100	78.1%	0.673	0.084	0.559	1.698
Sugar and confectionary	120	93.8%	1.134	0.031	0.868	2.771
Animal and vegetable fats and oils	128	100.0%	0.919	0.158	0.784	1.998
Fruit and vegetable juices	117	91.4%	5.854	0.249	3.924	17.371
Non-alcoholic beverages (excepting milk based beve	101	78.9%	7.918	1.678	6.900	17.373
Alcoholic beverages	43	33.6%	0.387	0.015	0.169	1.572
Drinking water (water without any additives except	127	99.2%	19.603	2.439	17.918	38.667
Herbs, spices and condiments	127	99.2%	0.343	0.021	0.232	1.135
Products for special nutritional use	3	2.3%	7.059	5.192	6.460	9.524
Composite food (including frozen products)	74	57.8%	3.397	0.559	2.755	8.739
Snacks, desserts, and other foods	82	64.1%	1.541	0.114	1.153	3.960

Figure 6.4: Chronic food consumption statistics (consumers only, ASNS children Survey) for Austrian children extracted from EFSA Comprehensive European Food Consumption Database in September 2016. Mean, P5, median and P95 are expressed in grams per day per kilogram of body weight (g/kg bw/day).

Foodex L1	Nr Consumer s	% Consumer s	Mean	P5	Median	P95
Grains and grain-based products						
	1,074	100.0%	3.380	1.500	3.240	5.864
Vegetables and vegetable products (including fungi)						
	1,072	99.8%	2.187	0.500	1.984	4.702
Starchy roots and tubers						
	912	84.9%	1.807	0.375	1.559	3.810
Legumes, nuts and oilseeds						
	849	79.1%	0.392	0.024	0.273	1.197
Fruit and fruit products						
	956	89.0%	2.889	0.245	2.412	7.051
Meat and meat products (including edible offal)						
	1,071	99.7%	2.599	0.939	2.381	5.078
Fish and other seafood (including amphibians, rept)						
	137	12.8%	0.951	0.286	0.806	2.116
Milk and dairy products						
	1,067	99.3%	3.722	0.406	3.156	9.234
Eggs and egg products						
	1,044	97.2%	0.381	0.038	0.278	1.034
Sugar and confectionary						
	1,042	97.0%	0.460	0.048	0.371	1.119
Animal and vegetable fats and oils						
	1,074	100.0%	0.647	0.267	0.597	1.144
Fruit and vegetable juices						
	617	57.4%	1.085	0.038	0.222	4.444
Non-alcoholic beverages (excepting milk based beve						
	989	92.1%	4.780	0.758	3.676	12.381
Alcoholic beverages						
	297	27.7%	3.276	0.214	2.143	10.526
Drinking water (water without any additives except						
	447	41.6%	4.673	0.870	3.333	12.963
Herbs, spices and condiments						
	1,074	100.0%	0.232	0.077	0.186	0.537
Food for infants and small children						
	1	0.1%	3.213	3.213	3.213	3.213
Composite food (including frozen products)						
	147	13.7%	0.512	0.037	0.178	1.713
Snacks, desserts, and other foods						
	64	6.0%	0.454	0.108	0.388	1.163

Figure 6.5: Chronic food consumption statistics (consumers only, National Repr Survey) for Hungarian adults extracted from EFSA Comprehensive European Food Consumption Database in September 2016. Mean, P5, median and P95 are expressed in grams per day per kilogram of body weight (g/kg bw/day).

Foodex L1	Nr Consumers	% Consumers	Mean	P5	Median	P95
Grains and grain-based products	10,398	99.8%	3.080	1.009	2.804	6.019
Vegetables and vegetable products (including fungi)	9,756	93.6%	1.704	0.170	1.356	4.385
Starchy roots and tubers	6,529	62.7%	1.256	0.296	1.052	2.783
Legumes, nuts and oilseeds	2,788	26.8%	0.477	0.038	0.320	1.522
Fruit and fruit products	8,700	83.5%	2.794	0.185	2.165	7.635
Meat and meat products (including edible offal)	9,708	93.2%	1.514	0.207	1.274	3.592
Fish and other seafood (including amphibians, rept)	2,652	25.5%	0.889	0.086	0.747	2.161
Milk and dairy products	10,097	96.9%	2.671	0.219	1.977	7.531
Eggs and egg products	2,504	24.0%	0.395	0.035	0.365	0.930
Sugar and confectionary	7,240	69.5%	0.428	0.024	0.279	1.333
Animal and vegetable fats and oils	9,971	95.7%	0.313	0.034	0.249	0.797
Fruit and vegetable juices	5,470	52.5%	5.715	0.877	3.750	17.287
Non-alcoholic beverages (excepting milk based beve	10,121	97.1%	12.171	2.862	10.417	27.906
Alcoholic beverages	5,557	53.3%	5.593	0.192	3.814	16.271
Drinking water (water without any additives except	9,408	90.3%	15.142	1.852	13.135	35.244
Herbs, spices and condiments	7,996	76.7%	0.562	0.029	0.376	1.685
Products for special nutritional use	53	0.5%	2.052	0.094	1.471	8.403
Composite food (including frozen products)	5,993	57.5%	1.733	0.099	1.148	5.159
Snacks, desserts, and other foods	2,818	27.0%	0.873	0.143	0.687	2.328

Figure 6.6: Chronic food consumption statistics (consumers only, National Nutrition Survey II) for German adults extracted from EFSA Comprehensive European Food Consumption Database in September 2016. Mean, P5, median and P95 are expressed in grams per day per kilogram of body weight (g/kg bw/day).

Foodex L1	Nr Consumers	% Consumers	Mean	P5	Median	P95
Grains and grain-based products	348	100.0%	8.943	3.290	8.117	16.433
Vegetables and vegetable products (including fungi)	346	99.4%	4.037	0.704	3.462	9.460
Starchy roots and tubers	324	93.1%	2.858	0.393	2.264	6.915
Legumes, nuts and oilseeds	187	53.7%	0.433	0.021	0.285	1.269
Fruit and fruit products	344	98.9%	8.465	1.264	7.832	19.547
Meat and meat products (including edible offal)	335	96.3%	2.763	0.469	2.345	6.794
Fish and other seafood (including amphibians, rept)	147	42.2%	0.949	0.068	0.770	2.197
Milk and dairy products	344	98.9%	21.111	2.260	18.037	46.959
Eggs and egg products	285	81.9%	0.692	0.034	0.472	2.120
Sugar and confectionary	331	95.1%	0.989	0.050	0.872	2.441
Animal and vegetable fats and oils	343	98.6%	0.615	0.102	0.563	1.317
Fruit and vegetable juices	289	83.0%	9.224	0.050	6.860	27.790
Non-alcoholic beverages (excepting milk based beve)	303	87.1%	19.916	1.778	13.644	63.579
Alcoholic beverages	29	8.3%	0.754	0.000	0.039	5.606
Drinking water (water without any additives except	342	98.3%	16.603	0.953	13.367	44.087
Herbs, spices and condiments	341	98.0%	0.812	0.020	0.520	2.756
Food for infants and small children	165	47.4%	16.089	0.194	9.922	54.132
Products for special nutritional use	12	3.4%	1.019	0.007	0.154	6.410
Composite food (including frozen products)	122	35.1%	0.991	0.035	0.442	3.328

Figure 6.7: Chronic food consumption statistics (consumers only, Survey Vels) for German toddlers extracted from EFSA Comprehensive European Food Consumption Database in September 2016. Mean, P5, median and P95 are expressed in grams per day per kilogram of body weight (g/kg bw/day).

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Biography

The author of this thesis obtained bachelor's degree in Chemical Sciences at University of Ljubljana, Faculty of Chemistry and Chemical Technology in 1985 and master's degree in chemical sciences at the same University in 1989, where she was employed as a young researcher. In the same year she started to work at National institute of Public Health in Ljubljana in Department for sanitary chemistry. Firstly she worked as an analyst researcher and then as a coordinator of Laboratory for consumer products, where she was dealing with analysis and evaluation of food contact materials, cooperation with Health inspectorate in planning of campaigns and cooperation with Slovenian Ministry of Health in legislative matters regarding food contact materials. In the period from 2000 – 2005 she was quality manager of the Department for sanitary chemistry as well. As a food contact materials expert she attended Food and Veterinary Office missions to Netherlands, Germany, Finland, Portugal and Belgium. She gave presentations about food contact materials for Technical Assistance and Information Exchange service of the European Commission (TAIEX) in Zagreb, Israel, Turkey and Belgrade.

Since 2013, she has been working at central unit of National institute of Public Health as a health researcher. She is a member of EFSA scientific network on food contact materials, EFSA scientific NANO network, member of Council of Europe Committee of experts on materials coming into contact with food, chair of Slovenian standardisation committee for food contact materials, member of food section of Slovenian Chemical Society and member of Slovenian Medical Association (Section for Preventive Medicine).