ENGINEERED NANOMATERIALS VIA AEROSOL ROUTES: FORMATION, CHARACTERISTICS AND SAFETY ASPECTS

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ABSTRACT

This thesis introduces the manufacturing of TiO$_2$, Si-C and carbon nanomaterials via aerosol methods. First, atmospheric pressure chemical vapour synthesis in a tubular flow reactor was used to produce nanosize TiO$_2$ and Si-C particles. Secondly, an atmospheric pressure annealing of the produced Si-C nanoparticles was performed in argon using an inductively heated furnace. The main objective was to investigate how process parameters and/or precursor properties affect nanomaterial characteristics (e.g., shape, crystallinity, crystal phase, primary particle size, agglomeration state, surface area, and surface structure) and to link the results with toxicity screening studies. The deposition kinetics, absorption and toxicological effects of the engineered nanoparticles depend on physicochemical parameters that are not commonly considered during toxicity screening tests. The parameters that were considered in this study, in addition to the particle mass and number size distributions, included the crystal phase, agglomeration state, surface area, surface charge, and surface activity of the produced particles.

The reaction temperature had the most prominent effect of the process parameters on the characteristics of the produced nanomaterials. A higher reaction temperature resulted in a smaller primary particle size and more crystalline particles. A decrease in the primary particle size is related to enhanced reaction kinetics and/or a change in the molecular structure of the particles. The decomposition or sublimation kinetics of the precursors at different reaction temperatures affected the composition of the vapour phase and, subsequently, the composition of the produced particles.

The solid-state processes that occurred in the nanomaterial synthesis, such as the selective evaporation of silicon from the solid precursor particles during annealing, affected the microstructure of the products, and the formation of new carbon nanostructures, i.e., carbon nanoflowers, was observed. The properties of the solid precursors, i.e., the chemical composition and degree of crystallinity, had an effect on the structure of the produced materials.

Depending on the process parameters and the properties of the precursors used, the primary particle size, agglomeration state, crystallinity, surface area, and surface activity of the produced particles changed. Thus, thorough characterisation of the administered nanomaterial is essential in toxicity screening studies if valid results are desired.

Keywords: Nanostructured materials, Nanoparticles, Titanium dioxide, Silicon, Carbon, Vapors, Aerosols, Microstructure, Molecular structure, Toxicity testing, Industrial safety
Preface

Tämä kirja on omistettu Hessulle.

Kiitos kärsivällisyystäsi!

Toivalassa, maaliskuussa 2014

Mirella Miettinen
LIST OF ABBREVIATIONS AND SYMBOLS

CNF  carbon nanoflower
HMDS  hexamethyldisilane C₆H₁₈Si₂
H₂N₂  formier (10 % H₂, 90 % N₂)
kₔ  gas-phase reaction rate constant (1/s)
kₛ  surface reaction rate constant (cm/s)
SiC  silicon carbide
TTIP  titanium tetraisopropoxide Ti(C₃H₇O)₄
TiO₂  titanium dioxide
APCVS  atmospheric pressure chemical vapor synthesis
BET  Brunauer-Emmett-Teller (method for measuring surface area)
DLPI  Dekati low pressure impactor
DLS  dynamic light scattering
DSC  differential scanning calorimeter
EDS  energy dispersive X-ray spectroscopy
FSP  flame spray pyrolysis
FTIR  Fourier transform infrared spectroscopy
MOCVS  metal-organic chemical vapor synthesis
NMR  nuclear magnetic resonance spectroscopy
OMCVS  organometal chemical vapor synthesis
SAED  selected area electron diffraction
SEM  scanning electron microscopy
SMPS  scanning mobility particle sizer
TEM  transmission electron microscopy
TGA  thermogravimetric analysis
XPS  X-ray photoelectron spectroscopy
XRD  X-ray diffraction
LIST OF ORIGINAL PUBLICATIONS

This thesis is based on data presented in the following articles, referred to in the text by Roman numerals I-VI.


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AUTHOR’S CONTRIBUTION

The research reported in this dissertation has been carried out in the Fine Particle and Aerosol Technology Laboratory at University of Eastern Finland during the years 2006-2012 under the supervision of Prof. Jorma Jokiniemi, Ph.D. Anna Lähde and D.Sc. (Tech.) Jorma Joutsensaari.

In paper I, an aerosol flow reactor for controlled titanium dioxide (TiO\textsubscript{2}) nanoparticle production from titanium tetraisopropoxide (TTIP) was developed for inhalation exposure studies. The author continued the development work based on the study of Dr. Ulrika Backman, tested and optimized the reactor for inhalation exposure studies, analysed the data, and wrote the paper. The influence of the process parameters on the powder characteristics, including the primary particle and agglomerate size, crystallinity, surface composition, and surface area was studied. The powder X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS) and specific surface area analyses were done by Dr. Joakim Riikonen and transmission electron microscopy (TEM) analysis was carried out by Dr. Unto Tapper.

In paper II, silicon-carbon nanoceramics were synthesised from hexamethyldisilane (HMDS) in high temperature aerosol flow reactor. The influence of the process temperature on the powder characteristics was studied. The author performed the experimental work, part of the analyses and wrote the article together with Dr. Anna Lähde. Scanning electron microscopy (SEM) and part of powder XRD and thermal gravimetric analyses (TGA) were done by M.Sc. Max Johansson, solid state nuclear magnetic resonance (NMR) analysis was done by Dr. Sari Suvanto, dynamic light scattering (DLS) and specific surface area analyses were done by Dr. Joakim Riikonen and transmission electron microscopy analysis was carried out by Dr. Unto Tapper.

In paper III, post-processing of the silicon-carbon nanoceramics, synthesised in paper II, was performed by annealing in argon at atmospheric pressure using an inductively heated furnace. An effect of the increasing crystallinity of the precursor powder and the induction temperature on the characteristics of the produced nanomaterials was studied. The author performed part of the analyses, collected the data, and wrote the paper together with Dr. Anna Lähde. An annealing of the precursor powders was done by Dr. Jouni Hokkinen, SEM and TEM analyses were carried out by M.Sc. Tiina Torvela and Dr. Unto Tapper, XRD analysis was done by M.Sc. Tommi Karhunen, and Raman analyses were done by M.Sc. Tommi Karhunen, Dr. Carsten Pfüller and Dr. Manfred Ramsteiner.

In papers IV-VI, the aerosol flow reactor developed and tested in paper I was used in inhalation exposure studies (both whole-body and head-only exposures) of mice as developed or with minor modifications. In paper IV, the author built up the experimental set-up, adjusted it to preferred exposure situations, used the reactor during the first exposures, and contributed to writing process of the corresponding sections of the article. In papers V-VI, the author participated the building up the experimental set-up and contributed to writing process of the corresponding sections of the articles.
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1 INTRODUCTION

According to the European Commission recommendation (COM 2011 696) “Nanomaterial means a natural, incidental or manufactured material containing particles, in an unbound state or as an aggregate or as an 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”. Furthermore, “fullerenes, graphene flakes and single wall carbon nanotubes with one or more external dimensions below 1 nm should be considered as nanomaterials”. Engineered nanomaterials are developed to exhibit novel properties in contrast to bulk of the same material, such as increased reactivity, conductivity or strength (SWD 2012 288 final). Engineered nanomaterials cover a wide range of substances from inorganic non-metallic (e.g., titantium dioxide) and metal nanoparticles (e.g., nanosilver) via carbon-based materials (such as carbon nanotubes or graphene) to organic macromolecules. The quantity of nanomaterials marketed annually is estimated to be approximately 11 million tons with a market value of approximately 20 bn € in 2012 (COM 2012 572 final). Currently, nanomaterials are used in a variety of fields, e.g., as a reinforcing agent for rubber, functional fillers in polymers, in energy storage, paints and coatings, catalysts, electronics, composites, cosmetics and biomedical applications (SWD 2012 288 final). Controlled tailoring of the properties of nanomaterials may enable new technological breakthroughs, e.g., in chemical sensing, drug delivery or energy harvesting.

Along with the growth in the applications of nanotechnology, the need to identify the physical and chemical features specific to these nanomaterials arises. Nano-specific properties, such as increased surface reactivity due to a larger surface-to-volume ratio, causes concerns over their potential harmful effects on humans and the environment (SCENIHR, 2010). The biological activity of nanomaterials depend on parameters that are not regularly considered in toxicity studies, i.e., the primary particle size, number size distribution, degree of agglomeration, shape, crystal structure, chemical composition, surface area, surface chemistry, surface charge, and porosity (Oberdörster et al., 2005; Borm et al., 2006; Johnston et al., 2009). Thus, systematic characterisation of engineered nanomaterials is essential for their proper safety evaluation and risk assessment (COM 2012 572 final). To date, the lack of comparability of the results from different studies hinders the identification of the metrics (e.g., mass or number concentration, surface area, etc.) best related to possible health effects (Johnston et al., 2009; Kuhlbusch et al., 2011).
In this thesis, the manufacturing of nanomaterials via aerosol routes is studied, and the effect of process parameters and precursor properties on product characteristics is explored, with links to toxicity screening tests. In addition, demands (e.g., generating a stable number and mass concentration over 6 h exposure runs with low concentrations of gaseous by-products) that inhalation exposure studies set on in situ nanoparticle production are discussed. The work in this thesis was published in 6 papers. In paper I, an aerosol flow reactor for titanium dioxide (TiO$_2$) nanoparticle production from titanium tetraisopropoxide (Ti(C$_3$H$_7$O)$_4$, TTIP) was developed, tested and optimised for inhalation exposure studies. In papers IV-VI, the aerosol flow reactor developed and tested in paper I was used in inhalation exposure studies of mice as developed or with minor modifications. In paper II, silicon-carbon nanoceramics were synthesised from hexamethyldisilane (C$_6$H$_{18}$Si$_2$, HMDS) with a high temperature aerosol flow reactor. In papers I and II, a systematic investigation of the effect of the process parameters and precursor properties on the product characteristics was performed, and the reaction by-products were analysed on-line. The nanopowder produced in paper II was also tested in inhalation exposure studies, and the results will be published in another thesis (Leppänen, M.). In paper III, the post-processing of the silicon-carbon nanoceramics synthesised in paper II was performed by annealing in argon at atmospheric pressure using an inductively heated furnace. The effect of the precursor powder properties and the induction temperature on the characteristics of the produced nanomaterials was studied.

This thesis is organised as follows. In chapter 2, a short literature review of the aerosol synthesis of nanomaterials with key gas-phase and solid-state processes and aerosol dynamics is provided. An introduction to the toxicological effects of nanomaterials is also included in the chapter 2. The objectives of the study are set in chapter 3. The experimental methods and characterisation techniques applied in this work are described in chapter 4. In chapter 5, the most important results are discussed and linked to toxicity screening studies of engineered nanomaterials. The conclusions are provided in chapter 6.
2 LITERATURE REVIEW

2.1 Nanomaterial synthesis

This chapter briefly introduces the most commonly applied nanomaterial synthesis routes but concentrates on the atmospheric pressure chemical vapour synthesis (APCVS) that was used in this work.

Nanomaterials can be produced by solid-state, liquid-phase or gas-phase (aerosol) routes. An aerosol is defined as a suspension of solid or liquid particles in a gas (Hinds, 1999). Solid-state manufacturing of nanomaterials normally implies mechanical milling that leads to inhomogeneous and degraded powders (Kodas and Hampden-Smith, 1999; Borm et al., 2006). Liquid-phase routes rely on chemical reactions (e.g., Chen et al., 2007; Wang and Li, 2011) that are usually performed at ambient temperature, which may result in poorly crystalline materials (Kodas and Hampden-Smith, 1999). In addition, impurities from the solvent, surfactants or other reagents in the system are undesirable for applications in which high purity nanomaterials are required. Compared with other routes, aerosol routes have the advantages that they are fast (the synthesis timescales are typically a few seconds), they do not involve large volumes of liquid byproducts or extensive post-processing, and the particles are usually formed at elevated temperatures, which enhances the formation of highly crystalline materials (Kodas and Hampden-Smith, 1999; Skillas et al., 2011). In industry, companies such as DuPont and Cabot have long used aerosol processes for the production of titanium dioxide (TiO\textsubscript{2}) pigment and fumed silica (SiO\textsubscript{2}) at massive scales (hundreds of tons per day) (Kodas and Hampden-Smith, 1999).

A prerequisite for nanomaterial synthesis via aerosol routes is usually a supersaturated vapour or chemical supersaturation in which it is thermodynamically favourable for the vapour phase molecules to react chemically to condensable products (Kodas and Hampden-Smith, 1999; Swihart, 2003). Proper characterisation and control of process conditions is essential for tailored nanomaterial synthesis. The primary adjustable variables in most aerosol reactor designs are the concentration of reactants, temperature profiles, residence times, and cooling rates (Kodas and Hampden-Smith, 1999; Tsantilis and Pratsinis, 2004; Strobel and Pratsinis, 2007). One way to classify aerosol methods is by the phase of the precursor and the source of energy used to achieve a state of supersaturation (Gurav et al., 1993; Swihart, 2003). Methods that exploit a solid precursor include inert gas condensation, laser ablation, and spark discharge generation. The basic principle of these methods is to vaporise a solid material into a background
gas and then cool the gas (physical process). In an alternative route, liquid or vapour precursors are heated to induce gas- or liquid-phase chemical reactions that result in the formation of solid particles (chemical process). This group includes several methods, such as chemical vapour synthesis (CVS), spray pyrolysis, plasma pyrolysis and flame or flame spray pyrolysis (FSP), which differ in the phase of the precursor (vapour/liquid) or in the source of energy (external heating in a hot wall reactor vs. in situ combustion reactions in a flame or decomposition of the precursors in thermal plasma). Flame aerosol processes are the most widely used in the manufacturing of nanomaterials at industrial levels (Skillas et al., 2011) and have been thoroughly reviewed by Pratsinis (1998) and more recently by Strobel and Pratsinis (2007). Spray pyrolysis, which utilises liquid precursors, is commonly used especially in the manufacturing of functional particles because multicomponent materials can be easily prepared using this method (Okuyama and Lenggoro, 2003). In spray pyrolysis, the reaction usually occurs in droplets followed by evaporation of a solvent. The primary advantages of the aerosol processes that use gaseous precursors compared with liquid ones are the resulting small primary particle size and narrow particle size distribution, solid particles and high purity (Gurav et al., 1993; Skillas et al., 2011). The main disadvantages are problems in producing multicomponent materials with homogeneous chemical compositions due to the different vapour pressures and chemical reaction rates of the precursors and the formation of hard agglomerates in the gas phase.

2.1.1 Chemical vapour synthesis

Chemical vapour synthesis in a broad view involves the dissociation and/or chemical reactions (e.g., pyrolysis, oxidation, reduction or hydrolysis) of gaseous reactants in an activated (heat, light, plasma) environment and can be performed from atmospheric pressure to high vacuum (Choy, 2003). The method has been used to produce ultrafine powders, films and coatings. It is a complex chemical system that is affected by thermodynamics, chemical kinetics and mass transport. The rate limiting step (usually the mass transfer rate) during the process can be altered using a reduced pressure. Thermally activated CVS is typically performed in a tubular flow reactor, which consists of a tube furnace oriented either vertically or horizontally (Fig. 1). Thermally activated CVS can be subdivided into atmospheric pressure (AP), low pressure (LP) or ultrahigh vacuum (UHV) CVS (Choy, 2003). In APCVS, temperatures are commonly higher than for reduced pressures but are typically less than 1700 °C (Kodas and Hampden-Smith, 1999). The method relies on the use of volatile precursors that are introduced into the reactor as gases or an aerosol of a solution. In the latter case, both the solution and the precursor in the droplet should evaporate in the reactor before the gas-phase
reaction occurs. Advantages of tubular flow reactors are that the temperature and flow velocity can be controlled precisely and the process can be easily scaled up to industrial levels (Kodas and Hampden-Smith, 1999; Skillas et al., 2011).

**Figure 1. Typical chemical vapour synthesis process in a tubular flow reactor**

### 2.1.2 Gas-phase reactions

Chemical reactions in tubular flow reactors are thermally induced and result in vapour-phase species that may react further with the gaseous precursor, each other or on a hot surface or nucleate and grow into particles (Kodas and Hampden-Smith, 1999) (Fig. 1). Gas-phase reactions compete with the chemical vapour deposition of the reactants on the reactor walls. The thermal decomposition pathway of a precursor depends on its chemical properties (volatility, reactivity, solubility) and may require an added reagent such as H₂O or H₂. It is crucial to understand the reaction pathway of a precursor and the role of intermediates and other reagents (e.g., carrier gas, solvent, dilution gas) in the system. Nakaso et al. (2003) stated that the chemical reaction rate is one of the most important parameters in controlling the size and morphology of the produced nanoparticles in APCVS. However, because CVS tends to be a non-equilibrium process, the exact determination of the reaction kinetics is difficult (Choy, 2003).

Metal-containing organic compounds have been used as precursors in CVS because many of them are sufficiently volatile. A single-source metal containing organic compound also enables the preparation of composite particles with a predetermined stoichiometry (Gurav et al., 1993; Kodas and Hampden-Smith, 1999; Lähde et al., 2011). Metal-containing organic precursors can be classified as metal-organic (MO) compounds that contain organic ligands but no metal-carbon bond and organometallic (OM) compounds that contain organic ligand and a metal-carbon bond (Kodas and
CVS processes can be divided into MOCVS and OMCVS according to the precursor used (Choy, 2003). In general, MO and OM precursors have lower decomposition or pyrolysis temperatures than the halides, hydrides or halohydrides that are commonly used in CVS processes (Hampden-Smith and Kodas, 1995; Choy, 2003).

Metal alkoxides are among the most frequently used MO precursors. The thermal decomposition of metal alkoxides may occur through many mechanisms, including intramolecular rearrangement according to the following equations (Kodas and Hampden-Smith, 1999):

\[ \text{M(OR)} \rightarrow \text{MOH} + \text{alkene}, \quad (1) \]

\[ 2\text{MOH} \rightarrow \text{M}_2\text{O} + \text{H}_2\text{O}, \quad (2) \]

and hydrolysis according to the equation:

\[ \text{M-OR} + \text{H}_2\text{O} \rightarrow \text{M-OH} + \text{ROH}. \quad (3) \]

For example, the gas-phase thermal decomposition of titanium tetraisopropoxide (TTIP, the precursor in paper I) proceeds via the above presented pathways and may be conducted in the presence of added water vapour to ensure that the organic ligands are completely removed as alcohol because the hydrolysis rate is much higher than the thermal decomposition rate due to the additional oxidising effect of water (Kodas and Hampden-Smith, 1999; Nakaso et al., 2003).

The gas-phase thermal decomposition of OM compounds (e.g., hexamethyldisilane HMDS, the precursor in the paper II) to desired products often requires the addition of a reducing agent such as H\(_2\) (Kodas and Hampden-Smith, 1999). Typically, the decomposition pathway of an OM compound is a multistep process that may begin, for example, with the reduction of a metal and the subsequent activation of C-H or C-C bonds in organic ligands (Eller and Schwarz, 1991; Kodas and Hampden-Smith, 1999) or a cleavage of an M-M bond by homolytic fission (Bullock et al., 1994) (Equation 4):

\[ (\text{RC})_n\text{M-M(CR)}_n \rightarrow 2 (\text{CR})_n\text{M} \cdot \quad (4) \]

followed by further decomposition of the formed radical species or reactions of these radicals with the added reagents and/or the gaseous precursor. The
2.1.3 Particle formation and growth

Aerosol dynamics in CVS start when the vapour becomes sufficiently supersaturated and nucleation occurs to form thermodynamically stable clusters (Fig. 1). Once clusters are formed, they immediately grow by coagulation as they collide and coalesce, by vapour condensation, and by surface reaction, in which the precursor reacts on the cluster surface (Kodas and Hampden-Smith, 1999). If coalescence (sintering) is faster than collision, spherical particles are formed. If coalescence is slow, and new collisions occur before coalescence has been completed, agglomerates are produced (Lehtinen et al., 1996). Surface reactions may affect the characteristics (e.g., the primary particle size or crystallisation stage) of the produced nanoparticles and have been observed to be important at high precursor concentrations and/or low reaction temperatures (Tsantilis et al., 2002; Nakaso et al., 2003).

Depending on the process conditions, primary particles in the agglomerates can be held together by weak van der Waal forces (soft agglomerates) or by stronger chemical or sintering bonds (hard agglomerates) (Tsantilis and Pratsinis, 2004). From the soft agglomerates the primary particles can be re-dispersed without a strong force in contrast to the hard agglomerates (Swihart, 2003; Büsser, 2011). Unagglomerated, spherical particles with a narrow size distribution are desired in many applications that require the compacting and sintering of particles (Okuyama and Lenggoro, 2003). Furthermore, the transportation kinetics and toxic effects of loosely bonded soft agglomerates in a human body may differ from those of hard agglomerates (Strobel and Pratsinis, 2007), thus information about the agglomeration state of the particles is essential for toxicity screening studies. In aerosol synthesis processes, where high nanoparticle number concentrations are typically desired, agglomeration is difficult to avoid (Swihart, 2003). However, the agglomeration state of the produced particles can be controlled by changing the process parameters, usually the precursor concentrations, reaction temperature, residence time and cooling rate (Tsantilis and Pratsinis, 2004). In general, an increase in the precursor concentration enhances both primary particle and agglomerate growth. A high reaction temperature accelerates the reaction kinetics and coalescence, promoting the formation of larger primary particles and smaller agglomerates. A longer residence time in the high temperature region also prolongs coalescence and results in a larger primary particle size and smaller
agglomerates. However, at low temperatures, the collision rate is usually faster than the coalescence rate, which results in a small primary particle size but larger agglomerates with increasing residence time (Lehtinen et al., 1996). By increasing the cooling rate, the hard agglomerate and primary particle diameters can be decreased, and an instant cooling ($-10^6$ K/s) to room temperature may hinder the formation of hard agglomerates. In tubular flow reactor set ups, the attainment of such high cooling rates is usually disabled.

2.1.4 Solid-state processes

In many aerosol processes, solid particles that contain precursors or mixtures of different reagents are formed (Kodas and Hampden-Smith, 1999). The conversion of the formed particles into the final product by solid-state chemical and physical reactions is one of the most complicated aerosol processes and may involve the following phenomena: heat transfer in the gas phase and in the particle, transport of volatile species in the particle and in the gas phase, phase transitions, solid-state diffusion (sintering, grain growth) and evaporation of the particle (Kodas and Hampden-Smith, 1999). The driving force for these processes is the minimisation of free energy ($\Delta G$), and the processes involve the cleavage and formation of bonds. Consequently, the chemical composition and morphology of the particles may change.

For example, the crystal phase and phase transition behaviour of TiO$_2$ and SiC depend on several process parameters, e.g., temperature, pressure, atmosphere, precursor composition etc. (Zhang and Banfield, 2000; Fissel, 2003; Li et al., 2004; Yakimova et al., 2006; Vasiliauskas et al., 2012). Descriptive phase diagrams for TiO$_2$ are illustrated in Fig. 2a (Levin and McMurdle, 1975) and occurrence probability of different SiC polytypes for the unseeded sublimation growth is illustrated in Fig. 2b (Knippenberg, 1963).
At low temperatures near atmospheric pressure \( \text{TiO}_2 \) has three main crystal structures, anatase, brookite and rutile. If an equal size of nanocrystals of the three polymorphs is assumed, anatase is thermodynamically the most stable at sizes less than 11 nm, brookite is the most stable at crystal sizes between 11-35 nm, and rutile is the most stable at sizes greater than 35 nm (Zhang and Banfield, 2000). With increasing temperature the possible phase transitions are: anatase to rutile, anatase to brookite to rutile, brookite to rutile, and brookite to anatase to rutile (Li et al., 2004). The direction of the phase transformations is determined, e.g., by initial particle size and phase, purity, reaction atmosphere, and synthesis method. Rutile is the most stable phase at high temperatures. SiC has over 200 polytypes; however, only 3C-, 4H- and 6H-SiC are commonly available today (Fissel, 2003). It is seen from the diagram presented above (Fig. 2b) that 3C polytype has not definite growth conditions, thus it is usually a metastable phase. Typical phase transitions of SiC include 2H to 3C, 2H to 3C to 6H, and 3C to 6H (Yoo et al., 1990).

Solid-state processes must be considered if nanomaterials with desired properties are targeted and they have been utilised, e.g., in the production of graphene via the thermal decomposition of SiC at high temperatures (Emtsev.
et al., 2009; Yazdi et al., 2013). The solid-state processes can also lead to changes in aerosol dynamics, e.g., the formation and subsequent growth of new particles if volatile species are released.

### 2.2 Toxicological effects of nanomaterials

Scattered data concerning the health effects of engineered nanomaterials remains inconclusive (Strobel and Pratsinis, 2007; Xia et al., 2013). Nanoparticles may enter the human body through different routes, e.g., by ingestion, inhalation or penetration of the skin (Kreyling et al., 2006). Inhalation exposure is currently stated as the most critical route (Kreyling et al., 2006; Wang et al., 2011). In addition to the material specific properties, uptake or deposition during inhalation exposure is affected by the respiratory system geometry and respiratory physiology (Wong, 2007). The physical deposition mechanisms of the particles in the respiratory tract include sedimentation, impaction, interception and diffusion (Hinds, 1999; Wong, 2007).

Typically, the biological activity of a material increases as the particle size decreases, thus hard agglomerates may have less health effects than soft agglomerates because the former behave as much larger particles and the latter behave as individual primary particles (Oberdörster et al., 2005; Strobel and Pratsinis, 2007). However, the shape, crystal phase, agglomeration state, surface area, surface charge, surface activity and hydrophobicity/hydrophilicity of the material may also affect the deposition kinetics, absorption and toxicological effects in the body (Oberdörster et al., 2005; Nel et al., 2009; Xia et al., 2013). Nanosize materials contain a large number of surface atoms and therefore exhibit increased reactivity, which enhances electron transfer, the binding of molecular species, and translocation across cell membranes (Pokhrel et al., 2013). The interaction of the nanomaterial with biological components (e.g., cell membranes, proteins, DNA) may, in addition to the above mentioned parameters, depend on the properties of the suspending media (pH, temperature, ionic strength) and the presence of large organic molecules or detergents (Nel et al., 2009). Therefore, the toxic effects of engineered nanomaterials may vary from organism to organism and from medium to medium (Pokhrel et al., 2013).

Animal studies routinely demonstrate an increase in pulmonary inflammation and oxidative stress upon respiratory exposure to inhaled or instilled nanomaterials (Oberdörster et al., 2005; Johnston et al., 2009). In oxidative stress, cells in contact with nanomaterials are activated and begin to synthesise free radicals and reactive oxygen species (e.g., hydroxyl radicals, hydrogen peroxide) that may damage the components of the cell (Kreyling et al., 2006). An inflammatory response includes both the activation
of the epithelium cells and the consequent activation and migration of other cells (particularly granulocytes in the case of a specific immunological response) from the blood into the airways. Toxicological studies of fibrous and tubular nanostructures have indicated fibrotic lung responses, inflammation and an increased risk of carcinogenesis at high doses (Oberdörster et al., 2005). For example, multi-wall carbon nanotubes may have dimensions similar to asbestos fibres and have been reported to induce asbestos-like pathogenicity and mesothelioma in mice and rats (Wang et al., 2011). Furthermore, the systemic translocation of nanoparticles after pulmonary exposure and subsequent uptake in the secondary target organs (e.g., brain, liver) have been reported; however, the transport mechanisms remain under debate (Oberdörster et al., 2005; Kreyling et al., 2006; Johnston et al., 2009). Some studies have proposed that particle surface characteristics may be a key factor in the generation of free radicals and reactive oxygen species and play an important role in systemic translocation (Oberdörster et al., 2005; Kreyling et al., 2006; Johnston et al., 2009). However, few studies have been published concerning the interrelationship between the physicochemical characteristics (e.g., size, shape, surface activity) and the dose of a nanomaterial and its biological effects (Oberdörster et al., 2005).
3 OBJECTIVES OF THE STUDY

The main objectives of this study are 1) to develop methods for the aerosol synthesis of nanomaterials with desired characteristics (e.g., size, shape, crystallinity or surface structure) and 2) to investigate how process parameters and/or precursor properties affect nanomaterial characteristics (papers I-III). An additional focus is 3) to manufacture thoroughly characterised nanomaterials for toxicity screening studies (papers I and II). Furthermore, 4) an aerosol flow reactor for controlled nanoparticle production in inhalation exposure studies is presented (papers I and IV-VI).
4 EXPERIMENTAL METHODS

4.1 Aerosol flow reactors

The aerosol flow reactors used in papers I and II consist of a bubbler placed in a water bath and vertical reactor tubes (Fig. 3). In the first reactor set-up (Fig. 3a), the reactor tube is composed of a special steel (W 1.484 H HEAT) that limits the maximum temperature of this reactor to 800 °C. In the second reactor set-up (Fig. 3b), a ceramic reactor tube (Alumina 99.7 % Al₂O₃) is used, which permits temperatures as high as 1400 °C. The heated section in both reactors is divided into two zones that can be controlled independently. The total lengths of the heated sections are 55 cm and 100 cm for the first and second reactor, respectively. Both reactors were operated at atmospheric pressure. The line from the bubbler to the reactor inlet was heated to prevent condensation of the precursor vapour before reaching the reactor. The properties of the produced particles can be adjusted by changing the bath temperature, residence time in the reactor, reactor temperature, and dilution air flows.

EXPOSURE CHAMBER

TEM

SMPS

FTIR

EXH = exhaust
FC = flow controller
PC = pressure controller
MFC = mass flow controller
CO = critical orifice
= heated line
In paper I, nitrogen (AGA Instrument nitrogen 5.0) was used to vaporise and carry the titanium tetraisopropoxide (TTIP) into the reactor, whereas in paper II, hexamethyldisilane (HMDS) was carried with formier gas (AGA 10 % H₂, 90 % N₂). The residence time in the heated section of the reactor was controlled in paper I using an extra nitrogen flow. In paper II, the residence time was not changed; however, an additional nitrogen supply ensured that the flow in the reactor was laminar, with no recirculation in the heating zone.

Immediately after the reactor, the product aerosol was diluted with dry, particle free air in paper I, and the desired mass concentration was adjusted by second, humidified air flow. In paper II, dilution was performed with nitrogen using a porous tube diluter.

**4.2 Annealing set-up**

In paper III, the post-processing of the silicon-carbon nanoceramics, synthesised in paper II, was performed by annealing in argon at atmospheric pressure using an inductively heated furnace. The precursor powders were designated according to the CVS temperatures (1000, 1200 and 1400 °C) as SiC-1000, SiC-1200 and SiC-1400, respectively.
The induction heating furnace is illustrated in Fig. 4. The sample crucible consists of two graphite pieces and was entirely enclosed except for a pyrometer view hole in the cover piece. The crucible was encased in graphite felt thermal insulation and maintained at atmospheric pressure. The annealing temperature was varied from 1900 °C to 2600 °C with a 30 min isotherm at the target temperature. An argon (AGA 5.0, purity 99.999 %) flow was used to maintain an inert atmosphere in the furnace.

![Figure 4. Schematic and a photograph of the induction heating set-up (Paper III)](image)

### 4.3 Characterisation techniques

The measurement of the gas-phase compounds in papers I and II was performed using a Fourier transform infrared (FTIR) spectroscope. The stability of the reaction and the concentrations of the decomposition products can be monitored on-line with FTIR spectroscopy. In paper I, the relative humidity of the administered aerosol was also measured using FTIR spectroscopy. The oxygen content of the administered aerosol was measured with a ZrO₂ cell that was incorporated in the FTIR portable sampling unit. On-line particle characterisation, i.e., the measurement of the number size
distribution and the total number concentration, was conducted using a scanning mobility particle sizer (SMPS). The mass concentrations were verified with gravimetric filter samples, and the mass size distribution was measured with a 13-stage cascade low pressure impactor (DLPI) used with baked and greased aluminium foils.

Transmission electron microscopy (TEM) analysis was performed to obtain information about the agglomeration state, shape and size of the produced agglomerates and primary particles. The primary particle size distributions were calculated from the TEM images in paper I. Selected area electron diffraction (SAED) was used to determine the crystallinity and the crystal structure of the produced particles. The crystal phases and crystallite sizes of the produced particles were analysed using X-ray diffraction (XRD). The surface chemistry and surface contamination of the TiO₂ agglomerates were studied with X-ray photoelectron spectroscopy (XPS) in paper I. The specific surface area of the produced particles was determined using the BET (Brunauer, Emmett and Teller) method (Brunauer et al., 1938). The zeta potential of the TiO₂ agglomerates was measured using electrophoretic light scattering in the pH range from 1 to 6 in paper IV with a Zetasizer Nano Series-Zen 3600 particle characterisation system (Malvern Instruments, UK). The TiO₂ particles were dispersed in a 0.1 M sodium chloride (NaCl) solution, and the pH was adjusted with hydrochloric acid (HCl). The surface structure and elemental composition of the particles produced in papers II and III were analysed with a scanning electron microscope equipped with an energy dispersive X-ray spectrooscope (SEM-EDS). FTIR spectroscopy with potassium bromide (KBr) tablets was used to determine the functional groups and chemical bonds in the produced compounds in paper II. Solid-state nuclear magnetic resonance (NMR) analysis was performed on the powder synthesised at the reactor temperature of 800 °C in paper II to obtain more information about the molecular structure of that powder. Thermal analysis was conducted to study the thermal stability, oxidation tendency and composition of the powders produced at various reaction temperatures in papers II (thermal gravimetric analysis TGA with differential scanning calorimeter DSC) and III (TGA). Raman spectroscopy analysis was used in paper III to determine the composition of the carbon phase of the powders after high temperature annealing.
5 RESULTS AND DISCUSSION

5.1 Effect of process parameters and precursor properties on nanomaterial characteristics

5.1.1 TiO$_2$ nanoparticles

The effect of bath temperature ($T = 30$-$50$ °C), residence time ($t = 2.3$-$4.3$ s) and dilution rate ($DR = 6$-$32$) on the characteristics of produced TiO$_2$ particles was studied in paper I at the reaction temperature of 500 °C. Changes in these process parameters did not affect the primary particle size distribution or the primary particle morphology of TiO$_2$. Larger agglomerates were produced with increased bath temperature or residence time in the reactor, whereas a higher dilution rate decreased the size of the agglomerates. In contrast, the reaction temperature had a clear effect on the characteristics of the produced nanomaterials. An increase of the reactor temperature from 500 °C to 800 °C resulted in a decrease in the primary particle size of TiO$_2$ from ~22 to ~12 nm. TEM images (Fig. 5) reveal that the primary particles produced at the reactor temperature of 800 °C are also fused together more tightly than the primary particles produced at the reactor temperature of 500 °C.

Figure 5. TEM images of the TiO$_2$ particles produced at reactor temperatures of a) 500 °C and b) 800 °C

The change in the primary particle size is related to the reaction rates of the TTIP decomposition pathways at different temperatures. The thermal
decomposition of the TTIP precursor can proceed via three mechanisms. The first mechanism involves intramolecular rearrangement according to the following equation (Okuyama et al., 1986):

\[
\text{Ti(C}_3\text{H}_7\text{O)}_4 (g) \overset{k_g}{\rightarrow} \text{TiO}_2 + 4\text{C}_3\text{H}_6 + 2\text{H}_2\text{O}. \quad (5)
\]

In the second mechanism, water vapour that is formed during the reaction (5) may induce gas-phase hydrolysis of TTIP according to the following equation (Seto et al., 1995):

\[
\text{Ti(C}_3\text{H}_7\text{O)}_4 (g) + 2\text{H}_2\text{O} \overset{k_g}{\rightarrow} \text{TiO}_2 + 4\text{C}_3\text{H}_7\text{OH}. \quad (6)
\]

The rate of the hydrolysis reaction is much faster than the rate of intramolecular rearrangement; however, the extent of the reaction is limited without the additional source of water vapour (Tsantilis et al., 2002). In the third mechanism, the surface growth rate was included (equation 7). Tsantilis et al. (2002) have stated that the best agreement between theory and experimental data of TiO₂ primary particle size evolution was achieved when the surface growth was included because depletion of TTIP also occurs at the surface of the formed TiO₂ particles.

\[
\text{Ti(C}_3\text{H}_7\text{O)}_4 \text{ (particle surface)} \overset{k_s}{\rightarrow} \text{TiO}_2 + \ldots \quad (7)
\]

After thermal decomposition of the TTIP, the TiO₂ molecules rapidly form stable clusters via nucleation due their low vapour pressure (Backman et al., 2004). These clusters grow by collisions with each other and coalesce as long as their size is sufficiently small (below approximately 10 nm, Kobata et al., 1991), i.e., the particles are not crystalline. At the lower reaction temperature (500 °C), only part of the TTIP precursor reacts (the process is kinetically limited), and the remainder of the unreacted precursor condenses on the formed clusters, inducing surface growth that results in a larger primary particle size (Nakaso et al., 2003). Once the primary particle size has reached a certain size, the particles do not sinter any more, and after that point, the particle growth is controlled by agglomeration (Lehtinen et al., 1996; Büsser, 2011). The primary particle size and reaction temperature affect the sintering process, and sintering is an effective phenomena when the sintering time for certain primary particle size is shorter than the residence time at that temperature (Nakaso et al., 2003). Based on Nakaso et al. (2003), the sintering time for 15 nm primary particles at 500 °C is longer than 10^6 s. The TiO₂ particles produced at the reaction temperature of 500 °C in paper I had a primary particle size greater than 20 nm, and thus, further growth was
governed by agglomeration, which explains why the change in the process parameters did not alter the primary particle size, but the agglomerate size increased with higher precursor concentration or longer residence time in the reactor. Due to the low process temperature and dominance of the surface reaction in the primary particle growth, agglomerates of partially sintered primary particles are formed (Fig. 5a insert). A decrease in the agglomerate size at higher dilution rates indicates that the agglomeration is quenched.

At a higher reaction temperature (800 °C), the decomposition reaction of TTIP is complete and occurs more rapidly, leading to a higher supersaturation and a faster nucleation rate, which results in more primary particles (Nakaso et al., 2003). The primary particle size remains small because surface growth is not dominant at this temperature. The small primary particle size leads to faster neck growth and enhanced sintering of the primary particles (Büsser, 2011) (Fig. 5b insert). And again, when the primary particle size has reached a certain size, the particles do not sinter further due to the still rather low process temperature, and hard agglomerates are formed.

5.1.2 Si-C nanoparticles

In paper II, the molecular structure of the produced Si-C nanomaterials was highly dependent on the reaction temperature (800-1400 °C). SEM images (Fig. 6) reveal that the size of the primary particles in the agglomerates decrease with increasing temperature (average sizes are 180 nm and 59 nm at temperatures of 800 and 1400 °C, respectively), which is related to the changes in the molecular structure of the particles. Based on SAED analysis, the produced particles were amorphous at 800 °C; however, the crystallinity of the particles clearly increased at higher reactor temperatures (Fig. 7). The atomic ratios of silicon and carbon in the particles were approximately SiC\textsubscript{1.40}, SiC\textsubscript{1.60} and SiC\textsubscript{1.05} at induction temperatures of 1000, 1200 and 1400, respectively.
Figure 6. SEM images of the Si-C particles produced at reaction temperatures between 800 and 1400 °C

Figure 7. SAED diffraction patterns of the Si-C particles produced at reaction temperatures between 800 and 1400 °C

NMR and powder FTIR analysis revealed that the particles synthesised at 800 °C contained SiC, Si-CH₂-Si and Si-H units. The DSC and TGA analysis results of these particles in synthetic air (N₂ 80 %, O₂ 20 %, H₂O 0 %) were also remarkably different from those of the powders prepared at higher temperatures (Fig. 8). The powder produced at 800 °C oxidised readily above 200 °C, and after the oxidation proceeded to a certain degree, a
The rearrangement of atoms released free carbon/hydrocarbons from the molecule. The powders produced at reactor temperatures of 900 and 1000 °C resulted in fairly identical TGA and DSC curves. The DSC plots exhibited exothermic peaks at approximately 900 and 1100 °C, induced by breaking off of hydrocarbon groups. The TGA and DSC curves of the powder produced at 1200 °C revealed a weight loss due to the release of free carbon at 600-650 °C with an intense exothermic peak in the DSC curve. This finding supports the conclusion that the transformation from an amorphous polymer network to an inorganic ceramics has proceeded further than at lower temperatures.

**Figure 8.** TGA (solid) and DSC (dashed) curves of the powders synthesised at temperatures of 800, 900, 1000 and 1200 °C (Paper II)

The decomposition kinetics of the precursor plays an important role in nanomaterial production from the HMDS and can explain the observed changes in the particle properties. Thermal decomposition begins with a homolytic fission of the Si-Si bond according to equation 8 (Bullock et al., 1994). Consequent unimolecular decomposition reactions (Equations 9-11, Bullock et al., 1994; Veintemillas et al., 1995) release methane, acetylene and ethylene.

\[
[\text{CH}_3\text{Si-Si}[\text{CH}_3]] \rightarrow 2[\text{CH}_3]\text{Si}^* 
\]

\[
[\text{CH}_3]\text{Si}^* \rightarrow [\text{CH}_3]\text{Si}^* + \text{CH}_4
\]
The Si-Si bond in hexamethyldisilane has a strong reactivity in the atomic hydrogen environment (Wróbel et al., 2002). Thus, the reaction of HMDS in a hydrogen-rich environment can also lead to the following reaction:

\[ \text{H}^* + [\text{CH}_3]_2\text{Si-Si}[\text{CH}_3]_2 \rightarrow [\text{CH}_3]_3\text{Si-H} + [\text{CH}_3]_3\text{Si}^* \]  

(12)

The major reaction pathways to SiC formation at high temperatures are (Veintemillas et al., 1995)

\[ \text{Si} + \text{CH}_4 \rightarrow \text{SiC} + 2\text{H}_2 \]  

(13)

\[ \text{H}_2\text{Si}[\text{CH}_3]_2 + \text{C}_2\text{H}_4 \rightarrow \text{SiC} + 3\text{CH}_4 \]  

(14)

The proportions of the concentrations of the main gaseous decomposition products methane (CH\(_4\)), acetylene (C\(_2\)H\(_2\)) and ethylene (C\(_2\)H\(_4\)) measured by gas FTIR at various temperatures are shown in Fig. 9. The proportion of acetylene increased when the reactor temperature increased. The proportions of ethylene and methane were the highest at the reactor temperature of 900 °C and decreased when the reactor temperature increased further.

**Figure 9.** Concentrations (normalised to total hydrocarbon concentration) of gaseous products of the HMDS decomposition reaction at reactor temperatures of 800, 900, 1000 and 1200 °C (Paper II)
If we examine the reaction pathway of HMDS, the formation of a large amount of methane at lower temperatures (equation 9) arises from the abstraction of hydrogen, which is abundant in the carrier gas. Furthermore, ethylene and acetylene (equations 10-11) are secondary products increasing in importance at higher temperatures (Bullock et al., 1994). Thus, at low reactor temperatures, the decomposition process produces mainly [CH\textsubscript{3}]\textsubscript{3}Si', [CH\textsubscript{3}]\textsubscript{2}Si' and [CH\textsubscript{3}]\textsubscript{3}Si-H species (equations 8, 9 and 12), and the formation of SiC proceeds through the reaction between carbosilanes and aliphatic hydrocarbons (Eq. 14), which is consistent with the analysed particle composition. Reaction (14) leads to the release of methane but consumes ethylene, which explains why the concentration of ethylene begins to decrease more rapidly than the concentration of methane. In our system, both reactions (13) and (14) most likely occur simultaneously, and higher temperatures result in reaction (13) becoming more dominant.

5.1.3 SiC and carbon nanostructures

In paper III, SiC and carbon nanostructures were prepared from solid Si-C precursor particles (synthesised in paper II) using high temperature annealing in an inductively heated furnace. The temperature of the induction furnace was increased step by step. Consequently, a series of solid-state processes occurred upon increasing the annealing temperature.

First, the formation of SiC crystals (Fig. 10a, number 1) with the 3C-SiC polytype dominating was observed at the lowest annealing temperature (1900 °C). It is generally accepted that the nucleation of the 3C-SiC polytype requires a lower temperature and higher Si/C ratio in the vapour phase compared with the hexagonal polytypes (Fissel, 2003; Yakimova et al., 2006; Vasiliauskas et al., 2010). The precursors used in paper III were amorphous or nanocrystalline SiC particles that contained an excess of carbon. The main reactions of dissociative evaporation of SiC are (Lilov, 1993)

\[
\text{SiC (s)} \rightarrow \text{Si (g)} + \text{C (s)} \quad (15)
\]

\[
2\text{SiC (s)} \rightarrow \text{Si}_2\text{C (g)} + \text{C (s)} \quad (16)
\]

\[
2\text{SiC (s)} \rightarrow \text{SiC}_2\text{ (g)} + \text{Si (liq., g)} \quad (17)
\]

\[
\text{SiC (s)} \rightarrow \text{SiC (g)}. \quad (18)
\]

At temperatures below 2273°C, an excess of free silicon in the vapour phase (reactions 15 and 17) and of solid carbon in the source powder prevails.
(reactions 15 and 16). With increasing temperature, the evaporation of the SiC, Si:C and Si:C$_2$ species is more pronounced, and the amount of free silicon in the gas phase should decrease because reaction 15 does not occur above 2273 °C. According to Sudarshan and Maximenko (2006), the vapour stoichiometry reaches a value of unity at 2527 °C. However, the dissociation and evaporation of Si-C based materials (e.g., a SiC powder or mixture of Si and C powders) are complicated phenomena that, in addition to temperature, depend significantly on the preparation conditions (e.g., mechanical milling vs. chemical vapour synthesis), properties (e.g., polytype composition, stage of crystallinity) and grain size of the precursor (Tairov, 1995; Sudarshan and Maximenko, 2006). Thus, the exact temperature limits for the existence of different sublimation species in the gas phase during evaporative dissociation cannot be given, and the composition of the gas phase and the vapour stoichiometry (Si/C ratio) may be significantly affected by the precursor properties.

Because 3C-SiC is a metastable polytype, a solid-state 3C to 6H transition is usually encountered at temperatures above 2000 °C (Yoo et al., 1990; Yakimova et al., 2006; Vasiauskas et al., 2010). The XRD results revealed that the solid-state transition from 3C to 6H-SiC occurred through higher-order hexagonal stacking sequences. At the lower induction temperatures, the higher-order hexagonal polytypes were more pronounced than 6H-SiC, whereas at the induction temperature of 2400 °C, the transition was nearly completed, and the 6H-SiC polytype was dominant. The increasing crystallinity of the precursor material promoted the formation of 3C-SiC, which can be explained by the smaller amount of free carbon in the more crystalline precursor particles (SiC-1400) that increase the Si/C ratio in the vapour phase. Additionally, because the precursor materials were powders that consist of amorphous preceramic or nanocrystalline nanoparticles, the evaporation of silicon may be enhanced, which further increases the Si/C ratio in the vapour phase.

**Figure 10.** a) SEM image of the structures formed during the annealing of Si-C precursor particles: 1) SiC crystals, 2) graphene sheets, and 3) carbon nanoflowers (CNFs); b) TEM image of the CNFs (Paper III)
Next, sublimation of silicon from the SiC precursor particles and from the formed large SiC crystals resulted in an excess of carbon on the surfaces, which induced nucleation of graphene layers (Yazdi et al., 2013) (Fig. 10a, number 2). The term graphene strictly means a flat monolayer of carbon atoms packed into a two-dimensional (2D) honeycomb lattice (Geim and Novoselov, 2007). However, practically, the electronic structure of graphene approaches the 3D limit of graphite at 10 layers. Consequently, three different types of graphene 2D crystals can be distinguished, i.e., single, double and few (3 to < 10) layer graphene. The formed graphene was mostly multilayered (typically < 10 layers) and extensively folded (Fig. 11); however, based on Raman analysis, the quality of graphene at its best was high (Fig. 12) and improved with increasing annealing temperature. This effect is most likely due to the enhanced surface diffusion at higher annealing temperatures, i.e., restructuring of the surface proceeded further before graphene formation (Emtsev et al., 2009). Thermal decomposition of SiC in argon at atmospheric pressure has been observed to improve the quality of the formed graphene layers compared with vacuum conditions (Emtsev et al., 2009; Virojanadara et al., 2009; Yazdi et al., 2013) because sublimation of SiC near atmospheric pressure leads to a reduced Si evaporation rate and conditions closer to equilibrium. In addition, sequestering the SiC source in the graphite enclosure with a small leak maintains a higher Si vapour pressure in the crucible (Heer et al., 2011). However, at the highest annealing temperature (2600 °C), the evaporation and escape of silicon from the crucible was complete, whereas carbon remained in the crucible.

Figure 11. TEM image of the graphene sheets and CNFs formed from the SiC-1400 precursor at the induction temperature of 2050 °C (Paper III)
Finally, the amorphous precursor particles that contained an excess of carbon enabled the formation of novel carbon nanostructures, i.e., carbon nanoflowers (CNF) (Fig. 10b) with a diameter below 60 nm. The CNF growth is induced by simple thermal decomposition of the Si-C precursor particle surface followed by the evaporation of Si (Yazdi et al., 2013). Stable curved carbon layers, the “flower petals”, are formed according to the mechanism proposed by Wang et al. (2007). The formation of surface-graphene C-C σ-bonds perpendicular to the SiC surface and the existence of the C-C π-bonds in the planar graphene sheet result in a repulsion in the centre between two adjacent dangling bond sites (Fig. 13). Once σ-bonds are generated, this bonding is preferentially maintained because σ-bonding is more stable than π-bonding (Kusunoki et al., 1999). The curved surface of the precursor particles causes the growth of the flower-like structure. The size of the precursor particles limits the size of the CNFs. Above an induction temperature of 2200 °C, no Si-C core remained in the structure.
TEM analysis demonstrated that the number of carbon layers in the CNFs produced from the SiC-1000 precursor powder decreased with increasing annealing temperature from 11 at temperatures below 2400 °C to 7 at 2600 °C (Fig. 14). This result may be related to the enhanced evaporation rate of the carbon atoms at the highest annealing temperature. In addition, roughening of the carbon sheets is visible in the TEM image of the powder produced at 2600 ° (Fig. 14 b, insert), which may indicate changes in the atomic structure.

The abundance of the graphene sheets compared with the abundance of the CNFs increased with increasing crystallinity of the precursor material. In addition, the number of carbon layers in the CNFs decreased with increasing crystallinity of the precursor material, and there were only 5 layers in the CNFs produced from the SiC-1400 precursor. This finding may be related to the smaller amount of free carbon and the already rather ordered structure of the remaining carbon-rich surface after sublimation of the Si atoms compared
with the disorder among the carbon atoms with the more amorphous precursor particles.

### 5.2 Characterisation of nanomaterials from the view of toxicity screening studies

The crystal phase, agglomeration state, surface area and surface activity of the produced TiO$_2$ (paper I) and Si-C (paper II) particles were studied using XRD, TEM, XPS and BET. Furthermore, to characterize the cell-permeation capacity, the zeta potential of the TiO$_2$ agglomerates was measured in paper IV. Based on the XRD analyses, the TiO$_2$ particles produced in paper I at 500 °C contained 77 vol% anatase and 23 vol% brookite with crystal sizes of 20 nm and 7 nm, respectively. The median size of the primary particles was ~22 nm (determined from TEM primary particle size distributions). The crystal phase distribution of the produced TiO$_2$ particles was different from commonly used commercial TiO$_2$ particles (Degussa P25). Degussa P25 contains 80% anatase and 20% rutile with the primary particle size between 20-30 nm (Oberdörster et al., 2007). The crystal phase and crystallinity of the particles may affect the toxicity of nanosize TiO$_2$. Typically, anatase has been stated to be more toxic than rutile (Johnston et al., 2009).

The primary particles in the TiO$_2$ agglomerates produced at 500 °C were quite loosely bonded compared with the agglomerates produced at the higher temperature (800 °C). This finding may affect the distribution and toxic effects of these particles in the body. Grassian et al. (2007) has suggested that the agglomeration state of TiO$_2$ nanoparticles may dictate their toxic potential, i.e., soft agglomerates might be more toxic due to better dispersion.

The produced TiO$_2$ powder had a specific surface area of 69 m$^2$/g, which is higher than that reported for Degussa P25 powder (48-55 m$^2$/g, Oberdörster et al., 2007). The surface area has been proposed to be crucial in the toxicity of nanoparticles (Johnston et al., 2009) due to enhanced reactivity. The composition and surface activity of the nanoparticles may also contribute to their toxicity (Johnston et al., 2009). Based on XPS analysis, the surface of the TiO$_2$ powder produced in paper I consisted of 55.4 wt% titanium (Ti), 41.4 wt% oxygen (O) and 3.2 wt% carbon (C). The observed carbon is most likely produced by the analysis method. XPS analysis is performed in vacuum and due to the evaporation of oil from the vacuum pump, adsorbed hydrocarbons will always be present on the sample surface. The XPS analysis revealed OH functionality on the surface of the particles. Because OH functionality on the surface only increases TiO$_2$ activity (Ardizzone et al., 2007) and no other impurities were present in our powder, the powder can be considered a rather pure composite and thus suitable for toxicological studies. The zeta potential was positive in acidic conditions, and the zero
point was reached at approximately pH 5.3. As the lungs of mice have neutral pH, TiO$_2$ particles are not taken easily into the cells because negatively charged particles are “rejected” by the negatively charged cell membrane.

In paper II, the specific surface area of the Si-C particles synthesised at the reaction temperature of 800 °C was approximately fourfold compared with higher temperatures (136 ± 3 m$^2$/g and 32-35 ± 0.5 m$^2$/g, respectively). This result contradicts the typical hypothesis that as the primary particle size decreases, the specific surface area increases. Based on the SAED diffraction pattern (Fig. 7) the particles produced at 800 °C were amorphous. However, the SEM and TEM images (Fig. 15) do not show clear porosity in the particles.

Figure 15. SEM (a) and TEM (b) images of the Si-C particles synthesised at 800 °C

The particles produced at 800 °C were also highly hydrophobic, settling on the surface of the water but dispersed readily in ethanol. The powders prepared at higher temperatures did not disperse without sonication in any solvent. The hydrophobic behaviour of the particles produced at 800 °C is connected with the Si-H or Si-CH$_3$ groups on the surface (Grundner and Jacob, 1986). In addition, the pronounced oxidation tendency of these particles compared with the particles produced at higher temperatures can be explained by the existence of the Si-H units on the particle surface. Hydrogen is a structure-breaking element and can facilitate the invasion of oxygen into the silicon lattice (Grundner and Jacob, 1986).

Based on the powder FTIR analysis, the particles produced at 900 and 1000 °C still contained Si-H bonds and CH$_3$-C linkages. Thus, the composition and functional groups of these particles differ from the more crystalline particles produced at 1200 °C, in which both Si-H and CH$_3$-C groups were absent. This may affect the behaviour and effects of these particles in toxicity screening studies. The particles produced at 1000 °C have been tested in inhalation
exposure studies, and the results will be published in another thesis (Leppänen, M).

5.3 Gas-phase nanoparticle production for in-situ inhalation exposure studies

Direct gas-phase processes for nanoparticle production followed by instant administration (paper I) have rarely been utilised in inhalation exposure studies. The administration of nanoparticles has usually been conducted using commercial nanosize powder and various powder feeders (e.g., Heinrich et al., 1995; Bermuzed et al., 2004; Wong, 2007). The problem with this set-up is that the particles have a tendency to stick together to larger agglomerates, which may result in a bimodal size distribution. A second alternative has been to use nebulisers, i.e., a commercial powder is dissolved or suspended in an appropriate solvent, nebulised and dried to produce an aerosol (e.g., Grassian et al., 2007). The problem is low dispersibility of the powders and the potentially harmful solvents that must be removed from the aerosol. Furthermore, the administration of nanoparticles with these methods is often poorly controlled. Proper characterisation at the point of administration is fundamental because of potential physicochemical changes between the produced bulk material and the administered material.

The main factors that must be considered in an inhalation study are the animal environment, exposure atmosphere, and inhaled dose (Wong, 2007). Parameters such as temperature, relative humidity (RH), oxygen content, waste gas content, and noise in the exposure facility are included in the animal environment. The recommended ranges for temperature, relative humidity and oxygen content are 22 ± 3 °C, 30-70 % and >19 % (OECD, 2009b). The temperature and oxygen content must be especially considered in long exposures. In the direct gas-phase methods, based on the chemical reaction to generate nanoparticles, the characterisation of the gaseous, possibly harmful by-products is essential. The exposure atmosphere must be monitored to assure a consistent exposure for each animal group. Demands that inhalation exposure systems set on the nanoparticle synthesis include a stable and easily adjustable number and mass concentration over a long period, together with a constant size distribution on the nanometre scale (Oberdörster et al., 2005). It is recommended that a minimum of three exposure levels and appropriate control(s) should be used (OECD, 2009a). The inhaled dose depends on the amount of test material that is taken up or deposited in the respiratory system from the exposure atmosphere. It is an imprecise parameter due to the difficulty of measuring the amount of air inhaled by the individual animal, changes in the respiration physiology, and
material-specific uptake factors but can be to some extent controlled by exposure-specific monitoring of respiration (Wong, 2007).

The aerosol flow reactor designed, constructed and optimised in paper I produces narrow number size distributions for TiO$_2$ at constant mass concentrations up to 30 mg/Nm$^3$ (geometric mean diameter 130 nm, geometric standard deviation 1.6) over six hours with low concentrations of reaction by-products at the reactor temperature of 500 °C. The concentrations of the by-products (propene C$_3$H$_6$, carbon monoxide CO, and nitrogen dioxide NO$_2$) increased approximately according to the decrease in dilution rate and were at maximum: 86 ppm C$_3$H$_6$, 5.1 ppm CO and 5.7 ppm NO$_2$. For comparison, the nasal and pulmonary irritation limits for C$_3$H$_6$ and NO$_2$ are 500 ppm and 349 ppm, respectively (Pottenger et al., 2007; Schaper, 1993). If needed, the by-products can be removed from the exposure atmosphere using, e.g., a denuder. In addition, the temperature (23-35 °C, depending on the dilution rate), the relative humidity (approximately 30 %), and the oxygen content (17-20 vol%) of the aerosol entering the exposure chamber were satisfactory. The highest temperature and the lowest oxygen content value were encountered only at the highest exposure concentration and were acceptable for short (< 2 h) exposures. If longer exposures at the highest exposure concentration are desired, adding oxygen and cooling the aerosol before the exposure can be performed. The major advantages of this device are that the test substance for inhalation exposure studies is directly in aerosol form and no pre-processing is needed, which eliminates possible physicochemical changes and contamination between the bulk and administered material, for example during storing or processing. The system is also easy to adjust to different experimental setups and precursors. Thus, well-defined test aerosols can be produced for inhalation exposure studies.

The inhalation exposure studies with mice (papers IV, V and VI) revealed that the TiO$_2$ nanoparticles produced using the presented aerosol flow reactor caused airflow limitation in the conducting airways (paper IV) and were deposited on the lungs of mice (paper V) but did not induce pulmonary inflammation (paper VI). More complete analysis of the toxicity screening tests of the TiO$_2$ nanoparticles and the results obtained will be included in the theses of the primary authors of the articles.
6 CONCLUSIONS

In this thesis, the manufacturing of TiO$_2$, Si-C and carbon nanomaterials via atmospheric pressure chemical vapour synthesis was studied. Post-processing of the produced Si-C particles by atmospheric pressure annealing in argon was also performed. The main objective was to investigate how process parameters and/or precursor properties affect nanomaterial characteristics (e.g., shape, crystallinity, crystal phase, primary particle size, agglomeration state, surface area, and surface structure) and to link the results with toxicity screening studies. The reaction temperature had the most prominent effect of the process parameters on the characteristics of the produced nanomaterials. A higher reaction temperature resulted in a smaller primary particle size and more crystalline particles. A decrease in the primary particle size is related to enhanced reaction kinetics and/or a change in the molecular structure of the particles. The decomposition or sublimation kinetics of the precursors at different reaction temperatures affected the composition of the vapour phase and, subsequently, the composition of the produced particles. The solid-state processes that occurred during the nanomaterial synthesis, such as the selective evaporation of silicon from the solid precursor particles in paper III, affected the microstructure of the products, and the formation of new carbon nanostructures, i.e., carbon nanoflowers, was observed. The properties of the solid Si-C precursors, i.e., the chemical composition and degree of crystallinity, had an effect on the formation and structure of the produced materials in paper III.

The deposition kinetics, absorption and toxicological effects of the engineered nanoparticles depend on physicochemical parameters that are not commonly considered during toxicity screening tests. The parameters that were considered in this study, in addition to the particle mass and number size distributions, included the crystal phase, agglomeration state, surface area, surface charge, and surface activity of the produced particles. The major drawback in the studies of health effects of engineered nanomaterials today is that the methods to produce and characterise tested nanoparticles are reported insufficiently. This issue hinders the comparison of results and sometimes begs the question: what has been actually tested? For example, in this study, depending on the process parameters and the properties of the precursors used, the primary particle size, agglomeration state, crystallinity, surface area, and surface activity of the produced particles changed. Thus, thorough characterisation of the administered nanomaterial is essential in toxicity screening studies if valid results are desired.

In conclusion, engineered nanomaterials with desired characteristics can be produced using the presented methods. The adjustable processes enable
the optimisation of the nanomaterial properties (e.g., the primary particle size, crystallinity or surface structure) for potential applications (e.g., composites, adsorbents, electrodes). The ability to synthesise solid precursor particles with well-controlled properties is crucial for advanced nanomaterial production via the sublimation route. Next, my research will focus on the exact formation mechanisms of the CNFs and graphene in our synthesis conditions and the optimisation of the process. The modification of the carbon structures, e.g., the generation of nanopores to enhance the adsorption capacity or functionalisation of the CNFs for biosensing applications, are also interesting study paths. Safety evaluation of the CNFs will be the issue for the future if the structure exhibits properties that are suitable for commercial applications.
REFERENCES


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