Applications of Nanosized Plant Particles in Medicine and Agriculture

Dissertation

zur Erlangung des Grades

des Doktors der Naturwissenschaften

der Naturwissenschaftlich - Technischen Fakultät

der Universität des Saarlandes

von

Dipl. –Pharm. Sharoon Griffin

Saarbrücken

2019

Tag des Kolloquiums:	15 Februar 2019
Dekan:	Prof. Dr. Guido Kickelbick
Prüfungsvorsitzender:	Prof. Dr. Johann Jauch
Berichterstatter:	Prof. Dr. Claus Jacob
	Prof. Dr. Marc Schneider
Akad. Mitarbeiter:	Dr. Stefan Boettcher

Die vorlegende Arbeit wurde von Februar 2016 bis Februar 2019 an der Universität des Saarlandes, Fachbereich Pharmazie, unter die Anleitung von Prof. Dr. Claus Jacob im Arbeitskreis für Bioorganische Chemie, sowie an der Philipps-Universität Marburg, Fachbereich Pharmazie, unter die Betreuung von Prof. Dr. Cornelia M. Keck im Institut für Pharmazeutische Technologie und Biopharmazie durchgeführt.

Diese Arbeit war finanziert durch Universität des Saarlandes, Philipps-Universität Marburg, das Landesforschungsförderungsprogramm Saarland (WT/2 – LFFP 16/01), das Erasmus+ staff mobility Programm und das ZIM Projekt: TOPAS (KZ16KN046424).

For the Lord gives wisdom;

from his mouth comes knowledge and understanding

— Proverbs 2:6

With love to my parents

&

brothers

Tables of content

ACKNOWLEGEMENTS

A person's life is a series of collective events that contribute to a larger illustration. A series of many such events made this work a reality from mere intent. This document would not have been possible without the support and encouragement of my mentors, colleagues and friends.

Among many, the most significant has been the support of Prof. Dr. Claus Jacob 'der Doktorvater' (University of Saarland). His vision, guidance and persistent devotion has been an essential part in developing this project and my research career.

I extend my deepest gratitude to Prof. Dr. Cornelia M. Keck (Philipps University of Marburg). Some of the studies in this thesis have only come into existence because of her unique expertise in Nanotechnology and I am delightful to be introduced to the 'nuts and bolts' of Nanosizing under her supervision.

The motivation and counselling provided by Prof. Dr. Marc Schneider (University of Saarland) was imperatively needed and goes beyond appreciation.

I would also like to acknowledge here the assistance of Prof. Dr. Karl-Herbert Schäfer (University of Applied Sciences, Zweibrücken), Dr. Muhammad Jawad Nasim, Dr. Adel-Marby and Muhammad Sarfraz (University of Saarland) for their numerous scientific insights.

In Marburg; I thank the colleagues at the Philipps University of Marburg. Among others, Florian Stumpf, Pascal Stahr, Natalie Geisel, Daniel Knoth, Olga Pelikh, Steffen Hartmann, Ralph Eckert, Verda Farida. In Saarbrücken, Yannick Ney, Polina Denezhkin, Reem Alkhayer, Irfan Masoon and Lisa Kiefer. In Italy, Paolo Zucca. In Brazil, Ethiene Castellucci Estevam. In Poland, Karolina Witek. In Togo, Dr. Nassifatou Koko Tittipina. In Nigeria, Dr. Azubuike Peter Eboaiwe and Dr. Chukwunonso ECC Ejike. In Pakistan, Prof. Dr. Jamshed Iqbal.

I would like to extend a very special thanks to Ken Rory (University of Saarland) for his continued support, guidance and the famous 'finger' to help this project keep rolling in the right direction.

Mere words would not do justice to the assistance and friendship I have developed with Achim, Manuela, Mattheo, Colleen, Zoé, and Naomie von Bodisco, Christian, Anne, Jasper, Noah, Jesse, Juni, Salomo Neddens and the members of the 'Frei-evangelischen-Baptistengemeinde Zweibrücken'.

Finally, I would like to thank my parents and my brothers for their financial support, for being there and for lending me their ears in hard times.

KURZZUSAMMENFASSUNG

Die neuesten Fortschritte in der Nanotechnologie machen es möglich, verborgene Schätze im Bereich der Pflanzen in einer neuen Perspektive zu erforschen. Nanosizing Techniken wie „High Speed Stirring“ und „High Pressure Homogenization“ helfen nicht nur, die aktiven Inhaltsstoffe aus den pflanzlichen Membranen herauszulösen - sie erhöhen auch die Bioverfügbarkeit der wasserunlöslichen aktiven Wirkstoffe der Pflanzen.

Diese Thesis erforscht die Möglichkeiten, Nanosizing zur effektiven Konvertierung von Pflanzenrohstoffen als Nanomaterial für geeignete Anwendungen im Bereich der Lebensmitteltechnologie, der Kosmetik, der Landwirtschaft und in der Medizin nutzbar zu gestalten. Basierend auf diesem Ziel wurden vergleichende Toxizitätsversuche von Nanonizierten *Pterocarpus erinaceus*, *Solanum incanum*, *Cynomorium coccineum* und ihren Extrakten gegen Mikroorganismen getestet, um die Vorteile und Herausforderungen des Nanosizing gegenüber den Extraktionsmethoden zu verstehen. In gleicherweise waren die biologischen Auswirkungen von Nanonizierten Pflanzenabfälle hilfreich, um ihr Potential in der Abfallverwertung aufzudecken. Zusätzlich wurde die Haltbarkeit des Nanomaterials durch die NaLyRe Sequenz, welche eine Gefriertrocknung in Verbindung mit Nanosizing benutzt, verbessert.

Schlussendlich eröffnet das Nanosizing von Pflanzen und deren Abfälle, u.a. als NaLyRe Sequenz, eine neue Möglichkeit für solche, die wasserunlöslichen Stoffe enthalten, um diese in Form einer grünen natürlichen Alternative dem Menschen bereitzustellen.

SUMMARY

Recent advances in Nanotechnology make it possible to study the hidden treasures within plants from a new perspective. The Nanosizing techniques of High Speed Stirring and High Pressure Homogenization not only help in the release of the active ingredients contained within the plant membranes, but also render insoluble active material more bioavailable.

This thesis probes the utilization of Nanosizing techniques to effectively convert raw plant material into nanomaterial, suitable for applications in the arena of nutrition, cosmetics, agriculture and medicine. In this context, comparative investigations of the toxicity of nanosized *Pterocarpus erinaceus*, *Solanum incanum*, *Cynomorium coccineum* and their respective extracts against microorganisms were beneficial in understanding the advantages and challenges of employing Nanosizing vs extraction technologies. Similarly, the biological impact of Nanosizing waste-plant materials (such as brewed coffee, grape seeds, walnut shells and tomato stems) helped uncover its potential in waste management. In addition, the nanomaterial was refined for long-term stability by means of the Nanosizing, Lyophilization and Resuspension (NaLyRe) sequence, which involves lyophilization in conjunction with Nanosizing techniques.

Ultimately, Nanosizing plant material or their waste, especially as part of the NaLyRe sequence, provides new possibilities for insoluble compounds within such materials as green natural alternatives for everyday life.

PAPERS INCLUDED IN THIS THESIS

The present thesis is a cumulative work comprising of four publications, which are referred to the coming sections with their respective letter:

A. Turning waste into value: Nanosized natural plant materials of *Solanum incanum* L. and *Pterocarpus erinaceus* Poir with promising antimicrobial activities.

Sharoon Griffin, Nassifatou Koko Tittikpina, Adel Al-marby, Reem Alkhayer, Polina Denezhkin, Karolina Witek, Koffi Apeti Gbogbo, Komlan Batawila, Raphaël Emmanuel Duval, Muhammad Jawad Nasim, Nasser A. Awadh-Ali, Gilbert Kirsch, Patrick Chaimbault, Karl-Herbert Schäfer, Cornelia M. Keck, Jadwiga Handzlik and Claus Jacob

Pharmaceutics, 2016, 8(2):11, ISSN 1999-4923, doi: 10.3390/pharmaceutics8020011.

Contribution of the Author♣: Nanosizing and characterization of the plant nanoparticles, designing of the corresponding figures as well as writing and formatting of the manuscript.

B. Nanosizing *Cynomorium*: Thumbs up for potential antifungal applications.

Sharoon Griffin, Reem Alkhayer, Seda Mirzoyan, Astghik Turabyan, Paolo Zucca, Muhammad Sarfraz, Muhammad Jawad Nasim, Armen Trchounian, Antonio Rescigno, Cornelia, M. Keck and Claus Jacob

Inventions, 2017, 2(3): 24, ISSN 2411-5134, doi: 10.3390/inventions2030024.

Contribution of the Author: Nanosizing and characterization of the Cynomorium nanoparticles, designing of the corresponding figures as well as writing and formatting of the manuscript.

C. No time to waste organic waste: Nanosizing converts remains of food processing into refined materials.

Sharoon Griffin, Muhammad Sarfraz, Verda Farida, Muhammad Jawad Nasim, Azubuike P. Ebokaiwe, Cornelia M. Keck and Claus Jacob

Journal of Environmental Management, 2018, 210:114-121, ISSN 0301-4797, doi: 10.1016/j.jenvman.2017.12.084.

Contribution of the Author: Selection and acquisition of the waste material, production and characterization of the nanosized material, designing of the corresponding figures, analysis of the data as well as writing and formatting of the manuscript.

D. Resuspendable powders of lyophilized chalcogen particles with activity against microorganisms.

Sharoon Griffin, Muhammad Sarfraz, Steffen F. Hartmann, Shashank Reddy Pinnapireddy, Muhammad Jawad Nasim, Udo Bakowsky, Cornelia M. Keck and Claus Jacob

Antioxidants, 2018, 7(2):23, ISSN 2076-3921, doi: 10.3390/antiox7020023.

Contribution of the Author: Production, lyophilization, characterization and stability studies of the chalcogen nanoparticles, designing of the corresponding figures, analysis of the data as well as writing and formatting of the manuscript.

♣ *Refers to the author of the thesis; Sharoon Griffin*

ABBREVIATIONS

C. albicans	*Candida albicans*
DPPH	1,1-diphenyl-2-picrylhydrazyl
E. coli	*Escherichia coli*
EL	External Layer
FD	Freeze-dried
GC	spent coffee ground
GS	Grape Seeds
h	hours
HPH	High Pressure Homogenization
HSS	High Speed Stirring
LD	Laser Diffraction
LM	Light Microscopy
LB	Luria-Bertani broth
NaLyRe	Nanosizing, Lyophilization and Resuspension
non-FD	non-freeze-dried
OD	Optical Density
OSCs	Organic Sulfur Compounds
PCS	Photon Correlation Spectroscopy
PBS	Phosphate Buffered Saline
PP	Peeled Plant
P. erinaceus	*Pterocarpus erinaceus*
RSS	Reactive Sulfur Species
RSeS	Reactive Selenium Species
RTeS	Reactive Tellurium Species
S. cerevisiae	*Saccharomyces cerevisiae*
SDA	Sabourand Dextrose Agar
SEM	Scanning Electron Microscopy
S. incanum	*Solanum incanum*
S. carnosus	*Staphlococcus carnosus*
S. feltiae	*Steinernema feltiae*

TS	Tomato Stem
WS	Walnut Shell
WP	Whole Plant
YPD	Yeast Peptone Dextrose
ZP	Zeta Potential

INTRODUCTION

Nanotechnology, first introduced in the early 1960s, has evolved rapidly and extensively in the recent years. Six decades down the road, the term "Nanotechnology" has a quite different meaning in our daily lives as envisioned by one of its pioneers, Norio Taniguchi [1,2]. Today, this term can be encountered in cosmetics (e.g., titanium dioxide, silicon dioxide), on the labels of beverages and in the $PM_{2.5}$ fraction in the exhaust fumes of cars and volcanoes. Therefore, it is appropriate to consider nanotechnology as a field inspired by nature [3-7].

Nature seen as a nanotechnologist comes forth with a range of exciting examples and techniques of transforming ordinary substances into nanomaterials. In volcanoes, combustion, for instance, is one natural nanosizing technique resulting in carbon nanotubes. Another example of physical processes commonly observed is abrasion, which is essentially nature grinding down large solid aggregates into fine particles. Other examples range from calcium sulfate and silicate particles in mineral springs to the anthropogenic impact in marine environment of turning macro plastics into micro or nano-plastics. From such examples one can conclude that nature's nanotechnology could be harmful for humans – giving rise to research into Nano-toxicological studies [8-13].

Besides these common natural nanotechnological processes there are some chemical ones, such as the oxidation of hydrogen sulfide gas (H_2S) and of hydrogen sulfide ions (HS^-) in mineral springs. As seen in the famous Elisenbrunnen in Aachen, the sulfide present in the spring's water is oxidized to element sulfur particles, which more often than not, precipitate and form deposits and thus, are available in large quantities. Their quality, nonetheless is poor and may contain contaminants. Yet they provide a sustainable source of nanomaterials which can be harvested and employed in agriculture. HS^- or H_2S can also chemically reduce selenite (SeO_3^{2-}) and sulfur which are abundantly present in mineral wells. Once oxidised, they could be transformed into a variety of selenium, sulfur and selenosulfur nanoparticles. These selenosulfur compounds, for instance, are added widely to shampoos for their pronounced anti-dandruff effect [14-17].

Whilst naturally occurring nanomaterials are interesting to discover, and indeed can be purified for better applications, there also exist nanoparticles of natural products which occur naturally. In order to accomplish this task, biological cells are involved. These being typically small, ranging from 100 nm for a virus cell to 50 µm for a plant cell, come across material in the nano range quite often [18,19]. Biological entities typically do not prefer a solid deposit as that may trigger apoptosis. Therefore, if cells (*e.g.*, bacteria like *Thiobacillus*) are exposed to inorganic salts (*e.g.*, S^{2-}, Au^{3+}), they tend to modify them by either an oxidative or a reductive pathway which unwillingly produces elemental nanoparticles [20,21]. Exposures like these are well studied in recent years and commonly referred to as biotransformations which produce particles of uniform size and good quality. This provokes a particular interest in the environmental sciences in terms of bioremediation and decontamination of toxic soils. By the state of the art approach known as nanobioremediation (NBR), pollutants are removed from a certain environment through nanotechnological processes by employing various microbes, plants and isolated enzymes [22,23].

Prof. Jacob's research group offers an example of particular importance on the generation of nanoparticles by exploiting microorganisms. Here, *Staphylococcus carnosus* was employed to reduce SeO_3^{2-} into selenium nanoparticles of around 80 nm in diameter [24]. The value of such approach stems from the notion that the generation of good quality nanomaterials by utilizing easily available bacteria is of much ecological and economical potential. Since the generation of solid particles places cells under stress resulting in apoptosis, harmful bacteria could be neutralized whilst generating nanoparticles which themselves can possess further antimicrobial activities. This double prospect of benefits adds up to a hat trick of applications in the agricultural arena [14]. By employing natural biogenic factories (microbes)

one could simultaneously annihilate pathogens, provide plants with nanomaterial to further defend them, and also enrich the soil with elemental selenium. As with all good things there is also a side-effect associated with such nanoparticles; the nanomaterial formed is not chemically pure and includes a protein coating representative of the biological source of its generation. Therefore, the final toxicity of these materials against microbes could be a mixture of the element itself and of the protein coating [24-26]. Whilst the presence of the coatings does provide additional stability to the nanoparticles, further studies are required to uncover the true mechanism of action of such nanoparticles.

A third avenue bringing nanotechnology and nature together could be visited by the direct production of nanomaterial out of natural products. Here, the techniques employed are inspired by the ones commonly found in nature (weathering, abrasion, etc.), yet technologically modified for optimization. The milling down of plant materials, which under normal conditions would be insoluble, results in nanosized materials which can be readily employed in a wide range of applications. In recent years, this approach has helped convert moderately soluble antioxidants directly isolated from plants, into nanocrystals with enhanced physicochemical properties. Products of this supreme technology are already on the market, while others show promise in laboratory experiments *e.g.*, danazol, celecoxib, meloxicam, nifedipine etc. [27-32] It is, therefore, paramount to understand the science underlying such approaches. This will be explored briefly in the following sections.

1. Basic concepts of Nanosizing

Nanosizing or nanonizing adheres to the so-called top-down technique for the formation of nanoparticles. In a layman's terms, it improves the biological activity of otherwise poorly water-soluble active compounds by reducing their particle size. Bioavailability has been a major hurdle in the drug development process for years, rendering the pursuit of high-bioavailable drugs costly and difficult to acquire. For most of these drugs, whether administered through the tropical, pulmonary or gastrointestinal route, the setbacks lie in their solubility and absorption [10,33]. Since the introduction of the concept of size reduction in the 90s, however, a new era has emerged focussing now on improving the effectiveness of drugs discovered by favourably altering their solubility to attain higher availability on the cellular level.

Whilst still heavily depending on the solubility and other physiochemical properties of the drug itself, absorption across biological membranes is also influenced by the physiological conditions of that particular absorption site. The diffusion mechanisms of the body require that the drug molecule be soluble in the local media -mostly water - for an effective transfer across the membranes. Size reduction is one approach which improve the solubility and dissolution rate of the drug to increase its *in vivo* concentrations [34]. This concept of bio-pharmacy can be explained using the Noyes-Whitney equation.

$$\frac{dc}{dt} = D \cdot A \cdot \frac{(c_s - c_0)}{h}$$

— (Noyes-Whitney Equation)

where dc / dt is the rate of dissolution, D the diffusion coefficient, A the total surface area of the particles, c_s the saturation solubility of the active ingredient, c_0 the concentration of dissolved active ingredient in the solvent and h the diffusion distance.

Nanosizing increases the total surface area of the coarse material, which in turn increases the dissolution rate dc / dt [35,36]. The consequences of this advantageous surge can be further explained by two other equations: Kelvin and Prandtl equations.

$$\frac{p}{p_0} = \frac{2\gamma M_r}{rRT\rho}$$

— (Kelvin Equation)

where p / p_0 is the change in dissolution pressure, γ the surface tension, M_r the molecular weight of the particle, r the radius of the solid particle, R the gas constant, ρ the density and T the temperature.

$$h_H = k \left(\frac{L^{1/2}}{V^{1/2}}\right)$$

— (Prandtl Equation)

where h_H the thickness of the hydrodynamic diffusion layer, k the constant, L the length of the particle surface in the direction of the flow and V the relative velocity of the flowing liquid surrounding the respective particles.

Firstly, interpreting the Kelvin equation suggests an increased solubility upon size reduction and thereby, higher dissolution pressure at the absorption site [37]. Secondly, the decrease in the diffusional distance is well clarified by the Prandtl equation [38]. Thus, the overall velocity of dissolution, as well as the saturation solubility are both enhanced and improved. Hence, and when compared to coarse materials, the gain in solubility of nanosized products results in a higher concentration gradient. The concentration gradient, as hinted earlier, is a driving force behind the uptake of active ingredients *via* passive diffusion at any absorption site. The higher the concentration gradient is, the more efficient the uptake. Conspicuously, one can simply deduce that the smaller the particle size is, the faster it will pass across membranes, and accordingly the more bioavailable a drug will be [32,39-42].

A higher dissolution rate is only one advantage of Nanosizing, as this technique also boosts particles' adherence to surfaces. The large surface area of nanoparticles provides more points of attachment *per* volume. Thus, no extra effort is required to stick them onto surfaces. A common example from everyday life is cake frosting, where icing sugar is used instead of table sugar [35,43]. To summarize, Nanosizing renders a drug molecule more vigorously adherent to surfaces and shortens the time required for across membrane penetration, and therefore enhances the bioavailability of active pharmaceutical moieties. The next section will focus on an overview into the different Nanosizing techniques utilized as part of this work.

2. Techniques involved in Nanosizing

Mechanical size reduction is one approach available today for top down production of nanoparticles. In the course of this thesis, this approach was achieved either through Bead Milling (BM), High Speed Stirring (HSS), High Pressure Homogenization (HPH) or a combination of them.

BM, also referred to in the literature as, "Pearl milling", involves interactions between beads and the coarse material in a confined chamber under high speeds [44]. Beads commonly used are made out of glass, zirconium oxide, ceramic or stainless steel. Within the milling chamber, the beads inflict friction and impact the coarse material through shearing forces resulting from bead to wall and bead to bead collusions. The size reduction depends on and can be controlled by the size of the beads and revolutions per minute (rpm) of the milling chamber [45,46]. This physical nanosizing approach has its unique advantages, yet also some disadvantages. The possibility of processing both dry and wet samples,

for example, is one BM advantage not offered by any other approach. Scaling up of BM, however, can be problematic as two third of the chamber volume has to be occupied by the beads and with larger volumes the weight of the machinery increases drastically. One other major critique of this method is the contamination resulting from the deterioration of the collided beads. A general rule in this context is that the beads employed need to be harder than the material being milled. Moreover, separating the processed sample and the beads is time consuming and considered a disadvantage usually tackled by adequate sieving. Developing improved machines to overcome such problems and produce nanoparticles of higher quality is being scrutinized. A BM involving planetary movements, for instance, inserts centrifugal and coriolis forces, in addition to the gravitational force, into the size reduction mix, rendering better 'nano-outcomes' [47]. Recent studies involving Ibuprofen and BM (with ceramic beads and 1,400 rpm) were successful in achieving particles having 2.5 percent the size of the original sample [48]. Other researchers have employed this technique to improve the oral bioavailability of Ketoconazole and Candesartan [49,50].

HSS, an industrial approach to nanosizing, revolves around processing of dispersing solids in a medium. One of the frequently employed mixer designs is represented by rotor-stator style mixers. These mixers have been employed to generate nanoemulsions and nanoparticles [51,52]. Rotor-stator HSS generates a vacuum when the rotor is spinning around the stator at speeds ranging between 10,000 and 30,000 rpm. This vacuum sucks in the sample suspension through the stationary stator slots, where it will be subjected to hydraulic and mechanical shear, as well as, radial and tangential forces. Due to the applied resistance of the stator slots, the suspended particles are torn apart and their particle size is reduced dramatically [53-55]. Although the rotor-stator HSS has been exploited to produce nanomaterial on its own, yet for the sake of homogeneity during Nanosizing, further processing with HPH is usually required.

HPH is a cutting-edge, recently introduced technique in nanotechnology, which has a tremendous impact on the production of nanoparticles. It revolutionized mechanical nanosizing because, in addition to the traditional utilization of shear forces, it made use of a phenomenon known as cavitation. In this context, the so-called Piston gap homogenizers serve as good practical example [34,40]. The cylinder of such homogenizer, 3 cm in case LAB 40 Homogenizer, is filled with liquid dispersions (suspensions or emulsions), which are then forced to pass through a narrow gap of 5-25 µm in diameter at a high velocity and pressure. The sudden changes in diameter impacts on the particles of the suspension twice. First, inside the homogenizer's gap the dynamic pressure increases whereas the static pressure falls, equalizing the vapour pressure of the liquid to the static pressure inside the gap. As a result, the liquid boils forming air bubbles. Secondly, due to the passage through the gap, the diameter of the particles and the surrounding pressure both change again leading to the implosion of the formed air bubbles. With the air bubbles imploding, the particles are cavitated at the weak points in the particle's structure (imperfections) resulting in size reduction. Subsequent application of more pressure or homogenizing cycles will lead to smaller particle sizes, as well as a decrease in the weak points as the particles become progressively perfect [56,57]. Eventually, an equilibrium is reached between the applied pressure and the interaction forces of the particles. Any further homogenizing cycle will not reduce the particle size unless of course the pressure is increased. The literature shows, however, that there does not exist a linear relationship between size and applied pressure. It shows also that the nature of the particles plays an important role because the imperfections in the particle's structure limit their size reduction under a given pressure. Further homogenization cycles at this stage are, nonetheless, helpful as they aid in decreasing the polydispersity of the nanosuspension [32,58]. There have been many studies where HPH has been employed as a sole method for nanoparticle production of rutin, hesperetin, and curcumin among others, or in combination with HSS, *e.g.* solid lipid nanoparticles of chrysin, luteolinon and quercetin [59-64].

3. Particle characterization associated with Nanosizing

Any procedure involved in scientific research requires a proper method of control to assess its advantages, as well as its limitations which, in nanosizing, are determined by the characteristics of its final product. The particle characterization is performed to investigate the particle size, polydispersity, shape, charge and stability of the nanosized particles. To obtain comprehensive and precise information, three different characterization techniques are employed: Photon Correlation Spectroscopy (PCS), Laser Diffraction (LD) and Light Microscopy (LM).

PCS, or dynamic light scattering analysis, is one of the most commonly used characterization method of nanoparticles. A main advantage for incorporating this technique is the efficient measurement of the sub-micron particles [65]. The basic principle lies in the detection of the scattered laser beam as it falls on the diffusing particles in a low viscosity dispersion medium. The fluctuations of the signals form a correlation function, which is interpreted as diffusion coefficient of the particles. The Strokes-Einstein equation is then applied to calculate the mean of the particle size (hydrodynamic diameter) from the diffusion coefficient. Additionally, the size distribution is also measured as a polydispersity index (PDI). This provides information regarding the range of particle sizes in a particular nanosuspension, where a value between 0.1 and 0.7 indicates a narrow range/distribution, while above 0.7 a broad one [66]. The size range, which can be measured using PCS, is between 3 nm and 3 µm, yet the upper limit variable depends on the density of the particles suspended in the dispersion medium. To obtain ideal PCS measurements, it is critical that the suspended particles only endure the Brownian motion which is then measured as the diffusion velocity. In dense suspensions, however, particles undergo sedimentation in addition to the Brownian motion and the diffusion velocity is then determined by the resultant of these two phenomenon [67,68]. Here, the densely packed small particles are treated as larger particles. Moreover, the size of larger particles present in the suspension increases the scattering intensity, resulting in a z-average not being a true representative of the entire sample. For this reason, PCS is optimal for collecting information about the size of small particles in diluted suspensions, where, theoretically, only Brownian motion exists. Both z-average and PI are usually measured over time to determine the crystal growth, for example, by Oswald ripening, or aggregations. Evaluating the crystal growth provides reliable data on the stability of the nanosized suspension [69,70]. Another related parameter which provides deeper insights into the charge and stability of the nanoparticles is the Zeta-Potential (ZP). The ZP, or the electrokinetic potential, is a measure for the effective surface charge on nanoparticles in suspension. It corresponds to the potential between the electric double layer of particles under an electric field and the dispersion medium. The electrical flow through the diluted nanosuspension, while using the same PCS instrument, induces electrophoretic mobility among charged particles. The Helmholtz-Smoluchowki equation can then be employed to convert electrophoretic mobility into ZP values. A low ZP value of an electrostatically stabilized particle suggests that the nanosuspension will aggregate and destabilize much faster than the one which has a higher ZP (ideally ± 30) [71-73].

In the case of nanosuspensions, it is sometimes crucial to include a secondary characterization technique, such as Laser Diffraction. LD, or static light scattering analysis, has gained its popularity in the last decade due to recent improvements and has broadened its spectrum of use from research laboratories to quality control in large-scale production lines [74-76]. This acceptance is on the grounds that LD is user friendly, time saving and able to characterize samples with broad ranges of sizes. Since the characterization results are presented as volume percentage concentrations of the nanomaterial in the dispersion system, it is possible to evaluate the efficacy of the production process by comparing the results at different Nanosizing stages. Principally, a particle under a laser beam will diffract light at a certain angle and intensity depending on its size; the smaller the size, the greater the angle of the scattered light. This size calculation requires the use of the Frauenhofer approximation to enable LD measurements of particles having sizes between 6 µm and 3 mm, depending on the wavelength of the

laser beam (633 nm for He-Ne laser) [77]. For the analysis of particles below 6 µm, however, LD follows the light scattering theory of Gustav Mie, which requires a proper knowledge of the particles' optical properties *i.e.*, imaginary and real refractive indices. The estimation of these properties is, nonetheless, difficult because both the Frauenhofer approximation and the Mie theory treat particles as being spherical. The latter fact constructs the main drawback of this technique limiting its employment for optimal particle characterisation [78-80].

The two characterization techniques described until now analyse the particles digitally. For this reason, it is difficult to visualize the nanomaterial produced optically. Optical imaging of nanoparticles using LM is the simplest and most available technique in any related area of research. Microscopy in particle analysis focuses on assessing texture, shape and size. It assists in identifying problems with surfactant used commonly in nanosuspension, as the possible development of agglomerates can literally be seen after each cycle of homogenization. Unlike LD, particles of non-spherical character can properly be characterized which provides a versatile edge. The imaging potential of LM is limited, however, and the main reason lays in its light source. Therefore, particles of about 300 nm or above can be observed readily, whilst in the case of smaller particles generally electron microscopy is recommended. Scanning electron microscopy, for instance, can be employed to visualize particles as small as 10 nm in size [81,82]. In short, the three characterization techniques in conjunction with one another, provide detailed information regarding the physical properties of the nanosized materials and can, hence, benefit in understanding their corresponding activities in biological systems.

Nanosizing natural products

Nature has always served humans as a fertile source of inspiration in improving their lives. The matter of survival against diseases and illnesses is an ongoing battle, where nature has generously and constantly equipped humanity with the necessary medical armaments. Chief among these survival kits are plants. Being a renewable and green natural asset, plants provide a multitude of pharmaceutically and nutritionally relevant compounds, generally more cost-effective than synthetic active ingredients [83,84]. Classically, a variety of sophisticated and often expensive technologies are incorporated for the isolation of these beneficial compounds. Moreover, a strong financial and scientific infrastructure is required to adequately test and formulate the isolated compounds into suitable dosage forms [85]. Such demands are, unfortunately, seldom available in the developing parts of the world, where many advantageous plants grow abundantly. It is therefore logical to focus future research on alternatives that are: a) easily available and accessible in a cost efficient manner and, b) competently capable of uncovering and exploring the hidden treasures of plants. In this context, it seems that Nanosizing can bestow a valuable approach in the pursue of feasible alternatives.

In order to experimentally assess both the advantages and challenges of Nanosizing, plant candidates were selected on the basis that they are known to contain biologically active compounds, not exotic as not to endanger their existence, and native to a developing country. *Solanum incanum* L., for instance, is found in northern Africa and known for its antimicrobial activity, especially in the agricultural arena [86]. In addition to the latter small weed, a commonly available tree from west Africa, *Pterocarpus erinaceus* Poir, was selected. Various parts of this tree have been used traditionally to cure minor ailments and fungal infections [87]. Both plants, however, have not been explored extensively for their practical applications and thereby present an excellent opportunity to be studied under the novel Nanosizing approach.

The investigation of the African plants revealed that their characteristics can play a vital role in the final particle size achieved, as well as the application possibilities. A subsequent investigation was performed in view of these findings involving a Mediterranean mushroom, *Cynomorium coccineum* L.

The composition of the parasitic plant is intriguing; an apparent lack of chlorophyll renders it dry and brittle. Furthermore, the inclusion of polyphenols and anthocyanins among other compounds enables *C. coccineum* to be utilized as antioxidant, antiemetic and antihypertensive [88-90]. The properties of this edible mushroom makes the plant a prime candidate in the Nanosizing exploration of entire plants and even comparable to the fruits and barks of the African candidates.

Human interactions with the plant kingdom are not limited to finding cures against diseases, rather they are primarily based on acquiring food for sustenance. Over centuries this acquisition has taken a more refined and industrialized prospect, which created the possibility for billions of human beings around the world to obtain nourishment either directly or indirectly through plant products [91]. This modern food processing, however, has its dark side. The amount of simultaneously produced waste for instance, projects a substantial ecological and environmental burden. Grape seeds, tomato stems, walnut shells and spend coffee ground represent good examples of such home-made kitchen waste. Unfortunately, this readily available waste, still rich in antioxidants and polyphenols, finds less alternative utilities and is often discarded into the environment [92,93]. The conventional applications, for instance as fertilizers, in vermicomposting or in coffee shampoos, do not fully exploit their potential and they require suitable additional processing [94-96]. Interestingly, Nanosizing of crude plants opens a door of possibilities to examine these abundantly generated waste materials under a new light.

The production of nanomaterial was subsequently followed by investigations in singular or multiple microbial systems including the Gram negative bacterium, *i.e.*, *Escherichia coli*, the Gram positive bacterium, *i.e.*, *Staphylococcus carnosus*, the Yeasts, *i.e.*, *Saccharomyces cerevisiae* and *Candida albicans* and a multicellular nematode, *i.e.*, *Steinernema feltiae.* These biological investigations served as a direct comparison between the activities of the extracts and nanosized particles of the previously mentioned plants. Carrying out these tests was also important to judge the feasibility of application of the nanosized waste. The suspensions of the nanosized materials, raised concern regarding the practicality of usage and stability. This liquid formulation requires special handling and storage conditions, thus limiting its transportation and application over extended periods. To tackle the stability issue, a number of studies were conducted focusing on the conversion of these nanosuspensions into a solid form. This led to a procedural sequence of Nanosizing, Lyophilization and Resuspension (NaLyRe). This sequence facilitated the attainment of nanosuspensions on demand with properties of the original suspension. Elemental Sulfur (S), Selenium (Se) and Tellurium (Te) were selected as test candidates for such studies. Organic compounds of these elements have shown antimicrobial and anticancer activities [97-99]. Interestingly, the similar chalcogen-chalcogen bonds responsible for their activities can also be found in the elemental forms of S, Se and Te [100]. Although they convey potential advantageous activities, the insoluble nature of these elemental forms render their many practical applications limited. Nanosizing, however can increase their bioavailability through particle size reduction.

AIMS OF THE THESIS

The research conducted within the framework of this thesis tends to exploit the modern advancements in nanotechnology to uncover the potential applications of plant parts or plant-based green by-products, towards finding alternatives, for instance, against less aggressive pathogens in the field of medicine, natural product-based nutrition, cosmetics and eco-friendly agriculture.

This encompassing objective was accomplished by the following research aims:

- Employment of Nanosizing techniques to mill down crude plant materials.
- Impact of Nanosizing techniques on chlorophyll-free plants.
- Application of Nanosizing techniques towards waste management.
- Improvement of Nanosized formulations for increased usability and prolonged stability.

RESULTS

In this section the four publications which are part of this work will be attached.

1. Publication A

Turning waste into value: Nanosized natural plant materials of *Solanum incanum* L. and *Pterocarpus erinaceus* Poir with promising antimicrobial activities.

Sharoon Griffin, Nassifatou Koko Tittikpina, Adel Al-marby, Reem Alkhayer, Polina Denezhkin, Karolina Witek, Koffi Apeti Gbogbo, Komlan Batawila, Raphaël Emmanuel Duval, Muhammad Jawad Nasim, Nasser A. Awadh-Ali, Gilbert Kirsch, Patrick Chaimbault, Karl-Herbert Schäfer, Cornelia M. Keck, Jadwiga Handzlik and Claus Jacob

Pharmaceutics, 2016, 8(2):11, ISSN 1999-4923, doi: 10.3390/pharmaceutics8020011.

2. Publication B

Nanosizing *Cynomorium*: Thumbs up for potential antifungal applications.

Sharoon Griffin, Reem Alkhayer, Seda Mirzoyan, Astghik Turabyan, Paolo Zucca, Muhammad Sarfraz, Muhammad Jawad Nasim, Armen Trchounian, Antonio Rescigno, Cornelia, M. Keck and Claus Jacob

Inventions, 2017, 2(3): 24, ISSN 2411-5134, doi: 10.3390/inventions2030024.

3. Publication C

No time to waste organic waste: Nanosizing converts remains of food processing into refined materials.

Sharoon Griffin, Muhammad Sarfraz, Verda Farida, Muhammad Jawad Nasim, Azubuike P. Ebokaiwe, Cornelia M. Keck and Claus Jacob

Journal of Environmental Management, 2018, 210:114-121, ISSN 0301-4797, doi: 10.1016/j.jenvman.2017.12.084.

4. Publication D

Resuspendable powders of lyophilized chalcogen particles with activity against microorganisms.

Sharoon Griffin, Muhammad Sarfraz, Steffen F. Hartmann, Shashank Reddy Pinnapireddy, Muhammad Jawad Nasim, Udo Bakowsky, Cornelia M. Keck and Claus Jacob

Antioxidants, 2018, 7(2):23, ISSN 2076-3921, doi: 10.3390/antiox7020023.

DISCUSSION

In essence, the studies conducted as part of this thesis support the concept of top down Nanosizing of natural products. On the one side, the results point out the benefits of utilizing such nanosized materials, together with the NaLyRe sequence, against common pathogens and shed some light on their promising applications in the fields of medicine and agriculture. On the other side, the results also reveal the limitations and the room for improvement in these techniques, especially when the ultimate objective is a product with optimal stability and biological activity. The highlights and challenges of nanosizing plant materials in view of these studies will now be discussed in more detail.

1. Optimizing the nanosizing techniques for plant parts

The introductory study and the results gathered thereof were essential in providing a scientific first view into the homogenization of crude material. The powders of plants, *S. incanum* and *P. erinaceus*, served as the model materials for the nanosizing technique and the evaluation of their activities against bacterial pathogens and nematodes pinpointed its advantages.

The results associated with the nanosizing technique indicated certain limitations related to the handling and storage of the nano-products. The methods of BM, HSS and HPH, however, were feasible to process the plant parts rapidly, in order to test their activities in biological systems. Once milled, the sizes and shapes of particles achieved from the fruits of *S. incanum* and barks of *P. erinaceus* were considered adequate for the bioassays. Nonetheless, the Nanosizing procedures may still require refinement to improve the physical features of the nanosized particles. Further improvements or adjustments in the technique should focus on enhancing the stability of the nanosuspensions, for instance, by incorporating more appropriate surfactants whether employed individually or in combination. Likewise, the inclusion of further nanonization methods could be worth considering in future studies. ART-Crystal, for example, is a new and successful approach to produce nanoparticles utilizing both lower pressure and fewer cycles. The integration of such cutting-edge technologies could be worth exploring, yet they might also increase the financial burden of production, which is counterproductive to the objective of the current study *i.e.*, finding cost-effective methods for developing countries. Therefore, any future enhancements of the Nanosizing technique need take into account the economical factor, while still attempt to refine the particles in terms of quality and stability.

Although the actual quantitative release of compounds was unclear, the antimicrobial assay exhibited toxicity after exposure to the nanosized suspensions. This crucial information could be necessary to understand ‘nanoparticle-mediated toxicity’, the pathways responsible for this toxicity, and also concerns of nano-safety. One possible explanation, after analysing the initial results, is that the particles might act as delivery systems of pharmacologically active compounds, *e.g.* carpesterol, incanumine, lupeol and epicatechin [101,102]. Cellular interactions between the particles and cell membranes or in case of nematodes the blockage of pores, might also be worth focusing on in later investigations.

Undoubtedly, the nanosizing approach in this study elucidated, to some extent, the toxicity of the chemical constituents stored within the various parts of the plants. Yet the effectiveness and feasibility of the approach still depended heavily on the characteristics of the plants themselves. These include, but are not limited to, the fibrous and fat content. Furthermore, the investigations of bioactivity for the nanosized material of the barks of *P. erinaceus* and the fruits of *S. incanum* showed similarities with their extracted counterparts. Hence, based on these findings, one can contemplate applications for

the plants and also for the Nanosizing techniques. The antibacterial activity observed for *P. erinaceus* can be favourable against minor topical or gastrointestinal infections, whilst the nematicidal activity of *S. incanum* could be useful in an agricultural context.

The preliminary investigation helped in understanding the simplicity of homogenization of different plant parts and the chosen model plants have provided essential insights for future employment. Here, common and indigenously found plants, especially from the developing countries, could now be selected. Medicinal plants from Togo, such as *Nauclea latifolia* and *Ocimum gratissimum*, with known anti-parasitic activity shine as promising candidates ultimately providing cheap alternatives for an economically strained population [103]. One may also consider parasitic plants which lack chlorophyll and other degrading compounds, yet in some cases include biologically active ingredients, *e.g. Cynomorium coccineum* [104].

Further exploration of the techniques' readiness will definitely be essential in comprehending if they can be a viable substitute of the extensive extraction processes of crude plants' potentials towards agricultural or medicinal applications. In addition, these studies will need to focus on the release of bioactive ingredients and the mechanisms of their particular biological action. The Nanosizing techniques investigated, nonetheless, emerge as a straightforward process encompassing the conventional extraction procedures and providing efficient formulation of natural products.

2. Nanosizing chlorophyll-free plants

The second part of this thesis focused on homogenizing entire plants, in particular the chlorophyll-lacking medicinal plant *Cynomorium coccineum* L. The procedures produced crude biologically active nanomaterial, which avoided lengthy and drastic solvent extraction and purification methods. The nanomaterial could be applied directly to bestow comparative bioactivity on the extracts obtained through conventional procedures. The observations and challenges, pertaining this study will now be discussed briefly.

Here, the notion that the brittleness of the material renders it more suitable for homogenization is demonstrated for different plant parts. In this context, the fibrous content has its impact on the Nanosizing process. *Cynomorium*, for instance, being devoid from chlorophyll is brittle and thus, more amenable to Nanosizing. The outer layer of the plant is hard, which poses less of a challenge when Nanosized as compared to fibrous leaves and fruits of other plants. In these plants, roots and barks could be more suitable for homogenizing, where the high pressures help obtain particles of uniform size and shape.

The biological activities of the nanosuspensions produced showed the beneficial potential of employing Nanosizing techniques for medicinal plants. The nanomaterial, especially the one obtained from the outer layer of *Cynomorium*, depicted superior toxicity against *C. albicans* when compared to other plant-based nanomaterial from *P. erinaceus* and *S. incanum* or that from elemental chalcogens, such as sulfur, selenium and tellurium [14,24,105].

Furthermore, the toxicity profile of the *Cynomorium* nanosuspensions against the microorganisms is comparable to the extracts of different parts of the same plant and in some cases even better. These findings are particularly intriguing, since extracts are usually considered more effective. They contain a concentrated proportion of the active molecules which are rapidly and entirely bioavailable to produce their respective effect. In comparison, nanosized particles are regarded inferior as they involve insoluble material. The results can be interpreted in two ways. Either the particles tend to release the active substance over time which are toxic to *C. albicans* obtained as part of this study, or

the fungal interaction with the particles results in the release of toxic material. In any case, the nanosuspensions produced improved toxicity compared to the extracts.

The exciting activities established by employing nansized *Cynomorium* particles on fungal cells have asked the question of whether different parts of the plant have similar activity. The parasitic, yet edible, mushroom can be divided into a brittle outer peel and more fibrous inner part. In fact, the hard outer layer is more amenable to nanosizing, resulting in better particles which, surprisingly, are more bioactive perhaps due to the presence of certain phytochemicals [106]. In comparison, the inner part is less active due to an apparent lack of active substances.

Moreover, there appears little to no difference in activities of the whole plant of *Cynomorium*; whether nanosized or extracted, when compared with those from the outer layer. This can be explained in the context of synergistic bioactivity. In this regard, the active constituents present within the inner and outer layer can function together as efficiently as from the outer layer individually. Hence, nanosizing reduces the processing steps and the resulting waste, while increasing the quality and yield of the active product.

Practical applications of these nanosuspensions, at the present time, will be futile and would definitely require further investigations into their stability, storage and, most compellingly, their mode(s) of action. Additionally, alternatives for the surfactant employed, *i.e.*, in this study Plantacare, could be beneficial. Improvement on the methodology to stabilize the particles and thereby eliminate the need for further surfactant utility should also be considered. The simplicity of Nanosizing with the techniques available to produce sterile nanomaterials, which are stable for days and active against common human pathogens, nonetheless, clears the way for other biologically active nanomarterials, in particular where more aggressive synthetic drugs are not an option. It is worth highlighting again that plants, such as *Cynomorium*, represent a renewable source for the production of the nanosized material and their applications can include widespread ecological and economic benefits.

3. Nanosizing for waste management and up-cycling

The third part of this project evaluates the utility of employing insights gained from the first two parts to handle a variety of waste from the food industry, with the aim to beneficially process them entirely without any further residues. Although the study focuses on novel up-cycling possibilities for specific applications, further considerations are required to ensure that the nanomaterials obtained are readily useable and effective against certain pathogens. This notion and its implications in the agricultural and medicinal arena will now be discussed in detail.

The waste materials, *i.e.*, spend coffee ground, grape seeds, tomato stems and walnut shells were collected and approached with suitable Nanosizing techniques. This circumvented the requirement for any pre-processing of the material *e.g.*, drying, cleaning or pressing. The microscopic images show a successive decrease in particle size and a successful conversion from bulky suspensions to fairly homogeneous nanosuspensions. Here, the grape seed samples were eventually the least size-reduced particles (≈400 nm), whereas that of the tomato stem reached around 200 nm. It is noteworthy that adequate techniques were employed to appropriately visualize these reductions in size. As the particle size went below the range of light microscopy, scanning electron microscopy was applied. Furthermore, the LD and PCS measurements already described were able to elucidate the particle characterization and particular size distribution. Interpretation of these measurements presented a remarkable result in the case of coffee samples. Being directly collected after brewing, these samples contained a high water content and were soft in texture. Still, the nanomaterial obtained by Nanosizing coffee was comparably monodisperse and with size of about 250 nm. In comparison, the dry and brittle grape seeds had

difficulty reaching sizes below 400 nm. It is, however, perceivable as the sample was not defatted and, thereby maintained a high lipid content. This is in agreement with our previous finding for fibrous and juicy materials being problematic during the Nanosizing procedures. A similar pattern can be observed for the nanosized tomato stems, mainly consisting of particles with smaller size, the presence of fibrous content, however, rendered the nanomaterial more polydisperse. Once again, the brittle nature of the material and its chemical composition are essential predictors of the possible outcomes of the nanomaterial. The focus of the study, nonetheless, remains towards finding of applications rather than achieving perfect nanoparticles. Therefore, the nanosuspensions obtained were subjected to biological evaluations against common microbes.

The nanosized coffee grounds demonstrates a growth promoting effect when tested against gram negative *E. coli* and Brewer's yeast (*S. cerevisiae*). Coffee grounds are traditionally used in kitchen gardens as a fertilizer, meaning that this waste, and even after being brewed, still contains valuable constituents. Polyphenolic compounds, for instance, are mostly poor water soluble, and nanosizing would help improve their bioavailability. Promoting the growth of microorganism such as *E. coli* might not be the desired effect in certain instances, hence, other areas of application could be considered. One such arena is microbe-based fermentation, where bacteria and yeast cultures are utilized in waste management. Modern approaches of waste management such as vermicomposting and anaerobic digestion could also benefit in a similar fashion.

Nanosized grape seed particles showed a somewhat different activity in biological systems. The presence of polyphenolic compounds in grape seed, similar to the coffee sample, have no toxic effect on *E. coli*. Both yeast candidates (*S. cerevisiae* and *C. albicans*), however, were affected in an inhibitory manner. Further investigations in the multicellular nematode model (*S. feltiae*) with more concentrated nanosuspensions resulted in statistically significant toxicity. In contrast to the previous studies with the entire mushroom (*C. coccineum*) and the plant parts (*P. erinaceus* and *S. incanum*), the concentrations of the nanosized grape seed suspension, which demonstrated a toxic effect, were particularly high. Moreover, the nature of compounds responsible for these activities is rather vague. The activities can be regarded either to the latent release of bioactive molecules from the nanomaterial, or to the physical interactions of the nanoparticles with the cellular structure based on their size, shape and charge leading to apoptosis.

Another possible reason for these activities might lie in the interplay with the redox processes within the cells themselves. This was briefly investigated experimentally by determining the antioxidant capacity of the nanosized material in contrast to the bulk material. The assays suggested a noteworthy aspect of Nanosizing as it appeared to liberate the anti-oxidative potential still stored within waste materials. The tomato stems, for instance, showed a staggering increase in the antioxidant activity when compared to the bulk material. This surge in the activity seems to be associated with the particle size reduction of the particles, thereby increasing their specific surface area, which in turn influences their antioxidant activity. The impact of size reduction is not limited to tomato stems and appears to be a common feature among the samples tested. Furthermore, the increased antioxidant behaviour, stemming from size reduction, could also help explain the growth promoting effect of some nanosized material *e.g.*, coffee grounds and grape seeds.

Green walnut shells also exhibited enhancement in their dyeing properties after being nanosized. Due to their specific tannin content of green walnuts shells are used in the textile industry as a dyeing agent [107,108]. Nanosizing, infact, is a capable and competent approach to reveal and uncover the hidden benefits of the so called "organic waste" in turning waste into value.

4. Nanosizing as part of the NaLyRe sequence

The final study focused on achieving the long-term stability of nanosized materials. The empirical results were promising and, to some extent, concurred with their objective. They supported the notion that combining Nanosizing techniques with lyophilization leads to easily reconstitutable powders. The sequence of Nanosizing, Lyophilization and Resuspension (NaLyRe) was analysed by employing elemental chalcogens, which resulted in improved physical properties and more effective antimicrobial activities. The insights of the study will now be discussed in more detail.

In contrast to our previous plant candidates, the refined chemical material was nanosized with comparable ease. A difference between the three chalcogens, *i.e.*, sulfur, selenium and tellurium, nonetheless, could be observed. In this realm, sulfur could only be reduced to 760 nm due to its compacting ability to form soft cakes. Selenium, showed better amenability and thus, the average size was reduced to 210 nm. This trend continued with tellurium nanoparticles of around 170 nm.

The findings of the study point out the advantages of employing the NaLyRe sequence as the products were soft, light-weight chalcogen nanomaterials. Moreover, they were easily and rapidly converted back into suspensions, ready to be tested in biological systems. Interestingly, the re-suspensions maintained the physical characteristics, *i.e.*, size, shape and charge of the original nanosized material. Here, the ZP experiments were critical as an indicator of colloidal stability. Unlike the nanomaterials from the previous studies, these lyophilized materials could be stored and transported conveniently. Therefore, NaLyRe diminished the need for precautions necessary with liquid samples against leakages and spills. This can be beneficial when looking for applications in the arenas of cosmetics, nutrition, agriculture or even medicine.

Besides highlighting the substantial benefits of the NaLyRe sequence, the study also explored improving its formulation. In general, the choice of excipients plays an essential role in determining the functionality of any formulation. In this study, the approach was aimed at overcoming complications associated with freeze-drying. Without the use of cryoprotectants, the highly concentrated nanosuspension suffers from agglomeration and caking and also excessive mechanical stress due to ice formation destabilizing the suspensions entirely. During the initial experiments of the study, the NaLyRe sequence was investigated with various concentrations of the cryoprotectant Mannitol. Whilst a 5 % concentration of mannitol had little benefit, a 20 % concentration was eventually optimal to maintain the size of the nanosuspensions. Cryoprotectants, such as mannitol, in these concentrations formed effective matrices around nanoparticles, thereby isolating them from freezing and agglomeration.

Mannitol as Cryoprotectant is not new and among others have formed part of previous studies involving nanoparticles [109]. Therefore, in consequent studies alternatives can be analysed. Trehalose, is one such alternative where the concentration needed for the cryoprotectant effect can be decreased. Still, Trehalose may interfere with the activities of the nanomaterial in biological systems and is more expensive when compared to mannitol [109]. Thus, it could be of value to search for excipients which can simultaneously function as stabilizers and as cyroprotectants.

In general, the nanomaterials investigated, after the NaLyRe sequence, were effective against all the microorganisms and to varying degrees. Here, tellurium samples were not only able to achieve the smallest size, but were also highly toxic in biological systems. This could be especially intriguing for applications against topical infections (*e.g.*, mucous, skin, nails, etc). Whilst tellurium salts may help re-sensitize resistant pathogenic strains, the nanomaterial may also have similar unwanted side effects upon humans [110,111]. Although such nanotoxicological concerns need specific investigations, still the slow release associated with nanoparticles could still be beneficial, for instance in the case of Reactive Tellurium Species (RTeS).

The concern for toxicity in practical applications is usually minimal in the case of selenium. In humans, selenium nanoparticles can be detoxified through various biological processes. This includes either the direct usage of the element or the release of Reactive Selenium Species (RSeS), such as selenides (H_2Se), selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}). In the current study, the resuspended selenium nanoparticles were particularly active against yeast cells where the growth of *C. albicans* was reduced by 40 %. This was rather appealing as some selenium containing shampoos are used for anti-fungal applications. In these personal care products selenium sulfide acts as sporicidal and thereby impedes further spread of the fungal infection [112,113]. The sulfur-selenium ring responsible for the activity in these shampoos is quite similar to the Se_8 ring structures in the selenium nanoparticles [17,114,115].

The activities of the chalcogen nanoparticles provide interesting insights into their antimicrobial applications. In order to be ready for human applications, detailed investigations about the mode(s) of action need to be conducted. As discussed earlier, some answers to these questions may lay in the physical interactions of the particles with the cells or, in the case of chalcogens, in the specific surface interactions. Moreover, the release of chalcogen-based molecules (*e.g.* inorganic polysulfides S_x^{2-}) could also be plausible.

Taking the example of colloidal sulfur usage in the treatment of grapevines, the nanosuspensions with improved bioavailability and physical characteristics can similarly be useful in agriculture [116,117]. Sulfur along selenium, apart from being protective to plants against microbes, enrich the soil and promote healthy plant growth with enhanced nutritional value. These applications can utilize both, the sterile nanosized original suspension and the resuspendable powder obtained from NaLyRe sequence [118,119].

CONCLUSIONS AND OUTLOOK

In essence, the entire project divided into the four included studies, gives a unique perspective of utilizing crude plant material to form readily usable nanoparticle-based delivery system in a few simple steps of Nanosizing. The techniques themselves do not pose any hindrance with regards to being modified and, when economically feasible, can be further fine-tuned to produce higher quality nanoparticles. By altering the physical characteristics into desired size, shape and charge, the release of active substances and the resulting biological outcome can both be controlled.

A wide range of plant candidates or their individual parts can be considered in subsequent studies. Their selection can be based on regional availability, or potential utility, for instance in the fields of cosmetics and nutrition. Since the plant, *P. erinaceus*, has previously shown potential as anti-inflammatory and anti-infective agent, its nanosuspension can be adopted against skin infections and dysentery by the local native community. Alternatively, applications in the agricultural sphere can be considered, where large amounts of these nanosuspensions can be employed with low risk of human toxicity.

Future studies would not only need to focus on refining the physical or nanotechnological aspects of Nanosizing, but also the chemical and pharmacological parameters which govern the biological activity of the nanosuspensions as seen in this project. This may uncover the absorption, distribution, metabolism and excretion of the particles, and also the mode(s) of action underlying their activities and the nanotoxicological issues related to the fibrous nature of some of the particles.

Waste management in a nutshell – quite literally in case of walnuts – becomes a realistic possibility, if one employs the Nanosizing techniques, Whilst the resulting nanosuspensions may not be as potent as some of the compounds on the market, there still remains potential in these valuable waste materials to be applied as natural anti-microbials, phytoprotectants, antioxidants or even as microbial growth promoters in large scale fermentation processes. This is rather intriguing as the Nanosizing approach, similar to anaerobic digestion and vericomposting, has the adequacy to process large quantities of waste material generated by the local food industries [94].

Apart from rendering waste into value, the approach itself produces no waste. It is worth mentioning that the products obtained through some up-cycling procedures available in the market, such as coffee shampoos, involve extensive refinements and extractions involving organic solvents, ultimately leaving residual waste. In contrast, up-cycling through Nanosizing is more practically attractive as it contributes to an economical residue-free waste management. Beside reduced generation of waste, there are other environmental and economic benefits. Firstly, being side-products of food, large concentrations of the consequential nanosuspensions would probably not be toxic for human use. Secondly, since obtaining and processing large amounts of such food side-product is feasible, this will contribute to a cleaner environment. Therefore, waste management under the umbrella of Nanosizing is simpler and more robust. Eventually the nanosized waste could be promoted as high quality, green and natural fertilizer or pytoprotectant in agriculture or as natural antioxidant skincare product in the local pharmacy.

Our preliminary study into waste management via the Nanosizing techniques has elucidated that there remains little difference between the biological activities of plants and their waste. For finding optimal applications, however, there still remains an urge to investigate the mechanisms and toxicity related to the nanosized material. From a more practical perspective, proper and cost-effective collection, storage and distribution of the house-hold and industrial food waste should be considered. This in turn could help developing countries or regions in optimizing their waste management. Furthermore, investigations to enhance the quality of nanomaterial produced, especially, in large scale

production lines and prolonging their storage stability is of particular interest. In this regard, employing dry milling and lyophilization could provide nanosized material which is both easy to handle and to transport.

In this project, we have tried to provide an answer to the question of long-term stability and the benefits of lyophilization. The consequent NaLyRe sequence was able to illustrate, in the chalcogen samples, that freeze-drying can be employed effectively to obtain nanosized materials in their solid form. These powders can be resuspended easily when needed and their resultant activities are similar to the ones of the original nanosuspensions.

In future, nanotoxicolocial concerns may require further investigation. Here, favouring alternative cyroprotectants and surfactants, over mannitol and Plantacare respectively, would be of particular interest to assist future large scale production of such nanosized materials [109]. This, along with other studies could foster our understanding of the physical, biochemical and physiological aspects of employing such nanosized materials in different arenas.

Against this background, the potential of the elemental chalcogens, can further be applied in practice with the assistance of nanotechnology. These elemental forms, without the additional load of organic groups, could be beneficial from a biological perspective, where less alterations of structural modification and conjugate formation exist. This is reflected by the recent interest in inorganic polysulfides, such as tetrasulfide (S_4^{2-}), which illustrates the biological importance of employing singular chalcogen molecules [120,121].

In summary, the use of NaLyRe sequence with integrated Nanosizing techniques converts insoluble materials, whether from parts or as whole plants, and also waste material to be more conveniently and effectively converted into nanosized materials with potential applications in field of cosmetics, nutrition, medicine and agriculture.

REFERENCES

1. Taniguchi, N. On the basic concept of nano-technology. *Proc. Intl. Conf. Prod. London, 1974* **1974**.
2. Whatmore, R.W. Nanotechnology—what is it? Should we be worried? *Occupational Medicine* **2006**, *56*, 295-299.
3. Kettler, K.; Krystek, P.; Giannakou, C.; Hendriks, A.J.; de Jong, W.H. Exploring the effect of silver nanoparticle size and medium composition on uptake into pulmonary epithelial 16hbe14o-cells. *Journal of Nanoparticle Research* **2016**, *18*, 182.
4. Kukkonen, J.; Bozó, L.; Palmgren, F.; Sokhi, R.S. Particulate matter in urban air. In *Air quality in cities: Saturn eurotrac-2 subproject final report*, Moussiopoulos, N., Ed. Springer Berlin Heidelberg: Berlin, Heidelberg, 2003; pp 91-120.
5. Brunner, T.J.; Wick, P.; Manser, P.; Spohn, P.; Grass, R.N.; Limbach, L.K.; Bruinink, A.; Stark, W.J. In vitro cytotoxicity of oxide nanoparticles: Comparison to asbestos, silica, and the effect of particle solubility. *Environmental science & technology* **2006**, *40*, 4374-4381.
6. I. Adawi, H.; A. Newbold, M.; M. Reed, J.; E. Vance, M.; L. Feitshans, I.; R. Bickford, L.; A. Lewinski, N. *Nano-enabled personal care products: Current developments in consumer safety*. 2018; Vol. 11.
7. Foss Hansen, S.; Heggelund, L.R.; Revilla Besora, P.; Mackevica, A.; Boldrin, A.; Baun, A. Nanoproducts – what is actually available to european consumers? *Environmental Science: Nano* **2016**, *3*, 169-180.
8. Nath Roy, D.; Goswami, R.; Pal, A. Nanomaterial and toxicity: What can proteomics tell us about the nanotoxicology? *Xenobiotica; the fate of foreign compounds in biological systems* **2017**, *47*, 632-643.
9. Griffin, S.; Masood, M.I.; Nasim, M.J.; Sarfraz, M.; Ebokaiwe, A.P.; Schafer, K.H.; Keck, C.M.; Jacob, C. Natural nanoparticles: A particular matter inspired by nature. *Antioxidants (Basel, Switzerland)* **2017**, *7*.
10. Müller, R.H.; Gohla, S.; Keck, C.M. State of the art of nanocrystals – special features, production, nanotoxicology aspects and intracellular delivery. *European Journal of Pharmaceutics and Biopharmaceutics* **2011**, *78*, 1-9.
11. Lähde, A.; Sæunn Gudmundsdottir, S.; Joutsensaari, J.; Tapper, U.; Ruusunen, J.; Ihalainen, M.; Karhunen, T.; Torvela, T.; Jokiniemi, J.; Järvinen, K., *et al.* In vitro evaluation of pulmonary deposition of airborne volcanic ash. *Atmospheric Environment* **2013**, *70*, 18-27.
12. Santillo, D.; Miller, K.; Johnston, P. Microplastics as contaminants in commercially important seafood species. *Integrated environmental assessment and management* **2017**, *13*, 516-521.
13. Cole, M.; Lindeque, P.; Halsband, C.; Galloway, T.S. Microplastics as contaminants in the marine environment: A review. *Marine pollution bulletin* **2011**, *62*, 2588-2597.
14. Faulstich, L.; Griffin, S.; Nasim, M.J.; Masood, M.I.; Ali, W.; Alhamound, S.; Omran, Y.; Kim, H.; Kharma, A.; Schäfer, K.-H., *et al.* Nature's hat-trick: Can we use sulfur springs as ecological source for materials with agricultural and medical applications? *International Biodeterioration & Biodegradation* **2017**, *119*, 678-686.
15. Berlo, K.; van Hinsberg, V.J.; Vigouroux, N.; Gagnon, J.E.; Williams-Jones, A.E. Sulfide breakdown controls metal signature in volcanic gas at kawah ijen volcano, indonesia. *Chemical Geology* **2014**, *371*, 115-127.
16. Ezoe, Y.; Lin, C.H.; Noto, M.; Watanabe, Y.; Yoshimura, K. Evolution of water chemistry in natural acidic environments in yangmingshan, taiwan. *Journal of environmental monitoring : JEM* **2002**, *4*, 533-540.
17. Cummins, L.M.; Kimura, E.T. Safety evaluation of selenium sulfide antidandruff shampoos. *Toxicology and Applied Pharmacology* **1971**, *20*, 89-96.
18. Neaves, W.B. Genomes, 2nd ed. T.A. Brown. Oxford, united kingdom: Wiley-liss, 2002, 600 pp., $97.50, cloth. Isbn 0-471-25046-5. *Clinical Chemistry* **2002**, *48*, 2300-2300.
19. Shors, T. *Understanding viruses*. Jones & Bartlett Publishers: 2011.

20. Stefess, G.C.; Torremans, R.A.M.; de Schrijver, R.; Robertson, L.A.; Kuenen, J.G. Quantitative measurement of sulphur formation by steady-state and transient-state continuous cultures of autotrophic thiobacillus species. *Applied Microbiology and Biotechnology* **1996**, *45*, 169-175.
21. Singh, P.K.; Kundu, S. Biosynthesis of gold nanoparticles using bacteria. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* **2014**, *84*, 331-336.
22. Duhan, J.S.; Kumar, R.; Kumar, N.; Kaur, P.; Nehra, K.; Duhan, S. Nanotechnology: The new perspective in precision agriculture. *Biotechnology Reports* **2017**, *15*, 11-23.
23. Yadav, K.; Singh, J.; Gupta, N.; Kumar, V. A review of nano bioremediation technologies for environmental clean-up: A novel biological approach. **2017**.
24. Estevam, E.C.; Griffin, S.; Nasim, M.J.; Denezhkin, P.; Schneider, R.; Lilischkis, R.; Dominguez-Alvarez, E.; Witek, K.; Latacz, G.; Keck, C., *et al.* Natural selenium particles from staphylococcus carnosus: Hazards or particles with particular promise? *Journal of Hazardous Materials* **2017**, *324, Part A*, 22-30.
25. Yazdi, M.H.; Mahdavi, M.; Varastehmoradi, B.; Faramarzi, M.A.; Shahverdi, A.R. The immunostimulatory effect of biogenic selenium nanoparticles on the 4t1 breast cancer model: An in vivo study. *Biological trace element research* **2012**, *149*, 22-28.
26. Tejo Prakash, N.; Sharma, N.; Prakash, R.; Raina, K.K.; Fellowes, J.; Pearce, C.I.; Lloyd, J.R.; Pattrick, R.A. Aerobic microbial manufacture of nanoscale selenium: Exploiting nature's bio-nanomineralization potential. *Biotechnology letters* **2009**, *31*, 1857-1862.
27. Al-Kassas, R.; Bansal, M.; Shaw, J. Nanosizing techniques for improving bioavailability of drugs. *Journal of Controlled Release* **2017**, *260*, 202-212.
28. Nekkanti, V.; Venkateswarlu, V.; Pillai, R. *Drug nanoparticles - an overview*. 2012; p 111-132.
29. Liu, Y.; Sun, C.; Hao, Y.; Jiang, T.; Zheng, L.; Wang, S. Mechanism of dissolution enhancement and bioavailability of poorly water soluble celecoxib by preparing stable amorphous nanoparticles. *Journal of pharmacy & pharmaceutical sciences : a publication of the Canadian Society for Pharmaceutical Sciences, Societe canadienne des sciences pharmaceutiques* **2010**, *13*, 589-606.
30. J Raval, A.; M Patel, M. Preparation and characterization of nanoparticles for solubility and dissolution rate enhancement of meloxicam. *Intl. R. J. of Pharmaceuticals* **2011**, *1*.
31. Hecq, J.; Deleers, M.; Fanara, D.; Vranckx, H.; Amighi, K. Preparation and characterization of nanocrystals for solubility and dissolution rate enhancement of nifedipine. *Int J Pharm* **2005**, *299*, 167-177.
32. Junghanns, J.-U.A.H.; Müller, R.H. Nanocrystal technology, drug delivery and clinical applications. *International Journal of Nanomedicine* **2008**, *3*, 295-310.
33. Müller, R.H.; Keck, C.M. Twenty years of drug nanocrystals: Where are we, and where do we go? *European Journal of Pharmaceutics and Biopharmaceutics* **2012**, *80*, 1-3.
34. Keck, C.M.; Muller, R.H. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. *Eur J Pharm Biopharm* **2006**, *62*, 3-16.
35. Möschwitzer, J.; Müller, R. *Drug nanocrystals—the universal formulation approach for poorly soluble drugs*. 2007; p 71-88.
36. Buckton, G.; Beezer, A.E. The relationship between particle size and solubility. *International Journal of Pharmaceutics* **1992**, *82*, R7-R10.
37. Digilov, R. Kelvin equation for meniscuses of nanosize dimensions. *Langmuir* **2000**, *16*, 1424-1427.
38. Chogale, M.; Ghodake, V.; Patravale, V. *Performance parameters and characterizations of nanocrystals: A brief review*. 2016; Vol. 8, p 26.
39. Moschwitzer, J.; Muller, R.H. New method for the effective production of ultrafine drug nanocrystals. *Journal of nanoscience and nanotechnology* **2006**, *6*, 3145-3153.
40. Junyaprasert, V.B.; Morakul, B. Nanocrystals for enhancement of oral bioavailability of poorly water-soluble drugs. *Asian Journal of Pharmaceutical Sciences* **2015**, *10*, 13-23.
41. Aguiar, G.P.; D. Arcari, B.; M.P.C. Chaves, L.; Dal Magro, C.; L. Boschetto, D.; Piato, A.; Lanza, M.; Oliveira, J. *Micronization of trans-resveratrol by supercritical fluid: Dissolution, solubility and in vitro antioxidant activity*. 2018; Vol. 112, p 1-5.
42. Tuomela, A.; Saarinen, J.; Hirvonen, J.; Peltonen, L. Chapter 11 - analytical tools for reliable in vitro and in vivo performance testing of drug nanocrystals. In *Nanoscale fabrication,*

optimization, scale-up and biological aspects of pharmaceutical nanotechnology, Grumezescu, A.M., Ed. William Andrew Publishing: 2018; pp 441-477.

43. Gao, L.; Zhang, D.; Chen, M. *Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system*. 2008; Vol. 10, p 845-862.
44. Martena, V.; Shegokar, R.; Di Martino, P.; Muller, R.H. Effect of four different size reduction methods on the particle size, solubility enhancement and physical stability of nicergoline nanocrystals. *Drug Dev Ind Pharm* **2014**, *40*, 1199-1205.
45. Niwa, T.; Miura, S.; Danjo, K. Universal wet-milling technique to prepare oral nanosuspension focused on discovery and preclinical animal studies – development of particle design method. *International Journal of Pharmaceutics* **2011**, *405*, 218-227.
46. Möschwitzer, J.P. Drug nanocrystals in the commercial pharmaceutical development process. *International Journal of Pharmaceutics* **2013**, *453*, 142-156.
47. Nakach, M.; Authelin, J.-R.; Agut, C. New approach and practical modelling of bead milling process for the manufacturing of nanocrystalline suspensions. *Journal of Pharmaceutical Sciences* **2017**, *106*, 1889-1904.
48. Sofwan Sinaga, A.G.; Karsono; Suwarso, E. Preparation and characterization of nanoparticles for dissolution rate enhancement of ibuprofen. *International Journal of PharmTech Research* **2015**, *8*, 545-550.
49. Basa, S.; Muniyappan, T.; Karatgi, P.; Prabhu, R.; Pillai, R. Production and in vitro characterization of solid dosage form incorporating drug nanoparticles. *Drug Development and Industrial Pharmacy* **2008**, *34*, 1209-1218.
50. Nekkanti, V.; Pillai, R.; Venkateshwarlu, V.; Harisudhan, T. Development and characterization of solid oral dosage form incorporating candesartan nanoparticles. *Pharmaceutical Development and Technology* **2009**, *14*, 290-298.
51. Urban, K.; Wagner, G.; Schaffner, D.; Röglin, D.; Ulrich, J. Rotor-stator and disc systems for emulsification processes. *Chemical Engineering & Technology* **2006**, *29*, 24-31.
52. Scholz, P.; Keck, C.M. Nanoemulsions produced by rotor-stator high speed stirring. *Int J Pharm* **2015**, *482*, 110-117.
53. Atiemo-Obeng, V.A.a.C., R. V. Rotor–stator mixing devices. In *Handbook of industrial mixing*, Edward L. Paul, V.A.A.O., Suzanne M. Kresta, Ed. 2004; pp 479-505.
54. Mortensen, H.H.; Calabrese, R.V.; Innings, F.; Rosendahl, L. Characteristics of batch rotor–stator mixer performance elucidated by shaft torque and angle resolved piv measurements. *The Canadian Journal of Chemical Engineering* **2011**, *89*, 1076-1095.
55. Tamminen, J.; Koiranen, T. Mixing performance comparison of milliscale continuous high-shear mixers. *The Canadian Journal of Chemical Engineering* **2015**, *93*, 2245-2252.
56. Liedtke, S.; Wissing, S.; Müller, R.H.; Mäder, K. Influence of high pressure homogenisation equipment on nanodispersions characteristics. *International Journal of Pharmaceutics* **2000**, *196*, 183-185.
57. Merisko-Liversidge, E.; Liversidge, G.G.; Cooper, E.R. Nanosizing: A formulation approach for poorly-water-soluble compounds. *European Journal of Pharmaceutical Sciences* **2003**, *18*, 113-120.
58. H. Müller, R.; Keck, C. *Second generation of drug nanocrystals for delivery of poorly soluble drugs: Smartcrystal technology*. 2008; Vol. 34.
59. Scalia, S.; Franceschinis, E.; Bertelli, D.; Iannuccelli, V. Comparative evaluation of the effect of permeation enhancers, lipid nanoparticles and colloidal silica on in vivo human skin penetration of quercetin. *Skin pharmacology and physiology* **2013**, *26*, 57-67.
60. Popa, A.; Niculae, G.; Meghea, A. *Co-encapsulation of a mixture of antioxidant and sunscreen agents into solid lipid nanoparticles*. 2014; Vol. 76, p 45-56.
61. Min Pyo, S.; Meinke, M.; Keck, C.; H. Müller, R. *Rutin—increased antioxidant activity and skin penetration by nanocrystal technology (smartcrystals)*. 2016; Vol. 3, p 9.
62. Mishra, P.R.; Al Shaal, L.; Muller, R.H.; Keck, C.M. Production and characterization of hesperetin nanosuspensions for dermal delivery. *Int J Pharm* **2009**, *371*, 182-189.
63. Rachmawati, H.; Al Shaal, L.; Muller, R.H.; Keck, C.M. Development of curcumin nanocrystal: Physical aspects. *J Pharm Sci* **2013**, *102*, 204-214.

64. Wang, Z.; Fan, H.; Li, Y.; Wang, Y. *Anti-hepatocarcinoma effects of chrysin loaded solid lipid nanoparticle against h22 tumor bearing mice*. 2015.
65. Carmichael, H.J.; Kochan, P.; Sanders, B.C. Photon correlation spectroscopy. *Physical Review Letters* **1996**, *77*, 631-634.
66. Stetefeld, J.; McKenna, S.A.; Patel, T.R. Dynamic light scattering: A practical guide and applications in biomedical sciences. *Biophysical Reviews* **2016**, *8*, 409-427.
67. Xu, R. Light scattering: A review of particle characterization applications. *Particuology* **2015**, *18*, 11-21.
68. Schmidt, P.C. Teilchengrößenmessung der laborpraxis. Von r. H. Müller und r. Schuhmann, wissenschaftliche verlagsgesellschaft mbh stuttgart 1996. 191 s., 98 abbild., 21 tab., dm 89,-, isbn 3-8047-1490-0. *Pharmazie in unserer Zeit* **1998**, *27*, 78-78.
69. Pecora, R. *Dynamic light scattering: Applications of photon correlation spectroscopy*. Springer Science & Business Media: 2013.
70. Petersson, K.; Ilver, D.; Johansson, C.; Krozer, A. Brownian motion of aggregating nanoparticles studied by photon correlation spectroscopy and measurements of dynamic magnetic properties. *Analytica Chimica Acta* **2006**, *573-574*, 138-146.
71. Bhattacharjee, S. Dls and zeta potential – what they are and what they are not? *Journal of Controlled Release* **2016**, *235*, 337-351.
72. Clogston, J.D.; Patri, A.K. Zeta potential measurement. In *Characterization of nanoparticles intended for drug delivery*, McNeil, S.E., Ed. Humana Press: Totowa, NJ, 2011; pp 63-70.
73. Lu, G.W.; Gao, P. Chapter 3 - emulsions and microemulsions for topical and transdermal drug delivery a2 - kulkarni, vitthal s. In *Handbook of non-invasive drug delivery systems*, William Andrew Publishing: Boston, 2010; pp 59-94.
74. Ma, Z.; Merkus, H.G.; de Smet, J.G.A.E.; Heffels, C.; Scarlett, B. New developments in particle characterization by laser diffraction: Size and shape. *Powder Technology* **2000**, *111*, 66-78.
75. Devaux, M.F.; Monredon, F.L.D.d.; Guibert, D.; Novales, B.; Abecassis, J. Particle size distribution of break, sizing and middling wheat flours by laser diffraction. *Journal of the Science of Food and Agriculture* **1998**, *78*, 237-244.
76. Clark, A.R. The use of laser diffraction for the evaluation of the aerosol clouds generated by medical nebulizers. *International Journal of Pharmaceutics* **1995**, *115*, 69-78.
77. Evaluation of a laser-diffraction-size analyzer for use with natural sediments. *Journal of Sedimentary Research* **1986**, *56*, 561-564.
78. Eshel, G.; Levy, G.; Mingelgrin, U.; Singer, M. *Critical evaluation of the use of laser diffraction for particle-size distribution analysis*. 2003; Vol. 68, p 736-743.
79. Boer, G.B.J.d.; Weerd, C.d.; Thoenes, D.; Goossens, H.W.J. Laser diffraction spectrometry: Fraunhofer diffraction versus mie scattering. *Particle & Particle Systems Characterization* **1987**, *4*, 14-19.
80. Mühlenweg, H.; Hirleman, E.D. Laser diffraction spectroscopy: Influence of particle shape and a shape adaptation technique. *Particle & Particle Systems Characterization* **1998**, *15*, 163-169.
81. Houghton, M.E.; Amidon, G.E. Microscopic characterization of particle size and shape: An inexpensive and versatile method. *Pharmaceutical Research* **1992**, *9*, 856-859.
82. Meloy, T.P. Particulate characterization: Future approaches. *Chapter* **1984**, *3*, 69-98.
83. Miller, J.S. The global importance of plants as sources of medicines and the future potential of chinese plants. In *Drug discovery and traditional chinese medicine: Science, regulation, and globalization*, Lin, Y., Ed. Springer US: Boston, MA, 2001; pp 33-42.
84. Kinghorn, A.D.; Seo, E.-K. Plants as sources of drugs. In *Agricultural materials as renewable resources*, American Chemical Society: 1996; Vol. 647, pp 179-193.
85. Balunas, M.J.; Kinghorn, A.D. Drug discovery from medicinal plants. *Life Sciences* **2005**, *78*, 431-441.
86. Beaman-Mbaya, V.; Muhammed, S.I. Antibiotic action of solanum incanum linnaeus. *Antimicrobial agents and chemotherapy* **1976**, *9*, 920-924.
87. Tittikpina, N.; Ejike, C.; E, C.-E.; Nasim, J.; Griffin, S.; Chaimbault, P.; Kirsch, G.; Atakpama, W.; Batawila, K.; Jacob, C. *Togo to go: Products and compounds derived from local plants for the treatment of diseases endemic in sub-saharan africa*. 2016; Vol. 13, p 85-94.

88. Wang, J.; Zhang, J.; Zhao, B.; Wu, Y.; Wang, C.; Wang, Y. Structural features and hypoglycaemic effects of cynomorium songaricum polysaccharides on stz-induced rats. *Food Chemistry* **2010**, *120*, 443-451.
89. Duke, J.A. *Duke's handbook of medicinal plants of the bible*. CRC Press: 2007.
90. Zucca, P.; Rosa, A.; Tuberoso, C.I.G.; Piras, A.; Rinaldi, A.C.; Sanjust, E.; Dessì, M.A.; Rescigno, A. Evaluation of antioxidant potential of "maltese mushroom" (cynomorium coccineum) by means of multiple chemical and biological assays. *Nutrients* **2013**, *5*, 149-161.
91. Busch, L.; Bain, C. New! Improved? The transformation of the global agrifood system*. *Rural Sociology* **2004**, *69*, 321-346.
92. Panusa, A.; Zuorro, A.; Lavecchia, R.; Marrosu, G.; Petrucci, R. Recovery of natural antioxidants from spent coffee grounds. *Journal of agricultural and food chemistry* **2013**, *61*, 4162-4168.
93. Idris, A.; Inanc, B.; Hassan, M.N. Overview of waste disposal and landfills/dumps in asian countries. *Journal of Material Cycles and Waste Management* **2004**, *6*, 104-110.
94. Manyuchi, M.; Phiri, A. *Vermicomposting in solid waste management: A review*. 2013; Vol. 2, p 1234-1242.
95. Yesudian, P. Can beverages grow hair on bald heads? *International Journal of Trichology* **2012**, *4*, 1-2.
96. Hardgrove, S.J.; Livesley, S.J. Applying spent coffee grounds directly to urban agriculture soils greatly reduces plant growth. *Urban Forestry & Urban Greening* **2016**, *18*, 1-8.
97. Jacob, C. A scent of therapy: Pharmacological implications of natural products containing redox-active sulfur atoms. *Natural product reports* **2006**, *23*, 851-863.
98. Jawad Nasim, M.; Ali, W.; Dominguez-Alvarez, E.; da Silva Junior, E.N.; Saleem, R.S.Z.; Jacob, C. Chapter 10 reactive selenium species: Redox modulation, antioxidant, antimicrobial and anticancer activities. In *Organoselenium compounds in biology and medicine: Synthesis, biological and therapeutic treatments*, The Royal Society of Chemistry: 2018; pp 277-302.
99. Hachmo, Y.; Kalechman, Y.; Skornick, I.; Gafter, U.; Caspi, R.R.; Sredni, B. The small tellurium compound as101 ameliorates rat crescentic glomerulonephritis: Association with inhibition of macrophage caspase-1 activity via very late antigen-4 inactivation. *Frontiers in immunology* **2017**, *8*, 240.
100. Mauro, J.C.; Loucks, R.J.; Balakrishnan, J.; Varshneya, A.K. Potential energy landscapes of elemental and heterogeneous chalcogen clusters. *Physical Review A* **2006**, *73*, 023202.
101. Yun-Lian, L.; Wan-Yi, W.; Yueh-Hsiung, K.; Chieh-Fu, C. Nonsteroidal constituents from solanum incanum l. *Journal of the Chinese Chemical Society* **2000**, *47*, 247-251.
102. Tittikpina, N.; Ejike, C.E.C.C.; Estevam, E.; Nasim, J.; Griffin, S.; Chaimbault, P.; Kirsch, G.; Atakpama, W.; Batawila, K.; Jacob, C. *Togo to go: Products and compounds derived from local plants for the treatment of diseases endemic in sub-saharan africa*. 2016; Vol. 13, p 85-94.
103. Tchacondo, T.; Karou, S.D.; Agban, A.; Bako, M.; Batawila, K.; Bawa, M.L.; Gbeassor, M.; de Souza, C. Medicinal plants use in central togo (africa) with an emphasis on the timing. *Pharmacognosy Research* **2012**, *4*, 92-103.
104. Zucca, P.; Argiolas, A.; Nieddu, M.; Pintus, M.; Rosa, A.; Sanna, F.; Steri, D.; Rescigno, A. Biological activities and nutraceutical potentials of water extracts from different parts of cynomorium coccineum l. (maltese mushroom). **2016**, *66*, 179.
105. Schneider, T.; Baldauf, A.; Ba, L.A.; Jamier, V.; Khairan, K.; Sarakbi, M.B.; Reum, N.; Schneider, M.; Roseler, A.; Becker, K., *et al.* Selective antimicrobial activity associated with sulfur nanoparticles. *Journal of biomedical nanotechnology* **2011**, *7*, 395-405.
106. Zucca, P.; Rosa, A.; Tuberoso, C.I.; Piras, A.; Rinaldi, A.C.; Sanjust, E.; Dessi, M.A.; Rescigno, A. Evaluation of antioxidant potential of "maltese mushroom" (cynomorium coccineum) by means of multiple chemical and biological assays. *Nutrients* **2013**, *5*, 149-161.
107. Alimohammadi, Z.; Younesi, H.; Bahramifar, N. Batch and column adsorption of reactive red 198 from textile industry effluent by microporous activated carbon developed from walnut shells. *Waste and Biomass Valorization* **2016**, *7*, 1255-1270.
108. Tutak, M.; Benli, H. *Colour and fastness of fabrics dyed with walnut (juglans regia l.) base natural dyes*. 2011; Vol. 23, p 566-568.

109. Abdelwahed, W.; Degobert, G.; Stainmesse, S.; Fessi, H. Freeze-drying of nanoparticles: Formulation, process and storage considerations. *Advanced Drug Delivery Reviews* **2006**, *58*, 1688-1713.
110. Estevam, E.C.; Witek, K.; Faulstich, L.; Nasim, M.J.; Latacz, G.; Dominguez-Alvarez, E.; Kiec-Kononowicz, K.; Demasi, M.; Handzlik, J.; Jacob, C. Aspects of a distinct cytotoxicity of selenium salts and organic selenides in living cells with possible implications for drug design. *Molecules (Basel, Switzerland)* **2015**, *20*, 13894-13912.
111. Pessoa-Pureur, R.; Heimfarth, L.; Rocha, J.B. Signaling mechanisms and disrupted cytoskeleton in the diphenyl ditelluride neurotoxicity. *Oxidative medicine and cellular longevity* **2014**, *2014*, 458601.
112. Allen, H.B.; Honig, P.J.; Leyden, J.J.; McGinley, K.J. Selenium sulfide: Adjunctive therapy for tinea capitis. *Pediatrics* **1982**, *69*, 81-83.
113. McGinley, K.J.; Leyden, J.J. Antifungal activity of dermatological shampoos. *Archives of dermatological research* **1982**, *272*, 339-342.
114. Lin, Z.; Wang, Z.; Chen, W.; Lir, L.; Li, G.; Liu, Z.; Han, H.; Wang, Z. Absorption and raman spectra of se8-ring clusters in zeolite 5a. *Solid State Communications* **1996**, *100*, 841-843.
115. Steudel, R.; Laitinen, R. Cyclic selenium sulfides. *Topics in current chemistry* **1982**, *102*, 177-197.
116. Young, H.C. Colloidal sulphur as a spray material. *Annals of the Missouri Botanical Garden* **1925**, *12*, 133-143.
117. Kwasniewski, M.T.; Sacks, G.L.; Wilcox, W.F. Persistence of elemental sulfur spray residue on grapes during ripening and vinification. *American Journal of Enology and Viticulture* **2014**, *65*, 453-462.
118. Considine, K.M.; Kelly, A.L.; Fitzgerald, G.F.; Hill, C.; Sleator, R.D. High-pressure processing--effects on microbial food safety and food quality. *FEMS microbiology letters* **2008**, *281*, 1-9.
119. Al Shaal, L.; Mishra, P.R.; Muller, R.H.; Keck, C.M. Nanosuspensions of hesperetin: Preparation and characterization. *Die Pharmazie* **2014**, *69*, 173-182.
120. Estevam, E.; Faulstich, L.; Griffin, S.; Burkholz, T.; Jacob, C. *Polysulfides in biology: From intricate chemistry to an astonishing yet hidden biological activity*. 2016; Vol. 20, p 211-217.
121. Kimura, H. Op6 - hydrogen sulfide and polysulfides as signaling molecules. *Nitric Oxide* **2015**, *47*, S6.

FURTHER PUBLICATIONS OF THE AUTHOR

The author was also part of the following publications, which are not part of the current thesis:

a. Togo to go: Products and compounds derived from local plants for the treatment of diseases endemic in the sub-Saharan Africa.

   Nassifatou Koko Tittikpina, Chukwunonso ECC Ejike, Ethiene Castelluci Estevam, Muhammad Jawad Nasim, Sharoon Griffin, Patrick Chaimbault, Gilbert Kirsch, Wouyo Atakpama, Komlan Batawila and Claus Jacob

   African Journal of Traditional, Complementary and Alternative Medicines, 2016, 13(1):85-94, ISSN 0189-6016, doi: 10.21010/ajtcam.v13i1.12.

b. Polysulfides in Biology: From Intricate Chemistry to an Astonishing Yet Hidden Biological Activity.

   Ethiene Castelluci Estevam, Lisa Faulstich, Sharoon Griffin, Torsten Burkholz and Claus Jacob

   Current Organic Chemistry, 2016, 20(2):211-217, ISSN 1385-2728, doi: 10.2174/1385272819666150724233028.

c. Natural selenium particles from *Staphylococcus carnosus*: Hazards or particles with particular promise?

   Ethiene Castelluci Estevam, Sharoon Griffin, Muhammad Jawad Nasim, Polina Denezhkin, Ramona Schneider, Rainer Lilischkis, Enrique Dominguez-Alvarez, Karolina Witek, Gniewomir Latacz, Cornelia Keck, Karl-Herbert Schäfer, Katarzyna Kieć-Kononowicz, Jadwiga Handzlik and Claus Jacob

   Journal of Hazardous Materials, 2017, 324(Pt A):22-30, ISSN 0304-3894, doi: 10.1016/j.jhazmat.2016.02.001.

d. Nature's Hat-trick: Can we use sulfur springs as ecological source for materials with agricultural and medical applications?

   Lisa Faulstich, Sharoon Griffin, Muhammad Jawad Nasim, Muhammad Irfan Masood, Wesam Ali, Salah Alhamound, Yousef Omran, Hyejin Kim, Ammar Kharma, Karl-Herbert Schäfer, Rainer Lilischkis, Mathias Montenarh, Cornelia Keck and Claus Jacob

   International Biodeterioration & Biodegradation, 2017, 119:678-686, ISSN 0964-8305, doi: 10.1016/j.ibiod.2016.08.020.

e. Natural Nanoparticles: A Particular Matter Inspired by Nature.

Sharoon Griffin, Muhammad Irfan Masood, Muhammad Jawad Nasim, Muhammad Sarfraz, Azubuike Peter Ebokaiwe, Karl-Herbert Schäfer, Cornelia M. Keck and Claus Jacob

Antioxidants, 2018, 7(1):3, ISSN 2076-3921, doi: 10.3390/antiox7010003.

f. The Small Matter of a Red Ox, a Particularly Sensitive Pink Cat, and the Quest for the Yellow Stone of Wisdom.

Muhammad Jawad Nasim, Polina Denezhkin, Muhammad Sarfraz, Roman Leontiev, Yannik Ney, Ammar Kharma, Sharoon Griffin, Muhammad Irfan Masood and Claus Jacob

Current Pharmacology Reports, 2018, pp 1-17, ISSN 2198-641X, doi: 10.1007/s40495-018-0152-3