

Pharmaceutical applications of
Pheroid™ technology

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Thesis submitted for the degree
Doctor of Philosophy Pharmaceutics
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POTCHEFSTROOM

November 2009

ACKNOWLEDGEMENTS

I would like to acknowledge and thank a number of people and institutions that played an integral role in the research and work that was required for the compilation of this thesis:

- My family, and especially my parents and my son, without whose constant and unquestioning support this study and thesis would not have been possible. Thank you also for teaching me the value of integrity, curiosity, creative thought and an analytical approach.
- My promoter and friend, Prof Awie Kotze for recognizing the potential of the Pheroid™ in the first place, for being promoter for this study, for your ideas about peptides and for your constant interest.
- My co-promoter and friend, Prof Jeanetta du Plessis for your contributions to this thesis, for your enthusiasm for cosmeceutical and topical science but mostly for your continuous support along the way.
- Piet Meyer, for your inventive outlook, for starting this whole business and allowing a curious mind to explore.
- The various colleagues that have acted interchangeably as advisors, peers and friends in the past and hopefully also in future. Here I specifically think of Pete Smith, Paul van Helden, Frans Kruger, Wilna Liebenberg, Hannekie Botha, Petra Engelbrecht, Seef Pretorius etc.
- The scientists that had an impact on the direction of this study: Proff. Paul van Helden, Pete Smith, Peter Donald, Jonathan Hadgraft, Johann Wiechers, Seef Pretorius, Leon van Rensburg. The following individuals are also included: Erica Koi, Riaan Buitendag and Liezl-Marië Nieuwoudt.
- My students from whom I learnt more than I taught them.
- The patent attorneys DM Kisch Inc., and more specifically Nico Vermaak for your friendship, valuable insights and for being always ready to consider a new avenue.
- My friends – I won't try to list you.
- The academic institutions that collaborated in conducting the required studies. These include specifically the MRC Centre for Cellular and Molecular Biology at the Stellenbosch University, the Department of Pharmacology of University of Cape Town, the Department of Agriculture of the University of the Free State, the State Vaccine Institute, BIOVAC and University of Pretoria.

- ➔ Financial support for various studies was supplied by the North-West University, the NRF, MeyerZall Laboratories, Sheckels Trading 11.
- ➔ God, through whom all is possible.

Ralph en Reuben, dié proefskrif is vir julle.

*“kan jy sien hoe ver het ons gekom
in die sirkels van die tyd
met ons medaljes en ons wonde
stap ons saam die drumpel uit
ongeskonde....”*

Uit Blou aarde; Johannes Kerkorrel (1985)

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Since Chapter 4 is in fact a reproduction of the book chapter, the numbering of the figures follows that of the book chapter.

- Figure 16.1. Confocal laser scanning micrographs of: (a) a mixture of liposome-like bilayer vesicles and nanospheres with a mean diameter of 200 nm, (b) a Pheroid microsphere of the reservoir type with a mean diameter of 35–40 μm, (c) colloids with three phases (w/o/w emulsions) with a mean diameter which is submicron, (d) colloids with three phases (w/o/w emulsions) with a mean diameter up to 15 μm, (e) a pH-dependent depot ranging in size from 5–100 μm, (f) Pheroids (red) containing on average 13 auto fluorescent active molecules.
- Figure 16.2 Contribution of N₂O to the miscibility of the oil and water phases (a). A section of the membrane of the Pheroid (b), as calculated by molecular modeling, based on Ab Initio, force field and energy theory as part of a Masters of Science study on Pheroid structure at the North-West University by J. Voges.
- Figure 16.3. Size distribution of a typical Pheroid formulation prepared through a low-energy manufacturing procedure. Pheroids were labeled with Nile Red and sizes were determined by CLSM.
- Figure 16.4. Theoretical interior volume for different sizes of vesicular Pheroids. The white freeform line indicates the size range of the average vesicular Pheroid.

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- Figure 16.5: The comparative depth of penetration of an auto-fluorescent active compound (coal tar) into human abdominal skin determined using confocal microscopy in a test and reference product. The Y-axis reflects the intensity of the fluorescence.
- Figure 16.6: Some of the parameters determined in the described proof of concept study. The baseline Pheroid product (reference product) and essential oil product were identical except for the addition of 0.1% (v/v) *Calendula officinalis* to the Pheroids in the test product.
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- Figure 5.1: Number of TB, pulmonary TB (PTB) and smear positive (Sm+) cases in SA, 1996-2002.
- Figure 5.2: The cure rate of tuberculosis in the nine provinces in South Africa during 2001.
- Figure 5.3: Scanning electron micrograph of *Mycobacterium tuberculosis*
- Figure 5.4: Schematic model of the mycobacterial cell wall.
- Figure 5.5: Target sites for relevant anti-tuberculosis drugs in mycobacteria.
- Figure 5.6: A schematic model of the fatty acid components of the membrane of the Pheroid™.
- Figure 5.7: The molecular composition of Chremophor RH40.
- Figure 5.8: The molecular structure of α -tocopherol showing reactive oxygen-based groups and the methyl group on the chromanol ring.
- Figure 5.9: Lipid peroxidation and reactions of α -tocopherol.
- Figure 5.10: Cellular uptake and transport of vitamin E in liver cells.
- Figure 5.11: A confocal micrograph of RMP entrapped in a Pheroid™ vesicle.
- Figure 5.12: Confocal micrographs of optical longitudinal sections through BacLight-labelled BCG bacteria.
- Figure 5.13: The *in vitro* dosage response curve of *M.tuberculosis* H37Rv.
- Figure 5.14: Impact of Pheroid™ on *M.tb* growth.
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- Figure 5.16: The impact of the presence of the Pheroid™ and various concentrations of antituberculosis drugs on the *in vitro* growth of *M.tb*.
- Figure 5.17: Time to positive growth of *M.tb* H37Rv cultures after the administration of various drug treatments.
- Figure 5.18: Growth responses of *M.tb* MDR strain T25 after being challenged with INH in the absence and presence of Pheroid™-entrapment.

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- Figure 5.19: The % inhibition of growth caused by the addition of EMB at various concentrations with and without Pheroid™ entrapment.
- Figure 5.20: Growth response of *M.Tb* strain TV79 treated with 0.5 µg/ml RMP and RMP entrapped in Pheroid™ at the same concentration.
- Figure 5.21: Micrographs of the growth response of BCA after treatment with PZA in the absence (left) and presence (right) of Pheroid™.
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- Figure 5.26: Pyrifitol P (green) and Pyrifitol C (red) soft gelatine capsules containing the four tuberculosis drugs.
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- Figure 5.28: An overlay of the time versus plasma concentration of INH for the test and reference drug in a 16 subject phase 1 trial.
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- Figure 5.32: The growth of the drug sensitive reference strain H37Rv and the drug resistant strain 1182 in plasma of patients.
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- Figure 6.1: The systemic immune response against DT as reflected by the titres of neutralizing antibodies against DT found in the blood after 4 and 6 weeks.
- Figure 6.2: The enhancement of specific antibody production due to the formulation of the DT antigen with the adjuvants. The positive control PBS-DT was used as reference and divider.
- Figure 6.3: The results confirm the importance of the size of the adjuvant particles: in contrast to the nasal administration, the systemic immune response was

enhanced 40-fold by the nano-sized particles [FAA-1(n)] of the present invention, but only 2-fold by the micro-sized particles [FAA-1(m)].

Figure 6.4: The survival of the mice after vaccination against rabies by administration of different serial dilutions of each vaccine.

Figure 6.5: The relative potencies; as determined for the groups reflected in figure 6.4. The potencies are based on the degree of survival and may be expressed as IU/ml according to the recommendations of the WHO.

Figure 6.6: The comparative efficacy of the proposed FAA-based vaccine against hepatitis B in mice.

Figure 6.7: The relative potency of the different vaccines, using the results obtained with the peptide antigen alone as divider. Rec FAA is the freeze-dried and reconstituted FAA- based hepatitis vaccine.

Figure 7.1: The comparative plasma profiles after administration of calcitonin.

Figure 7.2: The average plasma levels rhGH after administration.

Figure 7.3: The comparative average plasma levels of insulin after nasal administration.

Figure 8.1: The increase in number of nodes on cucumber plants treated by use of the plant support formulation of the invention.

Figure 8.2: The increase in leaf size of cucumber plants treated by use of the plant support formulation of the invention.

Figure 8.3: The numbers of medium to large cucumbers harvested at different times from plants treated with a plant support formulation according to the invention compared to untreated control plants.

Figure 8.4: The numbers of extra large cucumbers harvested at different times.

Figure 8.5: The total harvested cucumbers during each month from plants treated with a plant support formulation according to the invention compared to untreated control plants.

Figure 8.6: The numbers of green peppers harvested at different times from plants treated with a plant support formulation according to the invention compared to untreated control plants.

Figures 8.7 and 8.8 are micrographs of sections through the roots and a leaf of the control baby marrow plant (i.e. the plant that was not treated with the formulations according to the invention. In these micrographs, no Elementol was administered to the plant. Material is visualized because of autofluorescence.

Figures 8.9 and 8.10 are micrographs of sections of baby marrow plants treated with plant support formulations according to the invention (Plant 2). In the micrograph in

Figure 9, nearly all vesicles of the Elementol have permeated the cells of leaf itself, with few of the Elementol vesicles remaining in prominent veins of the plant.

- Figures 8.11: The comparative growth after 5 weeks as measured by leaf of the longest leaf of Clivia plants treated with different plant support formulations according to the invention.
- Figures 8.12: The growth over time as measured by leaf of the longest leaf of Clivia plants treated with different plant support formulations according to the invention.
- Figure 8.13: The average head diameter of Elementol R-treated lettuce plants versus control plants over a 12 week period after transplantation.
- Figure 8.14: The average comparative growth in plant height of Elementol R-treated lettuce plants versus control plants over a 12 week period after transplantation.
- Figure 8.15: A graph showing an example of a plant by plant comparison of Elementol R-treated lettuce plants versus control plants as described in Example 16, using plants with a similar number of leaves at 1st treatment.
- Figure 8.16: A graph that illustrates the average % enhancement in Fm:Dm ratios during the trial period caused by Elementol R-treatment of the lettuce plants versus control plants as described in Example 16.
- Figure 8.17: A graph that illustrates the difference in the Elementol R-treated lettuce plants and control plants in terms of the % moisture as described in Example 16.
- Figure 8.18: A graph that illustrates the respiration rate per mg protein for the study period in the Elementol R-treated lettuce plants and control plants as described in Example 16.
- Figure 8.19: Two graphs showing a comparison of the average chlorophyll A and B contents per mg of protein per fresh mass between Elementol R-treated lettuce plants and control plants for the period of the study as described in Example 16.
- Figure 8.20: A graph that reflects the chlorophyll A:B ratios obtained from the chlorophyll corrected for mg of protein and fresh mass as described in Example 16.
- Figure 8.21: A graph showing the changes in average number of flower buds formed during the first few weeks after transplantation (WAT) in Elementol R treated and control tomato plants as described in Example 17.
- Figure 8.22: A graph showing the average % enhancement in flower bud production of Elementol R treated and control tomato plants as described in Example 17.
- Figure 8.23: A graph that shows the linear increase of accumulative average yield for 3 tomato plants over the period of the study as described in Example 17.

- Figure 8.24: A graph that shows the average accumulative fruit to average accumulative bud ratio of tomato plants treated as described in Example 17.
- Figure 8.25: A graph that shows the average % of moisture found in the fruit of Elementol R treated tomato plants versus control plants as described in Example 17.
- Figure 8.26: A graph that shows the effect of ComCat[®] (CC), Elementol R (E) and combinations thereof on changes in accumulative number of fruit harvested from 3 plants per group over a period of 13 weeks as described in Example 18.
- Figure 8.27: A graph that shows the total accumulative fruit mass observed from plants treated with ComCat[®] that is entrapped in Elementol R as compared to the increase observed with Elementol R or ComCat[®] individually as described in Example 18.
- Figure 8.28: A graph that shows the increase in fresh fruit mass by the combination of Elementol R and CC as described in Example 18.
- Figure 8.29: A graph that shows the respiration rate per protein content after the first administration (week 5) and the second administration (week 9) of the Elementol R, Comcat[®] and combination treatment as described in Example 18.
- Figure 8.30: A graph that illustrates the comparative amounts of chlorophyll B per mg of protein as determined in week 13 of the trial described in Example 18.
- Figure 8.31: A graph that shows the comparative Brix readings in week 13 for Elementol R treated, CC treated and the combination treated plants described in Example 18 with HClO₄ as background.
- Figure 8.32: A photograph of germinating radishes on germination paper in the *in vitro* study described in Example 19.
- Figure 8.33: A graph that illustrates the comparative average length measured for coleoptiles of wheat for the fertilizer control, and the various dosages of Elementol R described in Example 19.
- Figure 8.34: A graph that shows the enhancement in the yield of grain from wheat by a single administration of Elementol R cultivated in field trials as described in Example 19.
- Figure 8.35: A graph that shows the average comparative plant, root and leaf weights of maize plants cultivated from seeds treated with the fungicide Captan, with a combination of Captan and Elementol R or with untreated seeds as described in Example 19.

ABSTRACT

For a drug to have a therapeutic effect, it has to reach its site of action in sufficient quantities. The Pheroid™ drug delivery system enhances the absorption of drugs in various pharmacological categories and is the focus of this study. A number of patents are registered in various countries to protect its application. Pheroid™ technology is trademarked, but may for ease of reading, be called Pheroid(s) only. The Pheroid™ itself is composed of an organic carbon backbone composed of unsaturated fatty acids with some side-chain interactions that result in self-emulsifying characteristics. The resulting vesicles and nano-sponges can entrap hydrophilic, hydrophobic or amphiphilic compounds for biomedical and agricultural application and can be manipulated as to loading ability, mechanical resistance, permeability, size and solubility.

Pheroid™ was investigated for its potential use in the areas of vaccines, peptide drugs, topical products and cosmeceuticals, antimicrobial treatments and agriculture. In all of these areas, the Pheroid™ has indeed shown applicability: the results showed improved uptake and/or efficacy of the entrapped chemical or biological compounds after administration by a number of administration routes. For oral administration, a precursor format, the pro-Pheroid™, was used, wherein the vesicles and/or sponges are formed post-administration.

Proof of concept studies on the *in vivo* absorption and bioavailability, as well as studies on *in vitro* efficacy of Pheroid-based formulations were carried out for antimicrobials, such as tuberculosis drugs, antimalarials and antiretrovirals. In all cases, the *in vitro* efficacy of the active compounds was increased, compared to well-known standard drug treatments. In a phase I bio-equivalence study, a Pheroid™-containing combination formulation was compared against the comparative market leader. The results demonstrated that the bioavailability of the active compounds in the Pheroid™ was at least as good but mostly significantly better than that of the comparative medication. In addition, the incidence of side-effects was decreased in the case of the Pheroid™ formulations. Furthermore, *in vitro* results indicate that drug resistance can at least partially be negated. Pheroid™ technology may also be capable of protecting labile drugs such as peptides against degradation and increasing efficacy so that lower dosages can be administered less frequently and with fewer side effects.

Based on *in vitro* and *in vivo* results, a number of products are currently in development. The application of Pheroid™ technology is potentially limitless and includes such areas as TB, malaria, cancer, AIDS, gene delivery, vaccines, patented medicines and generics and agriculture.

Keywords: delivery system, Pheroid™ technology, cosmetics, anti-infective, vaccines, peptides, plants.

UITTREKSEL

'n Geneesmiddel kan slegs terapeuties effektief wees indien dit sy teiken in genoegsame hoeveelhede bereik. Die Pheroid™ geneesmiddel afleweringssisteem verhoog die absorpsie van verskillende klasse geneesmiddels en is die kern van die studie. 'n Aantal patente wat handel oor die verskillende toepassings van Pheroid™ tegnologie is in verskillende lande ingedien en is reeds in etlike lande geregistreer. 'n Handelsmerk bestaan rondom Pheroid™ tegnologie, maar die ™-simbool word soms weens tegniese of ander redes weggelaat. Pheroid™ bestaan uit 'n organiese koolstof ruggraat wat opgebou is uit onversadigde vetsure. Syketting interaksies van die vetsure is vir self-emulsifiserende gedrag verantwoordelik. Die vesikels en nanosponsies wat so ontstaan is in staat om hidrofiele, hidrofobe en amfifiliese substansie vas te vang of te verpak vir biomediese en landboukundige toepassings. Die belading, meganiese weerstand, elasticiteit, penetrasie deur biologiese grense soos die vel en selmembrane, grootte en oplosbaarheid van die Pheroid™ kan gemanipuleer word.

Die potensiaal van Pheroid™ tegnologie vir gebruik op die gebied van entstowwe, peptied geneesmiddels, topiese produkte en kosmetiek, antimikrobiële middels en landboukundige middels is ondersoek. Op al die gebiede het die Pheroid™ wel bruikbaar blyk te wees: die resultate dui op verbeterde absorpsie en doeltreffendheid van die chemiese en/of biologiese middels onafhanklik van die toedieningsroete vir die Pheroid™-verpakte substansie of middel. Vir mondelinge toediening is 'n voorloper Pheroid™, die pro-Pheroid™, ontwikkel.

Konsep studies oor die *in vivo* absorpsie en biobeskikbaarheid, asook *in vitro* effektiwiteit van Pheroid™-gebaseerde formulasies van tuberkulose middels, anti-malaria middels en antiretrovirale middels is ondersoek. In alle gevalle het die *in vitro* doeltreffendheid van die geneesmiddels verhoog in vergelyking met standaard behandeling. 'n Pheroid™-gebaseerde kombinasie formule van tuberkulose middels is met die vergelykbare markleier in 'n fase 1 bio-ekwivalensie proef vergelyk. Die resultate wys dat die Pheroid™ net so goed maar in meeste gevalle beduidend beter as die markleier gevaar het. Daarby het die voorkoms van nuwe effekte in die geval van die Pheroid™ behandeling verlaag. *In vitro* resultate dui ook daarop dat geneesmiddel weerstandbiedendheid minstens gedeeltelik opgehef kan word. Die tegnologie mag ook bydra in die beskerming teen degradering van labiele geneesmiddels, soos peptiede.

Gebaseer op *in vitro* en *in vivo* resultate is 'n aantal produkte tans onder ontwikkeling. Die toepassing van Pheroid™ tegnologie is potensieel onbeperk en sluit sodanige gebiede soos tuberkulose, malaria, kanker, VIGS, geen aflewering, entstowwe, generiese medisyne en landbou in.

Sleutelwoorde: aflewering sisteem, Pheroid™ tegnologie, kosmetika, anti-infektief, entstowwe, peptiede, plante.

OUTLINE OF THESIS

This thesis documents the initial development of Pheroid™ technology. The technology started out as a single topical product for the effective relief of psoriasis. The psoriasis product was formulated and commercialized by P.J. Meyer and S. Zall as Exorex or Linotar. An analysis of the product showed that it contained submicron sized stable vesicles. These vesicles were subsequently characterized as a delivery system and trademarked as Pheroid™. It is the potential of this delivery system that is the focus of this study. In the course of the thesis this technology, the particles or vehicles contained within the system and its pro-form, may be referred to as Pheroid™, Pheroid, Pheroids, pro-Pheroid™, pro-Pheroid and pro-Pheroids. In most cases the symbol indicating a trademark, i.e. ™, is used but it is sometimes left out for ease of reading or other technical reasons. All of the work included in this thesis was performed by the applicant, unless specifically stated otherwise.

The use of delivery systems is common in the pharmaceutical industry and as several delivery systems are available, the development of another delivery system may be questioned. In Chapter 1, the problem statement and the motivation for this study are addressed. Some attention is given to the general industrial requirements for the production of pharmaceutical preparations containing delivery systems as inherent ingredient in the preparations. The need for novel customizable drug delivery systems is discussed, in conjunction with the specific objectives of the study.

The need for and type of delivery systems used are closely related to the route of administration of pharmaceutical preparations, the mechanism of absorption and the biological distribution of the absorbed active pharmaceutical ingredients (API's). These aspects and the physical, biological and production factors that influence the characteristics of delivery systems are addressed in Chapter 2.

As background for the development of Pheroid™ technology as a patentable product, Chapter 3 starts out with a discussion of the generation of an idea for a new product or technology and process and the requirements of patenting such an idea or technology. It then addresses the process of product development and identifies the similarities and differences of the various stages of the two processes. The chapter continues with a historical background of Pheroid™ technology. The reasons for and the development of the pro-Pheroid™ are described. An exposition of the ingredients, the parallel processes of manufacturing both Pheroid™ and pro-Pheroid™ and the equipment used in the manufacturing processes are discussed. The analysis required for quality control of the produced formulations is explained, with reference to quality assurance issues and regulatory requirements.

Chapter 4 contains a book chapter that was written on invitation. The chapter was published in the book "Science and Applications of Skin Delivery Systems" in 2008. It concerns the use of Pheroid™ technology in topical and cosmeceutical products and delves into the reasons for the use of specific ingredients of the Pheroid™. A comparison between the Pheroid™ and other systems are made and the unique character of the Pheroid™ system is highlighted. The design and clinical evaluation of a Pheroid™-based and comparative essential oil-based cosmeceutical product are discussed in the book chapter.

An exploration of the potential of Pheroid™ delivery technology in the treatment of infectious diseases is described in Chapter 5. The specific infectious disease chosen as a model for studying this application of the technology is tuberculosis. The causes of the high incidence of an ultimately treatable disease and the need for a new treatment regime for this disease are summarized. A theoretical discussion of the roles of two of the Pheroid™ ingredients, namely α -tocopherol and nitrous oxide, in this specific application is included. An investigation into the formulation, bioavailability and efficacy of the existing anti-tuberculosis drugs rifampicin, isoniazid, ethambutol and pyrazinamide in conjunction with the pro-Pheroid™ is described. Dosage form development and stability analysis are touched upon. The efficacy analyses are based mainly on bacterial efficacy studies and infection studies in an *in vitro* model. The bioavailability evaluation is based on single case volunteer studies and a phase 1 clinical trial, approved by both the South African Medicines Control Council and the South African Medical Association Research Ethical Committee. Studies such as clinical trials of necessity include a number of skilled people working as a team as will be indicated in Chapter 5.

The use of Pheroid™ as an adjuvant able to enhance the efficacy of vaccines is described in Chapter 6 in the following manner: Besides a short chapter summary, the research performed on Pheroid™ as vaccine adjuvant is contained in the complete patent submission to the European Patent Office under the Patent Cooperation Treaty. It is therefore presented as it was published by the World Intellectual Property Organization (WIPO). The vaccine patent has been granted in South Africa. The patent submission includes animal efficacy studies on several vaccine candidates – a rabies vaccine, a hepatitis B vaccine and a diphtheria vaccine. Morbidity and specific immune responses are studied, including systemic and mucosal responses.

In Chapter 7, the use of Pheroid™ technology in the effective administration of peptides is explored. As in Chapter 6, the full patent submission to the European Patent Office under the Patent Cooperation Treaty, and published by WIPO is included after a short summary. While the patent submission focuses on the *per-oral* and nasal administration of insulin, some additional material is included with reference to other model peptides. This patent has been granted in South Africa.

Although not strictly a pharmaceutical application, the use of Pheroid™ technology as a delivery system for plants is investigated in Chapter 8. As in the previous two chapters, the full patent submission to the European Patent Office under the Patent Cooperation Treaty, and published by WIPO is included after a short summary. The patent has been granted in South Africa. Since the arena may be less well known to the reader, a discussion of the applicability of Pheroid™ technology in agriculture is included. The product development, the trials, the design of manufacturing equipment and the legal and regulatory process involved in this chapter were mostly performed outside of the university in my private capacity. It has nevertheless been patented by the NWU as part of its Pheroid™ technology portfolio and forms as much part of unlocking the potential of Pheroid™ technology as any of the other applications described in the preceding chapters. It is therefore included in the thesis. This investigation, and the resultant commercialization of the product, has been one of the most satisfying experiences of my career as a scientist and of my life as a human being.

The last chapter of the thesis, Chapter 9, summarizes the results and proposes future directions and studies. The format of the thesis is not the usual format. The format has been specifically chosen to reflect the exploration of the applications of a unique technology. In the reader's perusal of this thesis, cognizance must be taken that the very nature of patents are conceptual and not analytical: A patent makes the original statement. Furthermore, one of the requirements of a patent is that it should be understandable and repeatable by persons skilled in the art. The granting of a patent is therefore an indirect recognition of the repeatability of the work, while the type of statistical analysis generally used in scientific writings may cloud the original concept of a patent.