

**The conceptual design of a
harvesting device
for obtaining taxanes in a sustainable way**

CH3812
Conceptual Product Design

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Preface

This report is written for the course 'Conceptual Product Design', CH3811, in the masters program of Chemical Engineering at the TU Delft. In a team of five students is worked for three months on the design of a device to harvest taxanes from yew trees. In this report the results of this three months during project are presented. The time was spent for coming up with a suitable product for the project, finding background information, making a basis of design and finally working out a conceptual design technically as well as economically. Hard work was necessary, but the weeks passed by quickly since most of the time we enjoyed working together on the project.

During the design process help was obtained from numerous people of whom we are very grateful. Help is received for many different aspects, which made the project better in some way. Therefore, we would like to thank the following people for their cooperation and support.

Each other	For good participation, good times and a nice working experience
Peter Appel	For guidance during the whole project, for being critical and helpful
Pieter Swinkels	For helping us with using our creativity, improving the cooperation among each other and focussing on the parts that were hardest for us
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Leendert Maat	For providing us with an article in which the main answers about the origin of the transport mechanism of taxanes should have been given
Rein Roos	For providing us with background information about electric fields related to natural occurring phenomena like thunderstorms

Further we would like to thank all the people who were so kind to help us in other ways.

Summary

Due to the increasing demand for the anti-cancer medicine Taxol and derivatives of this generic drug, there is a global shortage of taxanes, the raw ~~the~~ material of this pharmaceutical product. The aim of this project was to develop a product that could fill the gap between resources and demand. The product is a device that with the technique of electrospraying can harvest taxanes from yew trees. Our potential clients are yew tree plantation owners who can obtain extra amounts of taxanes by using our device on top of the taxanes that are obtained by clipping the trees. The design methodology for the project, based on chemical engineering, industrial design and sustainability design methods is devised. This is followed by thorough literature research, explanation of the design and economic evaluation.

The taxane milking machine is a movable device that consists of a row of horizontal plates that can be inserted in a hedge of trees. The plates are copper electrodes covered with a polyamide-imide insulator. The top insulator layer is a few millimetres thick which prevents short-circuiting and at the same time allows the electric field to pass through. The needles try to reduce the potential difference between the needles and electrode by spraying sap out the needle which contains taxanes. The extract is gathered on the insulator plate and this is periodically rinsed with ethanol and collected.

The conclusion of our design is that the product is not ready for the market. Potential users will make no profit with the product due to the long spraying times that are required. The speed of the milking has to be increased with a factor of 15 to make it an economically feasible product. The negative outcome is partially due to the lack of information about this subject, and therefore it is strongly recommended that more research will be performed regarding the transport mechanism of taxanes inside the needle before more steps towards commercialization of this product are considered.

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1 Introduction

It has been known for many ages that plant and tree saps contain chemical compounds, such as natural insecticides or medicines that can be interesting for mankind. The pain relieving properties of the natural occurring precursor to aspirin in the bark of willow trees was already known by the ancient Greek culture [1]. In recent decades, many more naturally occurring compounds with medicinal properties have been found. One of the most important ones is the compound paclitaxel (brand name Taxol), which was discovered in 1967, and can be found in certain species of yew trees [2]. Paclitaxel is a member of the taxane family, which is a group of diterpenoids. This compound is poisonous, but it was found that it could be used against breast and ovarian cancer because it stops the division of cells (mitosis), and thus inhibits the growth of cancer tumours. Because cancer is increasingly common under the human population (worldwide, about 1.05 million people are diagnosed with breast cancer and 192,000 people are diagnosed with ovarian cancer every year)[3], the demand for paclitaxel will most likely increase in the future.

From 1967 up to 1990 paclitaxel was harvested by cutting down Pacific yew trees (*Taxus Brevifolia*), because the bark of the Pacific yew contains the largest amount of paclitaxel [2]. The Pacific yew is now almost extinct, and therefore it is not allowed anymore to cut the tree. Currently, other means to get paclitaxel are practised. One of these methods is extraction of precursors of the medicine, which are retrieved by removing needles from other yew tree species, like the European yew (*Taxus Baccata*), which have a high content of precursors in the needles (Figure 1). After extraction the desired paclitaxel, or docetaxel, which is a similar compound with an even higher activity than paclitaxel, can be synthesized from these precursors [4]. Although the tree will stay alive and will regenerate its needles (the shoots that were removed will grow back), the extraction process is laborious and not very efficient [5]. Another way of obtaining paclitaxel is using cell cultures of yew trees or fungi that produce paclitaxel. Finally, there is the total synthesis; the total chemical synthesis has many steps, because the paclitaxel or docetaxel is completely synthesized by chemical reactions [6].



Figure 1 Stem with needles of a yew tree

Even though different techniques exist for the winning of paclitaxel, the yearly production of paclitaxel is still not high enough. Moreover, the production will need to rise in the future, because of the prospect of the rising number of people with cancer every year [3]. It is questionable from where this extra amount of paclitaxel must come, since the current methods cannot guarantee to answer

this increase in demand. Therefore the goal of this project is to make a conceptual design of a new device for the winning of paclitaxel and its precursors in a sustainable way (i.e. environmentally as well as economically), which can work beside the existing methods in order to increase the amount of medicine that can be produced every year.

The approach to this design problem was the following: First a design methodology for the project was devised, which is based on chemical engineering design methods, as well as industrial design and sustainability methods. Then a literature research was performed on the biological background of taxanes and yew trees and on existing methods for winning taxanes. After that new methods were devised, and one of these methods was chosen for the new design. The following objectives and selection criteria for the taxane collection method were formulated:

- Achieving a constant production of taxanes from an inexhaustible source
- Achieving a high purity of taxanes
- The price of the harvested taxanes is comparable or lower than with current methods
- The harvesting is safe for the people working with the method, as well as for the environment
- The harvesting method should not harm the trees

After the method was chosen, the design methodology was followed to come up with a conceptual design. Of this design, the technical aspects, the market opportunities and the economical aspects are discussed. Finally the design process itself is evaluated.

2 Design methodology

In this chapter the design methodology that is used for the product design will be devised. It is important to have a consistent design methodology because of two reasons. Firstly, it will be clear how the final product design was designed and in case of errors the design steps can be followed again to correct them. Furthermore, it will also be clear to people outside our group how the design was made. They can retrace the design steps that were taken and in such a way understand why certain choices were made and how all the elements of the final design were chosen.

Consequently, the design methodology is an important part of the design process, because it gives direction to the design and structures it in an understandable way.

In order to come to a good product design a design methodology consisting of four steps is devised. The formulated design methodology is a combination of several methods, tools, and design rules, namely:

- The four step chemical product design method of Cussler and Moggridge [7]
- The house of quality (quality function deployment) [8],[9]
- The design cycle, creativity tools, and selection tools used by industrial designers [8]
- The cradle to cradle design method, which includes rules and tools to design (chemical) products in a sustainable way [10]

The several parts of the different methods are integrated into one consistent and concise method that can be applied to our project (see Figure 3 at the end of the chapter). First the main characteristics of the different design methods, tools and rules used in our design method will be explained in Section 2.1. Then the design method for our CPD project will be pictured (Section 2.2).

2.1 Main characteristics of the design methods

2.1.1 *Chemical product design methods and the house of quality*

The book of Cussler and Moggridge [7] was specially written for the design of chemical products. Therefore the main points of their book will be used in our design method. The most important aspects that are addressed in the book are the needs of the customer that buys the product, how chemical product ideas can be generated and selected in a constructive way, and how chemical products are manufactured. Finally, there is a chapter on basic economic concerns that have to be taken into account.

Customer needs

In the chapter on customer needs the method to identify customer needs is explained. The customer needs are really important because they trigger the direction in which a product has to be developed. The customer requirements together with technical product requirements define the properties and specifications of the final product.

There are three steps that should be taken in identifying customer needs; interviewing customers, interpreting their expressed needs, and translating the needs into product specifications. Translating customer needs into product specifications means that general wishes of the customer on how a product should perform are translated into design parameters like viscosity of the product, weight, size, etcetera.

Implementing the house of quality in Chemical Product design method

The house of quality (properly called Quality Function Deployment)[8],[9] is a specific tool that can help with translating the needs of customers into product specifications and what the technical parts are that designers have to focus on during design. This method is not explained in the book of Cussler and Moggridge but it is consistent with their method of identifying customer needs and translating them in product specifications. The product requirements will probably contain some contradicting requirements that need to be resolved in the final design of the product. If the new design resolves the contradicting requirements of the product, this will be a competitive advantage of the final product.

Divergence and convergence: ideas generation and selection

The most important chapters of the Cussler and Moggridge book are about ideas generation and selection. One of the most important messages of the chapters is that the chances of being successful in finding a good product idea are much higher if there are many ideas, which can be screened in a structured way.

The design method in total should be consistent in the sense that a common format of approaching a design problem should be applied. In our design method an industrial design tool is used, which is called the design cycle. For the divergent step of generating ideas and for the convergent step of ideas selection several creativity and industrial design tools will be used. The divergent tools that are used are mind mapping and morphological charts. These tools are all described in detail in Section 2.1.2 below.

In the convergence step it is important to organise ideas in categories and choosing the most important factors by which the product can be evaluated, e.g. scientific maturity, engineering ease, minimum risk, low cost, safety, low environmental impact. Subjective judgements on the evaluation factors can be made in a consistent way when a complete list of independent criteria is used, which are defined tightly. For this purpose general selection tools like a SWOT-analysis and Harris profiles are used, which will also be described below. General and subjective idea selection strategies will sometimes not help because detailed chemical and engineering information is needed. In that case engineering tools like thermodynamics, kinetics, and transport phenomena are used to assess the quality of an idea or design.

The final result after the step of idea selection is a product description with preliminary product specifications.

Product specifications and manufacturing

After the selection of the best idea three important aspects of product manufacturing and product specification have to be addressed: the first consideration is whether to seek patent protection or not, because patents can bring about a competitive advantage in the sense that they give an exclusive licence to market the product. This will result in less competition, higher selling prices and quicker recovery of development costs. The second aspect is to find missing information that is required to finalise the product specifications. Finally the manufacture method itself should be considered, which builds mainly on chemical engineering tools like thermodynamics, reaction kinetics, and transport phenomena.

Steps in chemical product design methods

Thus from the above it becomes clear that the chemical product design method consists of four steps: (1) identifying consumer requirements, (2) divergence: ideas generation, (3) convergence: idea selection, and (4) the manufacturing process. These are also the four main steps that are considered in our design method (see Figure 3 below). The other design tools and design rules from industrial design and cradle to cradle were placed somewhere in this sequence of steps, as will be explained below.

2.1.2 Industrial product design methods: design cycle, divergence, and convergence tools

There are many design methods and tools that are used during industrial product design. Chosen were those tools that could help in the four steps of the chemical engineering design process. The elegance of the industrial design tools chosen for our design method is the fact that they combine creativity methods with a consistent selection mechanism. Using these tools will bring about a large number of good ideas in the first place, and well structured means of selecting the best ideas from the bulk of ideas.

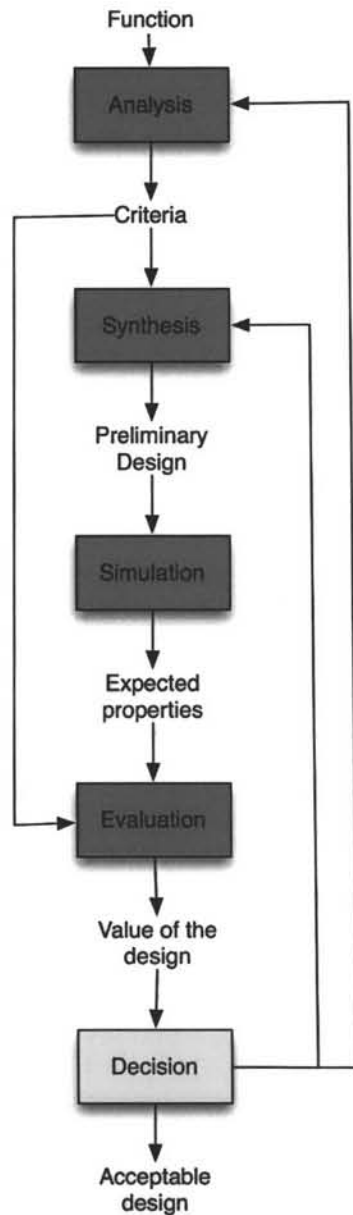


Figure 2 The design cycle, based on [8]

The design cycle

One of the most important design methods that is generally used by industrial designers is the design cycle (see Figure 2) [8]. It is similar to the chemical product design steps, but more fit for the detailed decisions made during the design process. Therefore it is proposed to use the design cycle at every one of the four design steps, every time a function of the product is defined. The reason is that in the design cycle, one applies logic from the goal or function of (a part of) the product to the means, i.e. the design.

Thus, the starting point is always the purpose of a product. Not only the technical purpose is considered, but also the psychological, social, economic and cultural functions that a product needs to have. In the cradle to cradle design method there is even an additional function of the product defined: the product has to be a nutrient to either the biosphere or 'technosphere'. This will become clear in Section 2.1.3.

The first step in the design cycle is to perform an analysis and getting an overview of the problems around the function of the product. Things that must become clear are what the problem in a certain situation is, what the origin of the problem is, and what possibilities there are of overcoming the problem. A clear list of required product properties (criteria) is also made. This has an overlap with identifying the contradicting requirements in the quality function deployment method.

In the synthesis step a preliminary design is made from several product requirements to get a new holistic design. The next step is to simulate or model the properties of the product, starting from some basic assumptions: from this model conclusions on the real behaviour of the product under certain circumstances can be drawn.

Then, the preliminary design is evaluated. The expected properties are compared with the desired properties (criteria), and it is decided whether the differences are acceptable. If not, one goes back to an earlier step in the design cycle and walks through the cycle again.

The design cycle is very helpful in the sense that it is a structured method, and will always deliver a useful result – either an acceptable design, or the information that the preliminary design is not yet acceptable. It does not tell designers, however, how a preliminary design should be synthesized. It just seems easy to pick a preliminary design and start simulating its behaviour, but it already became clear in Section 2.1.1 that one needs a large amount of ideas to select the best one out of the bulk of ideas.

Divergence methods

There are several divergence methods of which two methods were chosen. The first one is the associative creativity method called mind mapping. Mind mapping is mainly used to generate many ideas, for example about products or manufacturing methods. It is somewhat more structured than simply brainstorming, because a diagram is drawn with items linked to and arranged around a central theme [11]. Mind mapping is a very divergent method, so this method could be used best in the beginning of the design cycle when one is in need of many solutions to the problem.

These ideas can subsequently be structured in a morphological chart [8]. The idea of a morphological chart is to find all theoretical possible solutions to a problem. One searches for elements that are substantial to all solutions (the main parameters or elements), after which the theoretical possible realisations are inventoried. The main issue is to find all the parameters that can be in the final product design, and make a matrix of pictures, which contains all solutions to every element. Every row of the matrix contains a partial function or parameter of the product, while every column contains all the different solutions of the partial functions. Mind mapping can help in finding all these solutions.

Convergence methods

Once many solutions are found, the best ones have to be chosen (convergence). The Harris profile and SWOT-analysis can be used in this stage.

The Harris profile or product profile [8] is a graphical representation of the properties of alternatives in which strong and weak points are clearly presented: a product profile has four scales of performance: -2 (bad score), -1 (moderate), +1 (good), and +2 (very good). Every property of an alternative is scored, and the columns are coloured in (for an example see Chapter 10). For every alternative a profile is made, and a quick overview of the strong and weak points of a design is gained. A plus doesn't compensate for a minus because the properties aren't the same. Instead, one thinks about means to improve a design: it can either be adapted or merged with other alternatives. Finally the best alternative is chosen.

The SWOT-analysis is an extension of the Harris profile: it is a method to identify internal factors – strengths and weaknesses – and external factors – opportunities and threats – that are (un)favourable to a product design [12]. A way to use SWOT is to answer the questions on how the strengths can be used, the weaknesses can be improved, how the opportunities can be exploited and how the threats can be mitigated. This approach is strongly related to marketing strategies of the product.

2.1.3 The cradle to cradle design method: sustainability tools and design rules

One of the objectives that was stated in the introduction was to use the cradle to cradle design method [10] in our product design. Cradle to cradle is an approach to the design of systems in which materials are viewed as nutrients that can perfectly be recycled in either the natural cycles of the biosphere or the cycle of synthetic materials – 'technical nutrients' – in the technosphere. This is the larger picture of the phrase cradle to cradle, which was actually already coined in the 1970's [13]. In the last few years the term was reintroduced, and a design method for using cradle to cradle in (chemical) product and building design was devised in the book of chemist Michael Braungart and the architect William McDonough [10].

The advantages of using the cradle to cradle design method are multiple: there is an emphasis on creating healthy products with only positive instead of negative health effects. The products are designed in such a smart way that positive properties reinforce each other and bring about tremendous savings in costs (e.g. a roof with plants on it lowers degradation of the roof by UV-light and at the same time lowers the amount of water that needs to go to the sewage because it acts as a water buffer).

In the book five steps of cradle to cradle design are explained, as well as some general design principles, from which the best usable design rules are used in our design method.

Applying cradle to cradle

One of the cradle to cradle design rules that is applied in the first step of our design method is to carefully look at the purpose of a product, the function it should perform, and in later steps try to design a product that only performs that purpose without having negative side-effects (see below).

One of the most important design rules is to positively select the ingredients of which a product is made, and how they are combined. The purpose is to make a product healthy and safe. This rule can be implemented in the second step of the design method, where ideas are formulated on how the ingredients could be positively combined in the product.

To be able to have positive ingredients to select from and to combine, the tool of a "passive positive" list should be used, which is a detailed inventory of all the proposed chemicals and materials to be used. For every material the potentially problematic characteristics are identified. Once screened, substances are placed on different lists (problematic, problematic but no substitutes available, and positive/preferred), and substitutions are made where possible. Reinventing is a step further; in this case every part of the product is designed to become food for either biological or technical metabolisms. Several ideas on how to reinvent can be generated during step two of the design method.

Another rule is to respect diversity. This means several things: first of all there is a diversity of needs and desires among potential customers (step one of the design method). Respecting diversity in design means considering not only how a product is made, but also how it is to be used, and by whom (step two). A product should be developed that is fit for many cycles of use. Another aspect of diversity is the local tastes of customers: instead of promoting a "one-size-fits-all aesthetic" the product and packaging could be adapted to local tastes and traditions, without compromising the quality [10]. This is known as 'form follows evolution' and can be used in step three and four of the design method.

A last design rule that can also be used in step three and four is to follow informed personal preferences. This means to make choices on the best information available; if it is suspected that a part of the product will contain harmful materials, use the best alternative you have, even if the information is not complete. Ugly and possibly very environmentally friendly is not the way to go. If it doesn't appeal to you, it will probably not appeal to other people as well.

2.2 Integration for our design method

All the different design methods, tools and rules were successfully integrated and the result can be seen from Figure 3. The middle part of the figure is the most important part; it shows the four steps in chemical product design on which our method hinges. The core of the figure also shows that the design cycle is to be applied in every design step. To the left the most important tools that will be used to conduct the design steps are shown. These are mostly the industrial design and creativity tools, but also some cradle to cradle tools and chemical engineering tools. The right side of the figure shows the most important design rules that should be followed to come to a sustainable design of the product. Of course also some chemical engineering rules should be used, as the final product must comply with the laws of physics and chemistry.

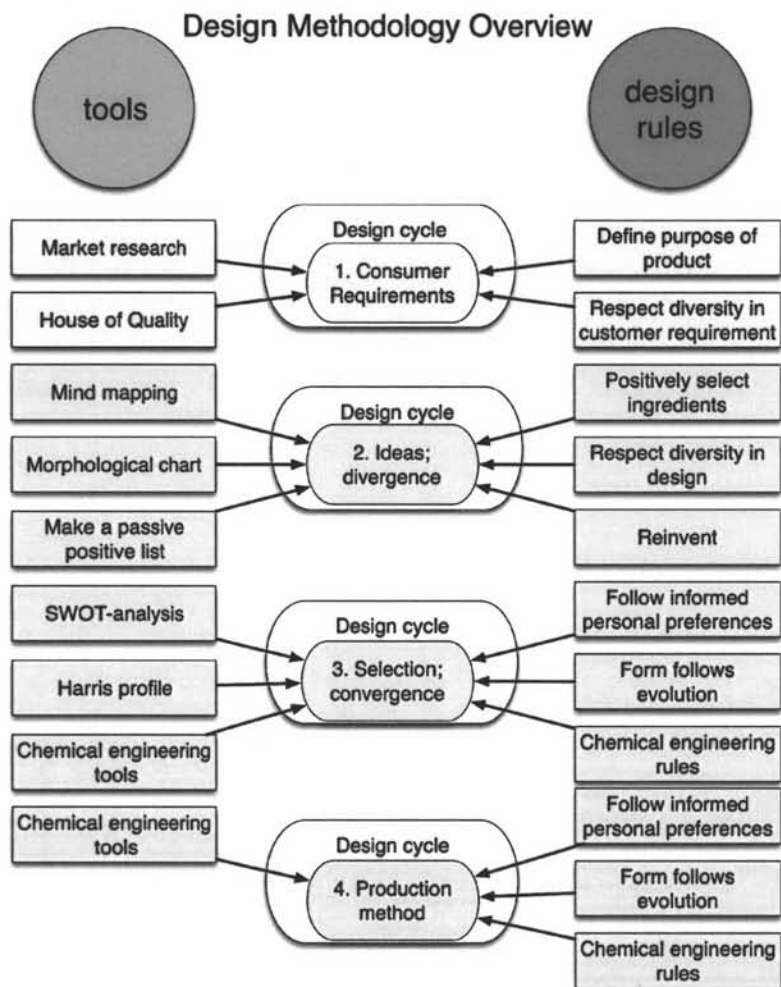


Figure 3 Overview of the design methodology. In the middle the four main steps or phases of the design can be found. At the left side the tools that are to be used at every design step are depicted. At the right the design rules of every design step can be seen.

3 Biological background of the yew tree

The yew tree is a natural source for taxanes, and its cells can also be used for plant cell fermentation, as will be explained in the next chapter. There also exists a total synthesis method for the production of taxanes however yew trees produce taxanes in a more efficient way. Because of this role of the tree, it is important to understand the biological basics of how the tree works. The main subjects that are discussed in this chapter are the yew tree species that exist, the leaf morphology and anatomy – i.e. the external and internal structure of the needles – and finally the chemistry of the secondary metabolites (phytochemistry) and the biochemical functions (physiology).

The information in this chapter will be used in Chapters 4 and 5, where existing methods of getting taxanes will be explained and where new possible methods are made up, keeping in mind the plant physiology and biochemistry. Some biological information will also be useful when explaining the working mechanism of the chosen method, as will become clear in Chapters 5 and 6.

3.1 Yew tree species

The yew tree is part of the genus *Taxus*, which can be divided in eight different yew tree species. These species grow mostly on the northern hemisphere. The species are divided in three main roots, but nowadays there are crossings as well, of which an example is given in Figure 4 (modified from [14]).

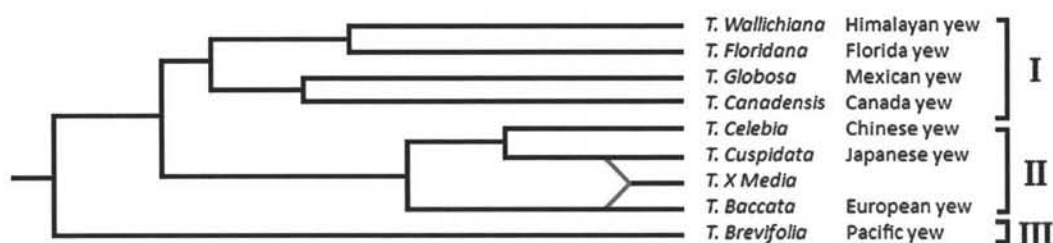


Figure 4 Family tree of Genus *Taxus* [14]

One of the species is *Taxus X Media*, which is a crossing of the European and Japanese yew. All these species have different properties, in the sense that the average needle length may differ, as will the concentration of certain compounds inside the tree, among others. For instance, the *Taxus Brevifolia* has the highest concentrations of paclitaxel in its bark. Some other species hardly produce paclitaxel, but produce compounds similar to it (precursors), like baccatin III or 10-deacetylbaccatin III. The *Taxus Baccata*, for example, is one of these species. The highest concentrations of these products can be found in its needles, not in the bark.

3.2 Plant morphology and anatomy

In this section on yew morphology and anatomy the focus lies on the needles, because one of the objectives stated in the introduction was that the method of obtaining the taxanes should not kill the tree. Therefore, harvesting from the bark of the tree is impossible, while getting the taxanes from the needle will not necessarily harm the tree. In some yew species the concentration of taxanes is highest in the needles, as can be seen from Table 1 in Section 3.3.

A yew needle can grow to approximately 25 mm in length and 2 mm in width [15]. A cross section of a yew needle can be seen from Figure 5; the width of the needle in this picture is approximately 2 mm. In the middle of the needle the vascular bundle can be seen with the phloem and xylem parts. The yew tree is part of the family of vascular plants or higher plants; higher plants have a vascular tissue, which is used for the transport of water, nutrients and metabolites in the plant. The transport of water and soluble inorganic nutrients to the leaves of the tree takes place via the xylem. The organic material is transported via the phloem, although the xylem can also contain some organic compounds [16]. The transport in the xylem is mainly forced by transpirational pull; inside the pores of a cell wall water forms a concave meniscus. This means that the water molecules are attracted more by the walls of the pores in the cell wall than by each other. When water evaporates from the surface of the cells in the leaves, the surface of the water retreats into the pores of the cell walls. The surface tension of the water causes a force which is large for water to be transported up the tree [17]. A detailed picture of the anatomy of the leaves can be found in Figure 5. The layer around the needle is called the cuticle. Just inside this layer we find the epidermal cells and in between the spongy parenchyma can be found. On the lower face of the needle, there are small channels to the outside, called stomata.

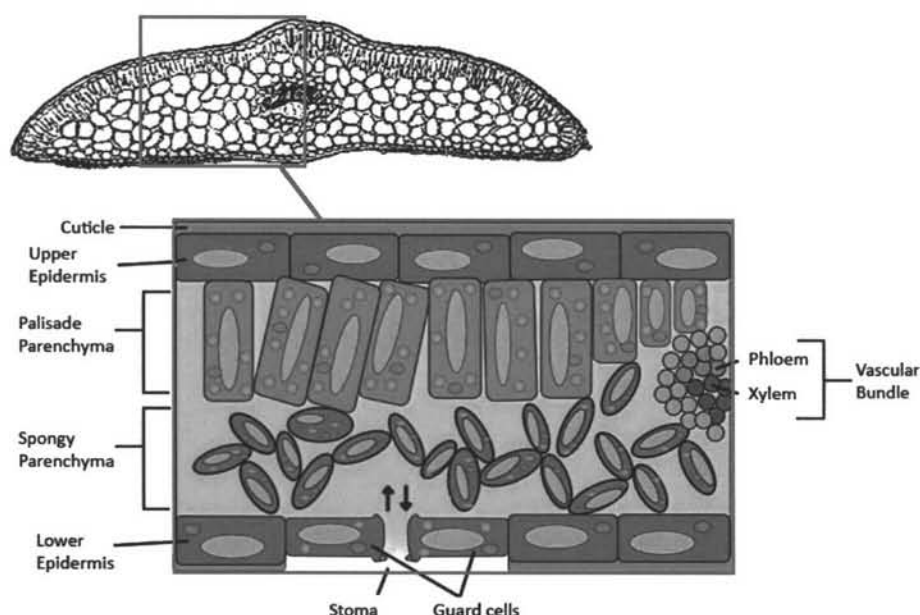


Figure 5 Cross section of a yew needle, with below a schematic representation [15], [18]

The stomata are arranged on the needle parallel to the midrib of the needle (see Figure 6). They are all located on the underside of the needle. The yew tree has less stomata than other trees, namely about 59 stomata/mm² [15]. Stomata are very small; from Figure 6 it can be seen that the diameter of the stoma is approximately 10 microns.

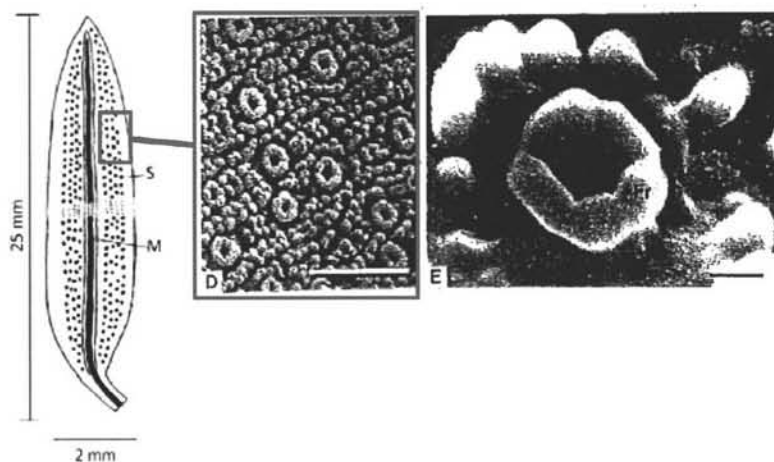


Figure 6 Underside of a yew needle, with a close up picture of stomata (D) and stoma with Florin's ring (E)[15]. S stands for stoma and M for midrib. Barsize D = 100 µm, E = 10 µm.

3.3 Phytochemistry and physiology

After the explanation of the internal and external structure of the needles the biochemistry of the plant will now be explained, including the biochemical functions (physiology), the biological pathway, and structural formulas of a few taxanes.

3.3.1 *Paclitaxel and its precursors*

Paclitaxel and the other taxanes produced by the yew tree family are terpenoid compounds, which are secondary metabolic products. That means that they are not necessary for the survival of the cell itself, however they can be useful for the plant as a whole. Frequently, the secondary metabolic products are used for communication between cells [19]. The plant can also communicate with the environment by means of secondary products, such as by pigments or aromas. The compounds are also sometimes used as reservoir for nutrients. Often secondary metabolic products of plants are highly toxic so they can be used as a defence mechanism against animals (e.g. insecticides), which is the case for the yew trees [19].

The structures of the most important taxanes are given in the following figures; from Figure 7, the molecular structures of paclitaxel and docetaxel (the actual medicinal compounds) can be seen. In Figure 8 the molecular structure of baccatin III, a precursor of paclitaxel and docetaxel, is shown and in Figure 9 the molecular structure of 10-deacetylbaccatin III (10-DAB III), another precursor of paclitaxel and docetaxel is given.

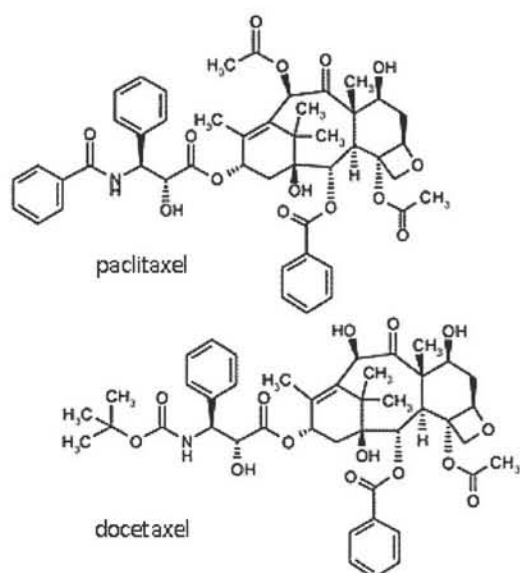


Figure 7 Molecular structures of paclitaxel and docetaxel

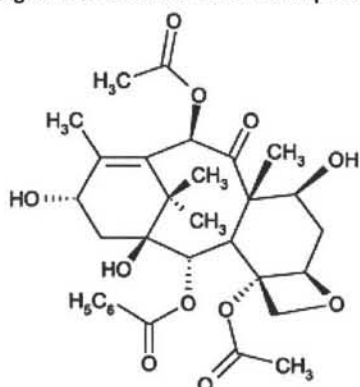


Figure 8 Molecular structure of baccatin III

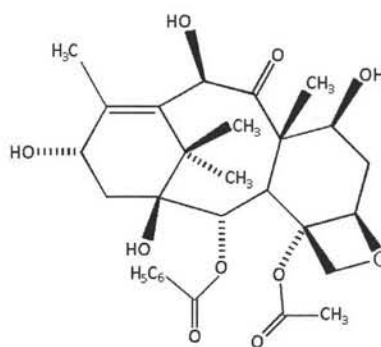


Figure 9 Molecular structure of 10-DAB III

It is evident that in all the structures a complex of rings is present, which is characteristic for diterpenoids. Terpenoids are organic structures that occur in nature, and they consist of five-carbon isoprene units. Diterpenoids consist of four of these isoprene units; hence their basic structure consists of 20 C-atoms [20]. The structure of baccatin III is practically the basic structure of the taxanes and has the structure of 20 C-atoms, with 3 acetyl side groups. Other taxanes can be created by substituting different side groups to this ring complex, as is visible for paclitaxel, docetaxel and 10-DAB III. Baccatin III itself is not active as a medicine against cancer, but adding side chains to create paclitaxel or docetaxel makes the structure active. Taxanes are very lipophilic structures, which results in the fact that they are hardly soluble in aqueous liquids [21].

Taxanes find their origin in 2 precursors; farnesyl diphosphate (combination of 3 isoprene units) and isopentenyl diphosphate (combination of 1 isoprene unit). When geranylgeranyl diphosphate synthase is added, the precursors are put together. After a ring-closing step, the basic structure of the taxanes is already visible (see Figure 10) [22].

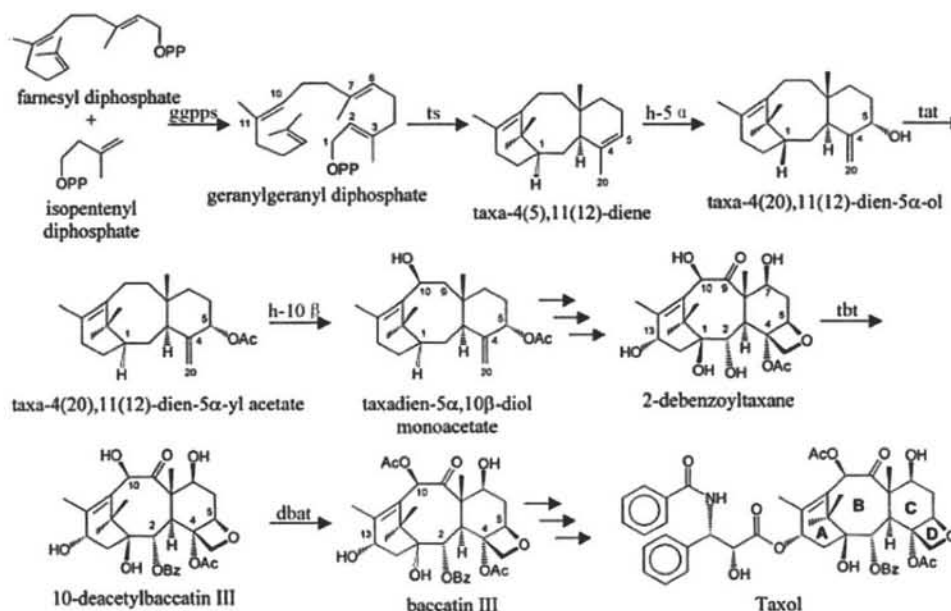


Figure 10 Biosynthetic pathway of Taxol; ggpps: geranylgeranyl diphosphate synthase, ts: taxadiene synthase, h-5 α : cytochrome P450 taxadiene 5 α -hydroxylase, tat: taxa-4(20), 11(12)-dien-5 α -ol-O-acetyltransferase, h-10 β : cytochrome P450 taxane 10 β -hydroxylase, tbt: taxane 2 α -O-benzoyltransferase, dbat: 10-deacetylbaccatin III-10-O-acetyltransferase. Multiple arrows indicate several as yet undefined steps. [22]

3.3.2 Storage and transport of taxanes

The taxanes are stored in the vacuoles of the cells and a small fraction is also present in the extracellular medium [23]. A picture of a plant cell is depicted in Figure 11 below, where a vacuole can be seen. The taxane molecules are very large and will not be able to diffuse through the cell membranes and vacuoles. For the transport of taxanes into the vacuole, a different mechanism is needed. The transport can be divided into two events; uptake of the alkaloid at the plasma membrane and transport from the cytosol into the vacuole. Several other kinds of compounds - especially a lot is known about alkaloids, which are nitrogen based secondary metabolites - appear to be transported across the cell membrane by ATP-binding cassette (ABC) transporters, which use ATP to transport the secondary metabolites across transport channels in the cell membrane. It is suspected that the transport mechanism is similar for diterpenoids [24]. In the second transport step into the vacuole it could be the case that an H⁺ antiporter is responsible for the transport from the cytoplasm to the vacuole. This means that the transport towards the inside of the vacuole is dependent on the proton gradient between the vacuole and cytoplasm.

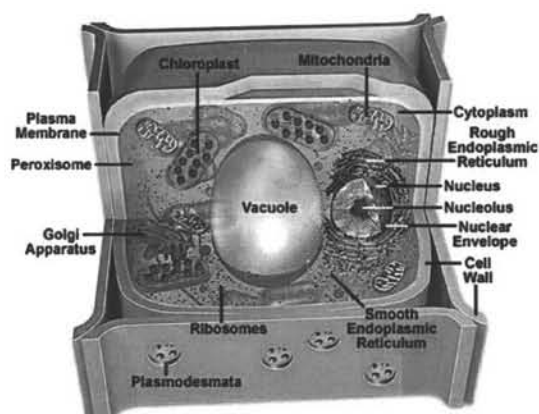


Figure 11 Plant cell with vacuole [25]

3.3.3 Content of taxanes in different parts of the tree

It was already said that the taxanes are most likely stored in parts of the tree that need to be protected from damage of insects or other animals. This counts for all taxanes that are toxic to these animals. There exist many types of taxanes, and the concentrations of these compounds differ per species and per part of the tree. In Table 1 and Table 2 below, the content of Taxol and other taxanes in different parts of the tree is shown. The data is based on experiments [2],[26]. These concentration measurements were done for different *Taxus* cultivars, and on different locations in the trees, so the different species and tissues can be compared.

Table 1 Weight percentage of Taxol in various parts of *Taxus* species [2]

Species	Plant Material	No. of Samples	Samplings Analyzed	Avg.% Taxol	Range of%
<i>Taxus Baccata</i>	Stem	1	2	0.001	0.0009-0.001
	Twig	2	4	0.0006	0.0004-0.0009
	Leaf	1	2	0.003	0.003
<i>Taxus X. Media</i>	Stem	1	2	0.002	0.002
	Twig	2	4	0.009	0.009
	Leaf	1	2	0.002	0.002
<i>Taxus Cuspidata</i>	Twig	1	2	0.0006	0.0002-0.0009

Table 1 shows that for *Taxus Baccata* the highest concentration of Taxol (paclitaxel) is found in the needles of the tree [2].

Rozendaal et al. [26] examined the concentrations of different taxanes in the needles of different *Taxus* cultivars. In Table 2 the results are presented. The different taxanes are paclitaxel (1), 10-deacetylpaclitaxel (2), cephalomannine (3), baccatin III (4), 10-deacetylbaccatin III (5) and brevifolol (6) [26].

Table 2 Average taxane concentration in different Taxaceae species [26]

Taxaceae Species	No. of Samples	Average taxane concentration (µg/g dried needles)						
		(1)	(2)	(3)	(4)	(5)	(6)	Total
<i>T. Baccata</i>	49	41	198	22	14	762	3	1039
<i>T. Baccata cv.</i>	473	63	179	42	10	468	6	768
<i>T. Brevifolia</i>	1	130	0	0	296	41	9132	9599
<i>T. Canadensis</i>	2	285	253	289	224	2665	77	3793
<i>T. Celebica</i>	6	26	81	0	0	70	46	223
<i>T. Cuspidata</i>	10	105	113	40	15	120	6	399
<i>T. Cuspidate cv.</i>	60	136	198	93	18	116	1	562
<i>T. Floridana</i>	1	516	515	0	0	1689	0	2720
<i>T. Globosa</i>	1	433	229	480	168	1395	0	2705
<i>T. X. Hunnewelliana</i>	9	41	100	0	0	63	0	204
<i>T. X. Media cv.</i>	108	211	205	131	36	230	6	819
<i>T. Wallichiana</i>	1	272	420	0	0	1092	0	1784

From Table 2 it is clear that the species *T. Brevifolia*, *T. Canadensis*, *T. Floridana*, *T. Globosa*, *T. Wallichiana* and *T. Baccata* contain the most taxanes in their needles. However, not only the species influence the amount of taxanes in the needles. This also depends on the month, season, soil and climate with which the tree has got to do. It appears that yew trees hibernate in the winter: In the months November to March there is stagnation of the liquid streams. The stomata close and there is less circulation of the liquids [27]. Plant life has an inside biological clock; yew trees are light sensitive and thus 'know' when it is day or night, and being able to distinguish the difference between day and night, they notice the length of the days. Depending on the length of the days, the tree goes in hibernation or blooms. When the tree is in hibernation, the production of secondary metabolites is slowed down drastically compared to when the tree is blooming [28]. The composition and yield differ per tree and soil (hence location). A tree in France can have totally different seasonal fluctuations compared to a genetically identical tree in Ireland [29]. According to Drs. W.M.J. Ursem [30], the highest yields of the 70 years old *Taxus Baccata* in the Botanical garden of the TU Delft can be obtained at the end of June and at the end of September.

3.3.4 Other compounds

Not only taxanes and their precursors (like farnesyl diphosphate and isopentenyl diphosphate) are present in the tree. The needles of the yew tree consist for about 65% of water [31]. Water can be excreted as transpirational fluid. In a hot summer day water evaporates out of the needles. An extract of *Taxus Baccata* needles was analysed by RP-HPLC by Theodoridis et al [32]. Besides familiar taxanes, like baccatin III and 10-DAB III, more than 18 taxines and cinnamates were detected. It was found that the fluid in the needles is a mixture of about 21 alkaloids of which 10 were structurally analyzed. There were 11 unknown taxanes and 3 unknown substances in the mix. The results can be found in Appendix A [32].

4 Existing methods to get Taxol

In 1967 paclitaxel is discovered as mitotic inhibitor and hence an effective active ingredient against cancer. Up to the 90's it was obtained from the bark of the Pacific yew tree [33], which required killing of the tree. As a result, the trees are almost extinct and are nowadays protected against being cut. Therefore, several other methods are developed and applied to get paclitaxel, and its precursors from which paclitaxel can be synthesized easily.

In order to meet the objectives, to design a device for the winning of taxanes in a sustainable way, it is essential to have a good understanding about the existing methods. This is important to compare the pros and cons of different methods, and finally with our newly proposed design.

It should be noted here that "obtaining" or "winning" paclitaxel refers to either getting paclitaxel from biomass or conversion of starting material into paclitaxel. The different methods to obtain paclitaxel can be divided into three categories: namely extraction from bark or needles, cell cultures and synthesis routes.

4.1 Extraction from bark or needles

Paclitaxel and its precursors are present naturally in bark, needle and other parts of yew trees. However the natural concentration of taxanes is very low, 0.01-0.05% of dry weight dependent on the yew species [33]. Bark and needles are freeze dried and grinded. The resultant powder is treated with various chemicals for extraction. After extraction, semi synthesis of the mixture to paclitaxel can be performed.

4.1.1 Extraction with chemical solvent

An extraction and purification process is developed whereby paclitaxel can be retrieved with a purity of 99.5% [5],[34]. Together with paclitaxel, its precursors like baccatin III and 10-deacetyl baccatin III (10-DAB) can be obtained in a similar way. The precursor will later be converted to paclitaxel or docetaxel through synthetic routes. The total extraction route from grinded needles to 99.5% pure paclitaxel consists of four main steps, namely extraction, adsorbent treatment, precipitation and HPLC (high performance liquid chromatography). The overall yield in this process is approximately 53.9%, in which yield is defined as the amount of paclitaxel before, divided by the amount of paclitaxel after the process step(s). The purities and yields of the different steps can be found in Table 3.

The extraction step makes sure that the taxanes which are present in the needles of the yew trees are separated from its biomass. The taxanes go from the solid phase into a liquid phase, after which purification steps can be applied. The process consists of two major steps, after which a dried extract is obtained with a yield of 98% and a purity of 6.3% [5]:

1. Extraction with methanol for four times. This is to remove the taxane mixture from the powder. The optimal extraction time per step is about 10 minutes, since there is no significant yield increase by longer times (see Figure 12)[5],[34]. After the four extraction steps the resulting taxane mixtures are collected together and concentrated before going to the next step.

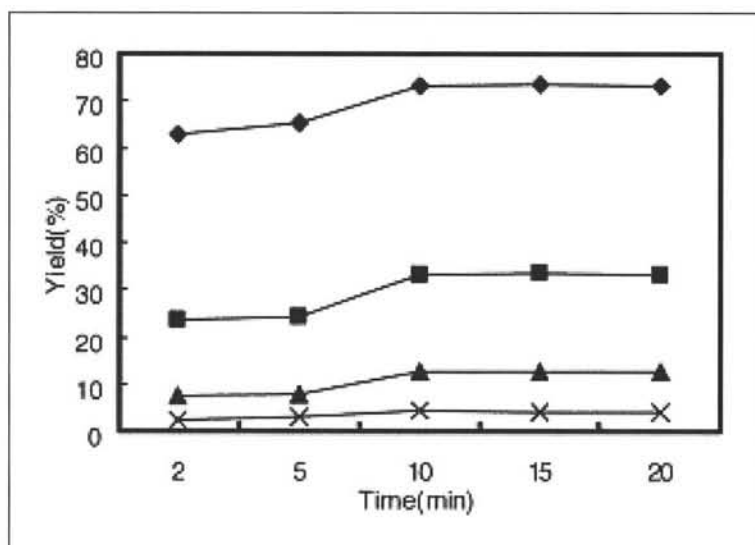


Figure 12 Effect of extraction times on paclitaxel recovery from biomass: first extraction (♦); second extraction (■); third extraction (▲); fourth extraction (X) [5]

2. Liquid-liquid extraction with methylene chloride (also called dichloromethane, DCM) for three times. This step removes most of the polar impurities. The crude extract is dried at reduced pressure by evaporation of the solvent.

The adsorbent treatment process is put after the first main step. Adsorbent treatment makes sure that impurities like tars and waxes, coloured compounds and compounds which are insoluble in DCM are removed. Synthetic adsorbents, like active clay and activated carbon, are used to remove these impurities from the crude extract. The mixture is then filtered to separate the purer extract from the adsorbent with impurities. Purities between 7.1% and 9.6% and step yields of 94.9% up to 98.9% can be obtained, dependent on the adsorbent material and the filtration rate [5].

The third step is precipitation of the taxanes. This removes phenolic compounds, like catechin, and non polar compounds. This step consists of two steps as well. First there is a precipitation using hexane. After that the precipitate is dissolved again in methanol for the 2nd precipitation. This second precipitation uses the difference of solubility of the different compounds. The purity after precipitation is 61.2% and the step yields are 95.2% for the first step and 81.3% for the second step [5].

After precipitation HPLC is used to remove other taxanes from the paclitaxel, to yield purer paclitaxel. First the precipitate is led down a hydrophobic resin column, e.g. ODS (octadecylsilylated C18), in which the elution fluid consists of water and methanol. A purity of 95% can be achieved, but the impurities which are left remain in the solid. Therefore the product is led through a second column; a silica column. This column uses isocratic elution with methanol and DCM. Isocratic elution is the "use of a constant-composition mobile phase in liquid chromatography" [35]. This can reach a purity of 99.5% [5]. Compounds similar to paclitaxel, like cephalomannine, are hard to remove, even by this technique. If the purity must be higher, a crystallisation step is needed.

Table 3 Purities and yields of extraction process [5]

	Paclitaxel (g)	Purity (%)	Step Yield (%)	Overall Yield (%)
Biomass	4.92		100	100
First extraction	4.88	0.6	99.2	99.2
Second extraction	4.83	6.3	99	98.2
Adsorbent treatment	4.62	8.8	95.6	93.9
First precipitation	4.40	27.1	95.2	89.4
Second precipitation	3.58	61.2	81.3	72.8
ODS-HPLC	3.06	95	85.5	62.2
Silica-HPLC	2.65	99.5	86.6	53.9

An advantage of the solvent extraction is that the technologies which are used are already mature and the step efficiencies are high. However, there are drawbacks as well. Since there are so many steps in the process, the overall yield is only 53.9%, which means that only about half the amount of paclitaxel is recovered. Another major drawback is the use of chemicals; for instance, methylene chloride is a suspected carcinogen. It seems counter effective that a medicine against cancer is retrieved using a substance that causes cancer. Other used chemicals, like methanol and hexane, have negative influence on the health as well, and large amounts of these chemicals are used in the process. Another drawback is the dependence on nature; needles of yew trees are needed and if too much needles are cut from the tree, it may be harmed. If, however, too little needles are cut from the tree, there may not be enough raw material for the process demand [5],[35].

4.1.2 Extraction using Supercritical Fluids

Alternative, supercritical fluid (SCF) can be used. Dried needles or bark are grinded to 700 µm [36]. The resulting powder is treated with n-hexane to remove waxy compounds and some other non-polar compounds to prevent clogging of the collection tubing under treatment of CO₂. After this treatment, the extraction is performed with super critical CO₂. Co-solvents (mainly small alcohols mixed with water) are often added to enhance the selectivity and yield towards paclitaxel and its precursors (Table 4). This effect is due to the fact that a co-solvent can help to release the taxanes from the matrix in the source material, since the taxanes have a naturally higher solvability in these substances [36].

Table 4 Taxol yields extracted depending on various compositions of co-solvents in the needles of *T. Cuspidate* (80°C, 350 bars) [36]

Extraction Method				Yields of extracted Taxol [µg/g]
Methanol Extraction				200
Supercritical fluid extraction: Composition of cosolvent (% v/v)				
CO ₂	Methanol	Ethanol	Water	
100	-	-	-	54
90	10	-	-	72
80	20	-	-	189
90	-	-	10	25
80	-	-	20	98
90	-	10	-	119
80	-	20	-	158
90	5	-	5	208
80	10	-	10	203
90	-	5	5	242
80	-	10	10	336

An immediate advantage of super critical extraction, compared to liquid extraction is that fewer chemicals are used. However, still, chlorinated and some potentially toxic compounds are used. Furthermore, high pressure of 100-300 bar and strict temperature control are required to maintain the critical state of the fluid, which are expensive and not altogether safe [36]. This would inevitably increase the cost. Finally, the selectivity towards precursors of paclitaxel is not very high (0.2% for paclitaxel and 0.7% for baccatin III) in comparison with of chemical extraction (2.4%) [36].

4.2 Cell cultures

Extraction of taxanes from bark or needles shows a dependence on natural resources. Due to the low Taxol content in yew tree, cell cultures become an attractive source for paclitaxel production. This can be done through either growth of plant cells or micro-organisms, which produce Taxol as a by-product.

4.2.1 Plant Cell Fermentation (PCF)

Currently BMS (Bristol-Meyers Squibb) obtains their raw material through plant cell fermentation (PCF) technology, developed by a German biotech company, Phyton [37]. PCF makes use of a particular *Taxus* cell line growing in aqueous medium. The winning of the taxanes produced by the cells can be done by direct extraction (i.e. without a drying and grinding step), of which the method is similar to that described for chemical extraction from the bark or needles [38]. The yield of the paclitaxel in cell cultures is about ten times higher than the yield in the bark of the yew tree. For example, a yield of 0.119% is obtained in a medium with a cell culture of *T. Yunnanensis* [39]. Furthermore, the production of taxanes in the cells may be enhanced by the addition of methyl jasmonate or by the application of ultrasound [23].

The apparent advantages of this technique, compared to the extraction from bark or needles, are that PCF saves the steps of freeze drying and grinding, and that a reduced amount of energy is required. The high yield is rather promising as well, but problems occur by scaling up the process. The cells get easily overgrown and will die; therefore no large scale industrial applications of this technique are known [40].

4.2.2 Fungus

In 1993, an endophytic fungus, *T. andeanae*, was found to be Taxol-producing. It is isolated from the outer bark of *T. Brevifolia*. After that, several other fungi were identified to be able to produce Taxol as well, among which the fungus *Nodulisporium Sylviforme*, which lives on the tree without causing apparent damage to it. The concentration of Taxol can be as high as 418 µg/l in culture and 1487 ng/l in fungi secretion [40].

Similarly to PCF, fewer chemicals will be used in obtaining Taxol from the fungi. However, this technique is still at research stage due to several problems. For instance, the yield in the culture medium is too low for the technique to be applied industrially. This yield should be in the mg/l scale, whereas it is now in the µg/l scale. On top of that, the amount of biomass produced is too low. Thirdly, since synthetic pathways, hence the intermediate products, are still unknown it will be difficult to improve the process, i.e. finding optimum conditions for production, find limiting steps and change them [40].

4.3 Synthesis routes

Paclitaxel is present only in minute amounts in biomass of yew trees. Together with increasing environmental concern of biodiversity, a synthetic route became a promising candidate for Taxol production [41]. There are mainly three synthetic pathways depending on the starting material.

4.3.1 Total synthesis

Paclitaxel can be made from available organic chemicals using total synthesis. It was a very attractive alternative around 1990, as scarcity of resources was a big concern. More than 30 groups proposed many different pathways to generate paclitaxel, docetaxel and other Taxol-derivatives [42]. One of these processes consists of 37 steps and has a low yield (2%) [4]. In 1994 the total synthesis of paclitaxel was achieved with fewer steps; however the yield was still very low. The starting material in the process is a petrochemically derived patchoulene oxide, which is easy to obtain. This is treated to obtain a 4-ring structure, after which paclitaxel and its derivatives can be made.

The biggest advantage of this method is that it eliminates the reliance on natural resources. It also opens the way to investigate other Taxol derivatives, which may be potential drugs like docetaxel [43]. However, one major drawback is that it consists of so many steps that the yield is low while the costs are high [44]. Hence total synthesis is more a means to gain more knowledge rather than a technique worth of industrial application.

4.3.2 Semi-synthesis from extracted precursors

Although the amount of paclitaxel is extremely low in yew trees, it can be made from baccatin III through a semi-synthetic pathway [45]. Baccatin III in turn can be prepared using 10-DAB. 10-DAB is present naturally in the European yew (*T. Baccata*) in much higher amounts (up to 1 g/kg) than paclitaxel and baccatin III [46]. The precursors are first extracted and purified to about 95% and then converted to paclitaxel [47]. Before the acetylation of the C-10 hydroxyl group, the C-7 hydroxyl group of 10-DAB is regioselectively protected. This protection can be applied and removed under mild conditions. After the acetylation of the C-10 hydroxyl group, a reaction takes place with a protected (2R,3S)-phenylisoserine side chain, which creates a protected paclitaxel molecule. The protective group is then removed, which results in paclitaxel. The overall yield of this process is approximately 26% [48].

An advantage of this method is that the starting material comes from the needles of the trees. This means that trees are a constant source for the raw chemicals, instead of only once. Since the semi-synthesis is performed after paclitaxel is removed from the mixture, it yields extra paclitaxel from the same amount of taxanes. Furthermore, other Taxol derivatives, for example docetaxel, which is another effective anti-cancer drug, can be produced in a similar way [4]. However reliance on natural resources still continues. The growth of trees in a plantation and subsequently the harvesting of the shootings are required [40]. One of the simplest pathways requires four steps and has a yield of 26% [48]. Besides that, the extraction and purification process are still required and further isolation of paclitaxel from the reaction mixtures is needed.

5 New proposals to get taxanes out of the tree

Currently the most used method for obtaining taxanes is extraction from the needles instead of the bark, since the yew tree is protected against being cut. The precursors are won by removing, and thereafter drying, grinding, and extracting the needles. The content of taxanes in the needles is very low and due to the many processing steps, this process is rather complicated [33].

By chemical extraction or cell culture, yew trees are no longer killed. Nevertheless, plant cells contain numerous other compounds, and steps to remove these chemicals are complicated, energy-intensive and costly [49]. Also, harmful chemicals are used in large quantity [34]. In a new design these disadvantages should be avoided. Therefore a brainstorm session was conducted, of which the detailed results can be found in Appendix B-1. Some of the ideas are partially based on existing methods, but new technologies, which have not yet been used on large scale, are looked into as well.

In order to make a concise decision for the new methods, the objectives stated in Chapter 1 are taken into account. This includes that the taxanes has to be obtained in reasonable amounts with a higher purity than with the existing methods in a sustainable way. It must be more profitable or bring an additional profit compared with the currently used methods.

From the list of ideas, four options remained and will be discussed in more detail. The four options are solvent extraction, supercritical fluid extraction, vacuum suction and electrospray, with which the needles will remain on the tree for all the options. The harvesting principle will be described shortly, including the feasibility, advantages and disadvantages.

5.1 Solvent extraction

Solvent extraction separates different compounds based on their relative solubility in organic and inorganic solvents. It is often combined with other extraction methods, such as expeller/press extraction, to reduce the solvent required for the extraction. With the postulated objectives, taxanes need to be obtained from the needles, leaving the needles on the tree. The use of an expeller is therefore excluded. The use of a solvent might be possible, although it may be necessary that the branches are immersed in the solvent solution to extract the Taxol and its precursors directly from the branch.

Methanol and/or ethanol can dissolve taxanes and they are therefore of interest as solvent. The solubility of taxanes in methanol is higher than in water, about 10 mg/ml compared with 0.01 mg/ml in water, and gives a clear colourless solution [50]. Paclitaxel has a melting point of 213-216°C. It is sensitive to heat and the temperature should be held between 2-8°C to slow down its degradation [51]. Therefore the solvent cannot be removed by increasing the temperature, but it can be removed from the taxanes by vacuum evaporation, because when the pressure is lowered the boiling point is lowered as well. For example, with vacuum evaporation at 0.04 bar a temperature of 5°C is high enough for the methanol to evaporate (see Appendix B-2) [52].

However, it is not clear if methanol can extract the taxanes out the needle, since it is questionable if the methanol gets in contact with the sap. Normally, the opening or closing of the stomata is controlled by turgor pressure, which depends on the amount of water present, however it is not known what the effect of methanol is on the stomata [53]. In addition, the used solvents might harm the needles and trees by suffocation, since the branches need to be immersed. The consequences of applying organic solvents to living systems based on aqueous environments, such as trees, are

harmful. The treatment of organic solvents causes a decrease of shoot length growth or even stops the growth of the tree [54].

Another important fact is that normally the needles are crushed before the extraction takes place. Crushing may facilitate the release of cell content and it creates smaller particles with a higher surface area to volume ratio. In this new concept, there is no opportunity to crush them. It is not known if the extraction can take place equally efficiently. With this given, extraction directly from the branches is not suitable as new method to harvest Taxol from the yew tree.

5.2 Supercritical fluid extraction

A pure supercritical fluid (SCF) refers to a pure compound which possesses the properties of both liquid and gas when temperature and pressure are above its critical point (grey part in Figure 13). It dissolves other compounds like liquid but moves through a medium and transports other materials like gas. Hence SCF is able to extract compounds in a similar way as conventional chemical extraction. During SCF extraction, more volatile components will tend to move into the SCF phase. After that, the SCF, which contains the compounds of interest, is removed from the starting mixture. The SCF can be separated from the target by adjusting the temperature or pressure to ambient. The advantage of a SCF is that only a minute amount of SCF will be found in the extract because the evaporation of the solvent at lower pressure [55].

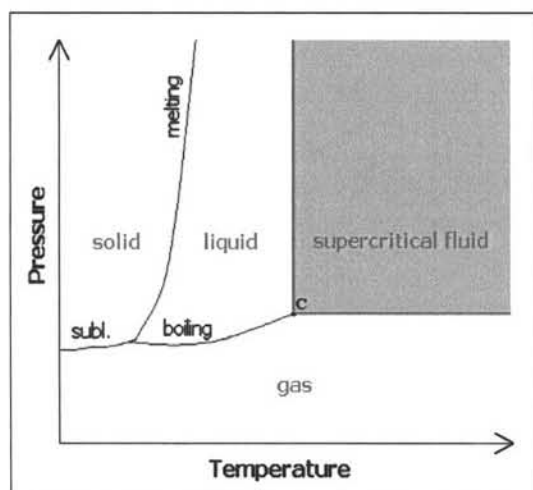


Figure 13 Pressure vs. temperature phase diagram [55]

SCF is popular in the pharmaceutical industry because of its low toxicity and good controllability over the dissolving power. However, a high pressure in the range of 100-300 bars is required. To create such a pressure in an open area where trees are growing will be a major challenge, leaving alone the issues concerning costs and SCF recovery. In addition, the high mobility inside the plantation is a required quality of the machine. Compression and regeneration units, together with the space needed to create high pressure will lead to a bulky unit. Furthermore, temperature control is also strict to keep the fluid in supercritical state.

Supercritical fluid extraction is already used to obtain taxanes. However, the first step is grinding needles into powder to release the taxanes. So diffusivity through intact needles is again a challenge here, same as that suggested for extraction.

5.3 Vacuum suction

Vacuum suction is another option to harvest taxanes from the needles of the tree without clipping them. Suction means creating lower pressure in a certain environment. Due to the pressure difference that is created by the suction device and the surrounding pressure, matter is pushed towards lower pressure region and out of the interior of the plant. One analogy is sucking lemonade through a straw; when suction is applied on the stomata of the needle, the liquids will flow out of them (see Figure 14).

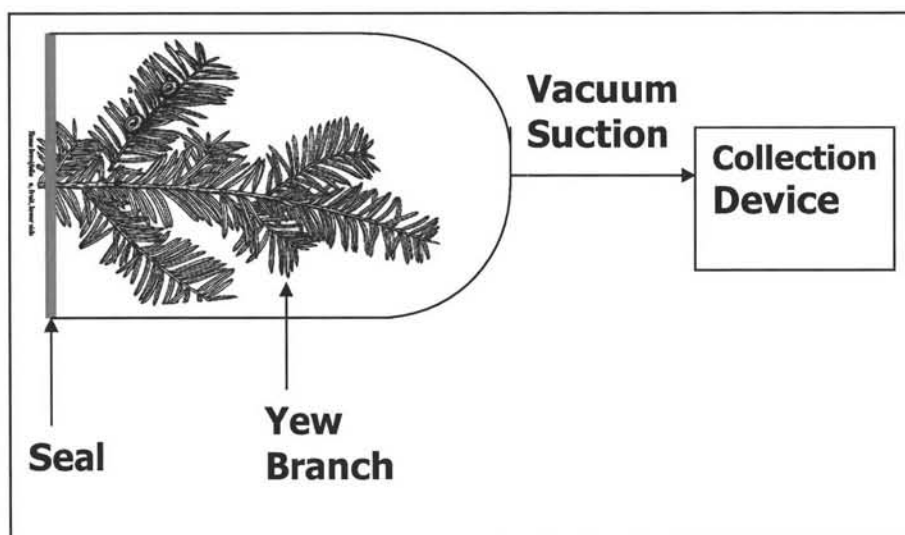


Figure 14 Vacuum suction setup

Problems that can be foreseen with this technique are for instance the amount of taxanes that can be released at a particular vacuum pressure; the sealing has to be closed air tight over the branch, otherwise the air sucked out will be replaced. It will be hard to put this seal over the branch without damaging it. Moreover, when the harvesting is done, the chamber has to be removed again. This will cause the same problems regarding the survival of the needles. Another disadvantage is that the chambers should be small, because vacuum suction takes quite some energy, since the air inside the chamber needs to be removed. The amount of air that must be removed from the chamber before enough vacuum is created should be as small as possible. Using small chambers automatically means using a lot of these chambers, causing difficulties in application. For these reasons, this option does not seem very attractive.

5.4 Electrospray; applying an electric field

Electrospraying is a technique where a liquid stream can be atomized by inducing an electric field. The electric field is applied by placing an electrode near the liquid stream (see Figure 15). This causes a potential difference between the electrode and the liquid stream. The charge carriers in the liquid stream (opposite of the charge of the electrode) are attracted to the electrode and move to the surface of the liquid stream. As a consequence, the charge density at the surface increases and due to the repulsion forces between the equal charged particles the jet explodes in very small droplets [56].

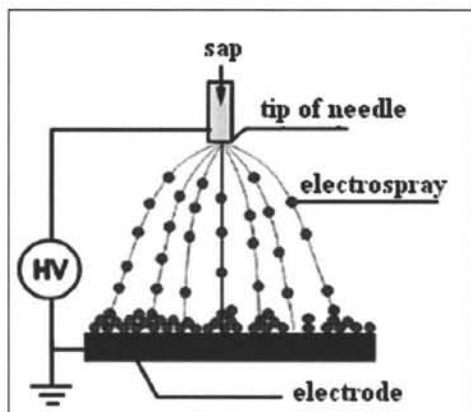


Figure 15 Basic setup of electrospray [57].

It was discovered that yew trees spray droplets from their needles when placed in an electric field. This discovery was done during a thunderstorm. When a thunderstorm approaches, the electric field strength increases dramatically from 100 V/m up to about 10,000 V/m. In 1999, two scientists used this finding and performed experiments where this thunderstorm situation was mimicked, in the hope to collect taxanes and find an alternative, sustainable way to obtain taxanes. An electrode with a high voltage was placed nearby a needle from a yew tree and after some time they observed the spraying. Analysis of the collected product showed that indeed it contained fractions of valuable taxanes [58],[59].

Since the experiment showed the promising result that the sprayed droplets contain taxanes, research was started at the TU Delft about this subject and is currently still going on. The following information is obtained from the small scale experiments [30],[59],[60]:

- The time it takes to electrospray the needles is about 30 min. from which only the last 20 min. spraying takes place. In the first 10 min., the tips of the needles move slowly toward the electrode. It is unknown why there is a delay of 10 min.
- There is one data set available where the composition of the product is analyzed. The results can be viewed in Appendix B-3. In short, about 1% of the taxanes that are present in the needles can be removed by electrospraying.
- Up to today there is no agreement on the maximum frequency at which electrospraying can take place without damaging is permanently. Dr. Ir. Marijnissen, one of the scientists who performed the first experiments, believes that it is possible to spray 10 times a year, while Dr. Ursem, assistant director of the Botanic Garden in Delft, believes that it is possible to spray only twice a year.
- The voltage for the electrode used by the small scale experiments is 4000 V with a distance between the electrode and the needles of about 1.5 cm.

Based on experiments that have been done, there seems to be no damage done to the tree. This technique does not harm the tree and the operating conditions are mild as well. The amount of chemicals used is also minimal; however the question remains whether it is applicable at large scale.

5.5 Choice between different techniques

The four different alternatives are described, together with their pros and cons, and a choice has to be made for one of these options. To do this, all four options are subjected to the objectives stated in Chapter 1. The taxanes have to be obtained in reasonable amounts with a higher purity on a sustainable way. The new method must be more profitable than the methods used today or it must bring an additional profit. People involved should not be negatively affected by the new method, not due to the use of toxic chemicals nor due to taking larger risks. The environment should suffer less by the new technique, which includes the new method should not harm the tree.

Further it would be desirable if isolation from chloroplast and waxy materials can be avoided in the design, because this will simplify the extraction, reduce the amount of chemicals used and save energy, hence money. Ideally, paclitaxel and its precursors may be obtained in higher concentration. The crude extract will have a higher market value and downstream processing costs can be lowered as well.

Besides subjecting the new proposal with the objectives, some principles most likely will not work if investigated more thoroughly, like the SFC extraction and the vacuum suction. Solvent extraction and supercritical fluid extraction are harmful for the trees and vacuum suction, besides the difficulty that is expected in application, will most likely be damaging the tree as well. Since from the small scale experiments it seems that electrospray is not harmful for the trees, and since electrospray actually seems to get the taxanes out, this option will be chosen. Hence there is searched for more information about electrospray to investigate if a device can be designed, which will get taxanes out of the trees in a sustainable (economically as well as environmentally) way using this principle.

6 Electro spraying – general information

Electrospraying is a method whereby small droplets can be produced from a liquid jet under influence of an electric field. The droplets can be in the range from nanometres up to tens of micrometers. The technique of electrospraying is also known under the name of Electrohydrodynamic atomization (EHDA). An advantage of this technique is that it can be used for a wide range of products. There is much flexibility with regard to the composition of the particle. On top of that, it is a very gentle method. No extreme conditions are used such as high temperature or pressure, which is a pre for the processing costs and also for the stability of the products. Also, electrospraying is known for its very narrow size distribution. This is also the reason why this method is used for powder and drug particle production. Other applications are for mass spectrometry, the production of thin films, and in agriculture [56],[61].

In this chapter first the electric field around a needle is discussed, then the mobility of the taxanes inside the needle, and in the following sub-chapter the mobility to the tip of the needle where the electrospraying takes place will be explained. Lastly, the electrospraying model applicable for the taxane electrospraying, the cone-jet mode, will be specified and the model is described in detail.

6.1 Electric field

It is found that in nature yew trees release some of their saps with taxanes before thunderstorms, when the electric field strength around the tree is enlarged. To mimic this situation, an electric field is induced around the needles by placing a positive electrode near the needles [59]. A map with the equipotential and field lines of this situation around one needle is shown in Figure 16.

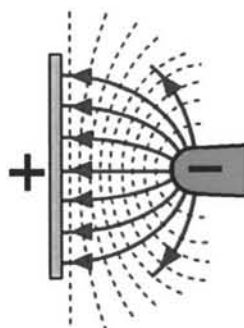


Figure 16 Field lines and equipotentials between electrode and needle, adapted from [62]

The plot above is for the situation when the needle is placed perpendicular to the electrode. The solid lines are the field lines, the dotted lines correspond to the equipotentials where each of the lines corresponds to a certain potential. The equipotentials going from the electrode to the needle are in the range of the potential of the electrode to zero (surface of needle). The distance between the equipotentials is the smallest at the tip of the needle, indicating that the field strength is the strongest in this area [63].

6.2 Movement of taxanes inside the needle

The mechanism behind the mobility of taxanes under influence of electric field is not well understood. No literature information was found on this subject. Different theories that were brought up by various people were investigated. The theories where the mechanism is derived from were found to be the most plausible. These are based on the thinking that the release of droplets from the needles by electrospraying can be compared with evaporation of water from the needle, with the only difference that by evaporation only water (in gas phase) leaves the needles, and that by electrospraying water is released in liquid phase with dissolved compounds in it. First, some research was done about the solubility of the different taxanes, then a thoroughly explanation about the mobility of taxanes within the needle by applying an electric field and the movement of droplets via the surface to the tip of the needle is given.

6.2.1 Solubility

Before it can be understood why taxanes are coming out needles when an electric field of a certain strength is induced around them, it is important to know where and in what phase the taxanes are present in the needles. In Chapter 3 it was discussed that a large part of the taxanes are present in the vacuoles of the cells and a small fraction in the extracellular medium. In this section, the phase of the taxanes is studied.

Taxanes are not well soluble in pure water. In Table 5 the maximum solubility of paclitaxel and two main precursors in water are listed. The latter two are based on calculated values found by SciFinder, hence these numbers can be a factor off from the real value. The maximum concentration of taxanes present in the needles of different yew species was calculated based on the screening of needles of different yew species done by van Rozendaal et al. [26]. The calculation for the conversion of the solubility in the needles from $\mu\text{g/g}$ dried needles to mg/ml can be viewed in Appendix C-1.

Table 5 Solubility of taxanes and their maximum concentrations in needles of yew trees.

Compound	Mass solubility in water	Molar solubility	Max. concentration found in yew needles in $\mu\text{g/g}$ dried needles [26]	Calculated max. concentration found in needle
Paclitaxel	0.01 mg/ml [64]	1.2E-5 mol/l	516	0.28 mg/ml
Baccatin III	0.0094 mg/ml [65]	1.6E-5 mol/l	296	0.16 mg/ml
10-deacetyl baccatin III	0.0045 mg/ml [65]	8.2E-6 mol/l	2665	1.4 mg/ml

The calculated concentrations of the taxanes found in the needles exceed the solubility limits. It is not very likely that taxanes are in the cell in solid phase therefore there is most likely a mechanism that increases the solubility, since it cannot solely be present in the aqueous phase.

A possible mechanism that could increase the solubility of taxanes is either by the formation of micelles or by the formation of colloids. Micelles are small aggregations of molecules that are dispersed in a liquid mixture where one substance is dispersed homogeneously throughout another. However, the molecular structure of Taxol and its precursors do not have a noticeable hydrophobic and hydrophilic part, and this is a requirement for the formation of micelles [66].

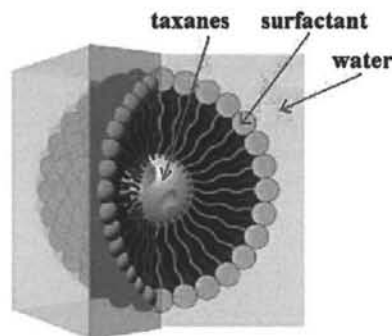


Figure 17 A colloid with taxanes inside, adapted from [67].

The option of the formation of colloid to increase the solubility of taxanes is left over. When other molecules are present that can form micelles, such as surface-active agents (surfactants) they can transport the taxanes through the sap. Since surfactants are amphiphilic, e.g. they have both a hydrophobic and a hydrophilic part, they can form spheres in a way such that the hydrophobic 'tails' are completely surrounded by hydrophilic 'heads'. Taxanes are non-polar and therefore they can cluster in the centre of the sphere. This is then called a colloid (see Figure 17). This theory about the solubility of taxanes seems the most reasonable way to explain the increase in solubility and therefore it is assumed that taxanes are present in the sap of the needles in the form of colloids [66].

6.2.2 Conductor in electric field

In general, when a conducting object is placed in an electric field, charge carriers inside the conducting object respond to the external electric force. By metals and in water, respectively the electrons and ions rearrange in a way to cancel out the electric field. Negative charge carriers move toward the electric field, while positive charges move in the opposite direction (see Figure 18)[68].

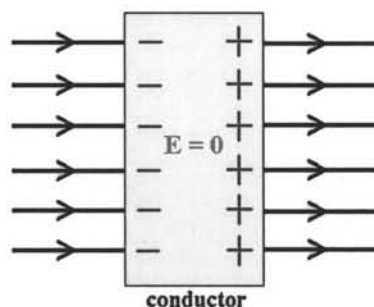


Figure 18 The rearrangement of the charge inside a conductor, where the arrows point in the direction of the electric field, adapted from [68].

By the rearrangement of the charge carriers a current is generated, however this current does not last long. When the relocation is finished, the electric field inside the conductor is zero.

6.2.3 Needle in electric field

When a needle is placed in an electric field, rearrangement of charge takes also place. A needle consists of 65% water [31], and due to the presence of free ions in the water, the needle can be seen as a conductor. The blocking of the electric field can be explained by the rearrangement of ions in the sap of the needle. In the next paragraphs the mobility of taxanes under influence of the movement of ions is discussed.

Free ions

When no electric field is applied, the free ions inside the needles are homogeneously dispersed. Once a positive electrode is placed nearby, the negatively charged ions are drawn toward the surface of the needle (see Figure 19) [69]. By the movement of the negatively charged ions to the outer sides of the needle, the positive ions are also moved somewhat in that direction since they are attracted to the negative ions. It is assumed that the repulsion between electrode and positive ions is much weaker than the attraction between the positive and negative ions, and that therefore the positive ions stay close to the negative ions.

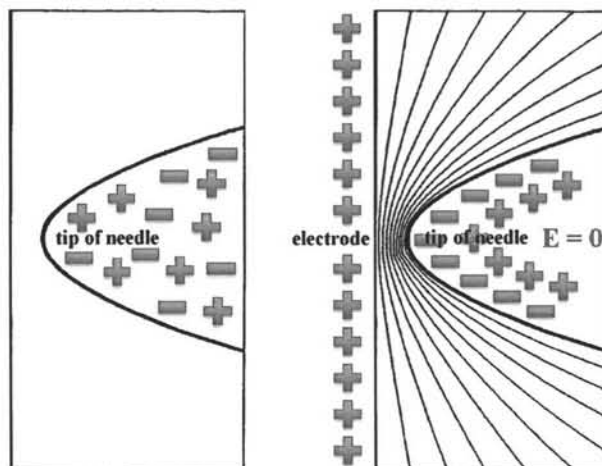


Figure 19 The distribution of free ions in the needle with (left) and without (right) applying an electric field

Using ionic mobilities and by making a force balance over the ion between the electric field and drag force gives an estimate of the velocity with which the ions move inside the needle. The calculations in Appendix C-2 show that the velocity of ions in the sap of the needle is at least around 0.007 m/s. From here it follows that the rearrangement is finished in less than 1 second, and that this is not the limiting factor in the spraying process. Once the rearrangement is done, the electric field inside the needle is zero.

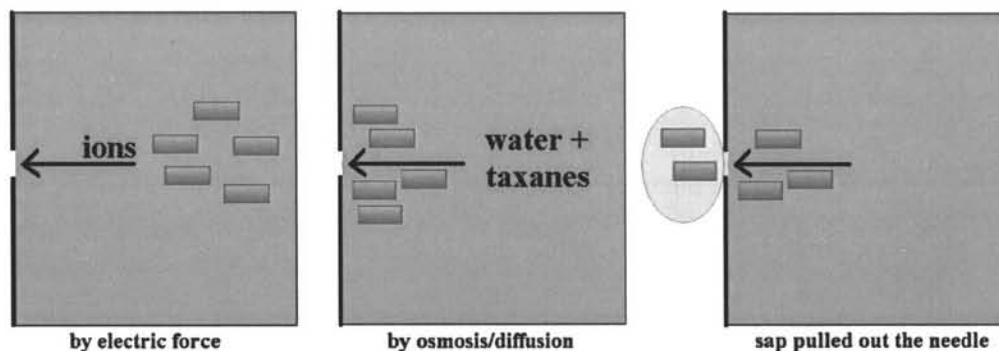


Figure 20 The mechanism of sap being pulled out of a needle by an external electric field

In Figure 20, the influence of the electric field on the movement of ions, and therefore on the mobility of water and dissolved material is explained graphically. Once the ions are moved under influence of the electric field, the concentration of ions close to the surface is much higher than inside the needle. Since there is locally a high concentration of ions, water moves towards the surface to disperse the solute uniformly in the solvent in order to maximize entropy.

In the water all kinds of compounds are dissolved, including taxanes in colloidal form. While water moves to the surface of the needle, the concentration of dissolved material inside the needle becomes higher than at the surface. Thermodynamically the most favourable situation is when the solution is homogeneous, since the entropy is then maximal [70]. Therefore, dissolved materials, together with taxanes that are present in the sap in colloidal form, are dragged with the water to the surface of the needle. This process can be seen as diffusion, since particles are moving from a high to a low concentration.

Positive charge

It is clear that the liquid leaving needle consists of more negative charge, since it is attracted to the positively charged electrode. The droplets will have a net negative charge, leaving the needle behind with a net positive charge. This raises the following questions about what happens with respect to the charge of the needle over time when the needle sprays droplets, since electric neutrality has to be maintained:

- Is there a mechanism that keeps the net charge zero while the needle is spraying, or does the needle stop spraying once a certain amount of negative charge is released?
- And if there is a mechanism to keep the total needle neutral, are negative charged particles replenished or do positively charged particles move away from the needle?

The needle will keep its neutrality during electrospraying. The needle is grounded, since it is part of a tree, and the tree is in contact with the earth. This means that whenever the needle loses or has a surplus of some charge, the earth, a huge reservoir of charges, will replenish or take it up via the tree. The question if the needle stops spraying after a while, in other words, if the time to replenish or take charge up is the limiting factor after the needle has sprayed for some time period is questionable.

From experiments it is found that after some time (± 30 min.) the tree indeed stops spraying. However, the researchers who performed these experiments believe that the limiting factor that stops the needle from spraying is the replenishing of water instead of charge [59],[60].

Most likely, the loss of negative charge is refilled by the tree during the electrospraying, since if the negative ions are not replenished, the concentration negative ions in the needle would decrease over time. It is thought that the needle is favoured by keeping the ion concentration about the same over time, and that since more negative ions are lost these ions will be replenished.

To get a better understanding about the mechanism more research related to this subject needs to be done. This information is important to find ways to optimize the release of taxanes by the electrospraying process in the future.

6.3 Movement of taxanes from the stomata to the tip

When the electrostatic attraction between the positive cathode and negative ions are higher than the forces that prevent the sap to leave the needle (capillary), the sap leaves the needle through the stomata. There is no confirmed theory about whether the sap is only sprayed from the tip of the needle, or that the electric force is that high that the sap is sprayed out of several stomata. Observations from Caner Yurteri indicate that sap is only been sprayed from the tip of the needle, since only one spray is observed from each needle. Nothing could be said about the number of stomata that are opened to release the sap [60], however some hypothesis could be made by looking at the electric field distribution.

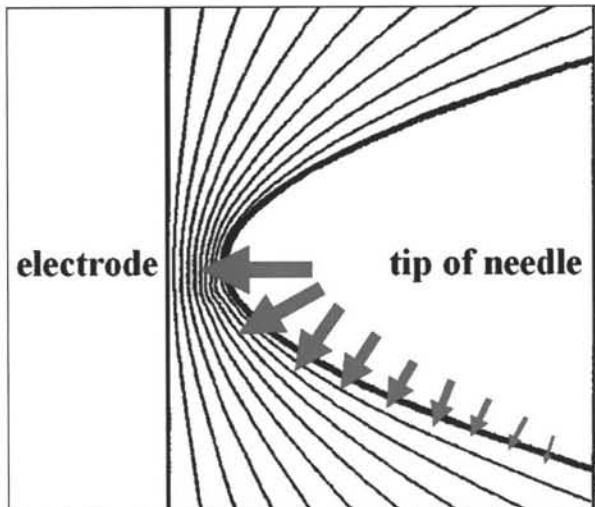


Figure 21 The predicted distribution of sap leaving the needle

The stomata are equally distributed along the bottom part of the needle (see Chapter 3). Since the electric field is the strongest at the tip of the needle, it is expected that most of the sap leave the needle at the stomata of this part. The electric field strength decreases when moved further from the tip, and it is thought that the amount of sap decreases also in this direction (see Figure 21). Droplets that leave the needle at other place than the tip are thought to move along the surface to the tip. The surface tension between the surface and the droplet keeps the droplet from falling (counteract the gravity) and the electric force pulls the droplet to the tip. The sap is collected at the tip of the needle from where it is been sprayed.

6.4 Spraying of the sap

Electrospraying of liquid can take place in different mode. A short overview on the different electrospray modes is given, continued by a more extended explanation of the mode which is used for the modelling of the electrospray of yew trees, the cone-jet and the forces on the droplets during the spraying.

6.4.1 Modes

There are two electrospraying modes, the dripping modes and the jet modes. With the dripping mode, liquid fragments are released from the nozzle. Just after ejection of the nozzle the droplets can be large (dripping), fine (microdripping), lengthened ((multi) spindle mode) or irregular of shape [57].

The jet modes can be recognized at its very thin long jet from the nozzle. The jet leave the nozzle in different movements, for example rotating (precession mode), or oscillating (oscillation mode). The most observable mode is when the jet is stable. This is called a cone-jet. It is also possible to have several cone-jets, the multi-jet mode (see Figure 22) [57].

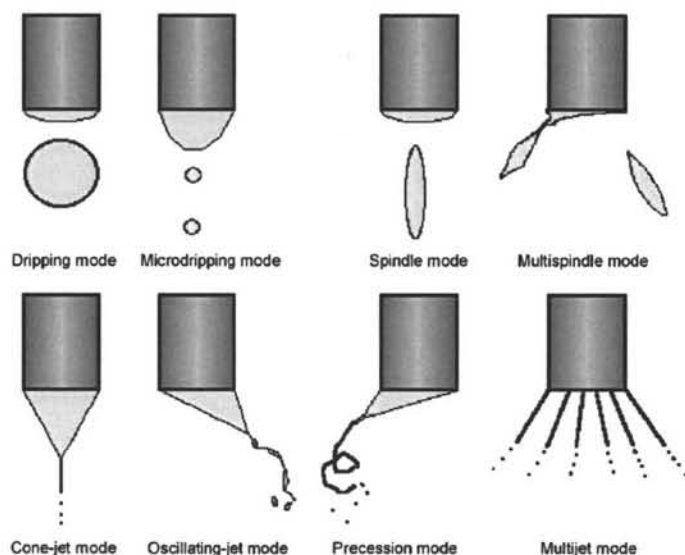


Figure 22 Different modes of electrospraying [57]

For the electrospraying of taxanes out the yew tree it can be assumed that the sap is spraying in the cone jet mode based on observations from experiments [60].

6.4.2 Specifications of cone-jet mode

The cone-jet mode is a stable jet along the capillary axis. The maximum deflecting is about 10° . At the end, the jet breaks up and forms droplets. The jet can be split in two instabilities, the varicose and the kink instability. By varicose the jet continues along the capillary axis, at a certain point the constant jet becomes interrupted and starts to form droplets. By kink instability the jet start to spin along the axis of the capillary also causing the jet to break up into fine droplets (Figure 23) [71].

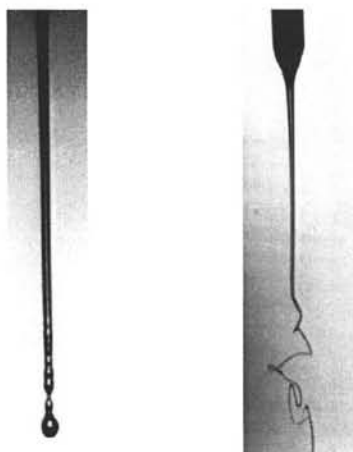


Figure 23 Types of jet instability with varicose (left) and kink instability (right) [71]

The diameter of a cone-jet is dependent on the voltage. Typically the diameter is smaller than $100\text{ }\mu\text{m}$; however when the voltage is increase the diameter can decrease up to a few micrometers, while its length shortens. The fine droplets, mean size 30 micrometers up to fractions of microns, are spread out with a spray cone apex angle of about 60° to 90° [71], [72].

In Appendix C-3, the forces on the cone-jet are shortly discussed. This model is not discussed in this report in detail due to the fact that the exact forces and dimensions of the Taylor cone are not of interest for this project. More interesting is the model of the spraying of droplets, since the parameters during the spraying are significant for the design of the harvesting apparatus.

6.4.3 Forces on the droplets

The maximum size of the droplet beyond which droplets break into smaller droplets is determined by the balance between Coulomb interaction forces inside the charged droplets and the surface tension. The Coulomb interaction force is dependent on the charge on the surface of the droplet. Once the droplets have such a high surface charge that it overcomes the surface tension, the droplet will explode. This is called the Rayleigh explosion, and the maximum charge as a function of the droplet size and surface tension is given by the following equation:

$$q_{\max} = \pi \sqrt{8\epsilon_0 \gamma d^3} \quad [56]$$

where q_{\max} is the maximum charge of the droplet (Coulombs), ϵ_0 is the permittivity of free space (C^2/Nm^2), γ is the liquid surface tension of water (N/m), and d is the droplet size (m). The droplets produced by electrospraying in the cone-jet mode carry approximately 70% of this charge [56].

The droplets in the ambient air experience four forces:

- electrostatic attraction forces due to the external electric field between the negatively charged droplets and the positively charged electrode
- Coulomb electrostatic repulsion forces between the charged droplets
- drag force due to the movement of the droplets
- gravitational force

The forces do not all contribute in the same magnitude to the movement of the droplets. The electrostatic attraction force is for example much greater than the force due to the gravity. In the paragraph about modelling, the relative magnitude between the forces is discussed.

6.5 Rate limiting step

For the design of the taxanes harvesting device it is important to know what the rate limiting step is in the electrospraying process. The optimization of the device should be focused on the rate limiting step, since the most can be gained by improving this aspect.

The extraction of the sap can be divided in four steps:

- the transportation of the sap from the capillary vessels to the stomata
- the transportation of the sap through the stomata to the surface of the needle
- the movement of the sap from the stomata via the surface to the tip of the needle
- the electrospraying of the sap from the needle toward the collection bag/plate

The rate limiting step is determined by elimination. Below, the reasoning behind the elimination is discussed.

6.5.1 Movement of sap to the tip of the needle

It is thought that the movement of sap at the outside of the needle from the stomata to the tip of the needle is not the rate limiting step is because the bottom side of the needle is covered with a thick wax layer of cutin to prevent loss of large amounts of water by evaporation [27]. The sap that

comes out the stomata consists mostly of water [60]. Cutin is a hydrophobic, and has therefore wetting properties. Research about the contact angle on needles also shows this. The contact angle between water and needle is on average around 100°, which indicates that there is less interaction between needle and liquid. Therefore there is a low resistance of flow and water drains easy [73]. The mobility of water on the surface of yew needles was also tested with a small experiment. A branch was immersed in a cup of water. When it was taken out the water quickly (few seconds) moved to the tips of the needles from where it dripped off.

The information written above is based on movement of water in the absence of electric field. The presence of electric field will increase the speed due to the attraction forces between the sap (negatively charged) and the electrode (positively charged). Based on the information of the research about contact angles and by the experiment performed by ourselves it is thought that it is reasonable to say that the movement of the sap to the tip takes place at a sufficient high speed so that it will not be the rate limiting step.

6.5.2 Electrospraying of the sap

The electrospraying of the sap from the needle toward the collector is eliminated as rate limiting step because the mode in which electrospraying from yew trees takes place. In Table 6 the results are given of a study where the electrospraying modes were studied with a hexane solution when the voltage was increased.

Table 6 Electrospraying modes for hexane solution by different voltages [74]

Voltage (V)	Description
3500	A blunt cone is formed, but no jet appears.
3750	Discontinuous jet. Microdripping of small droplets.
3875–4125	A continuous jet is formed. Cone-jet mode.
4250	Unstable atomisation.

Electrospraying from the yew tree takes place in the cone-jet mode (see Section 6.4.1). If the electrospraying was the limited step, an electrospraying mode which requires a small electric field should have been observed, such as the formation of a blunt cone, a discontinuous jet or the appearance of microdripping.

6.5.3 Transportation of the sap from the capillary vessels to and through the stomata

Since the other two steps are dropped out, in the mechanism of the transportation of the sap from the capillary vessels to and through the stomata of the sap are left over. There is no evidence to determine which of the two mechanisms is the slowest, however for the design of our harvesting device the both outcomes will lead to the same design. In either way, the rate limiting steps lies inside the needle, and there should be searched for a method to increase the speed to get the sap out the needle. No further research is done to get a better understanding about the rate limiting step. There was not much information available about this subject and due to the limited time it was decided to stay away from making a model. Our recommendation would be to investigate this in the future.

6.6 Modelling of electrospraying

Once the mechanism about the mobility of taxanes inside the needle is clarified, the electrospray process can be modelled. In the following paragraph the assumptions that are used for the modelling of the electrospray process are listed. By estimating the droplet size, other important parameters such as the flow rate and velocity of the droplet are calculated. This information is needed by the designing of the taxanes harvesting apparatus.

6.6.1 Assumptions

The reports from experiments done in the past regarding electrospraying to harvest Taxol and its precursors do not give information about basic information needed for modelling like the droplet size, flow rate, or composition of the droplets. Assumptions are made to start with modelling. These assumptions are based on thorough discussions, literature research, or calculations. The model that is made for electrospraying is a simplified version of the electrospraying model that is proposed by K. Geerse [56].

The movement of the droplets is only viewed in the direction between the needle and the electrode. Below a list is given with the assumptions that are made to model the electrospraying process. The explanation behind each assumption can be found in Appendix C-4.

- The droplet has the properties of water
- No evaporation of the droplet takes place
- All droplets have the same size
- The charge on the droplet is constant, e.g. there is no discharge during the flight
- 20% of the sap in the needle can be removed in 20 min., by removing more liquid the needle will be damaged
- Electric field attraction force and drag force are the main forces; the gravitational force and the Coulomb interaction forces can be neglected
- The air velocity around the electrospray is zero

6.6.2 Calculation

Flow rate

The maximum liquid flow from one needle was calculated from the amount of water present in a needle. Maximum 20% of the water can be removed to keep it still functioning in about 20 min. [30]. From the wet weight of needles (determined by weighting of needle), dry weight was calculated (see Appendix C-5). The flow rate was found to be 9 $\mu\text{l/h}$.

Droplet size

The droplet size was calculated from the flow rate. The following equation shows this relationship:

$$d = \left(\frac{16\rho_l \epsilon_0 Q^3}{\gamma K} \right)^{\frac{1}{6}} \quad [75]$$

Where d is the droplet size in metres, ρ_l is the density of water in kg/m^3 , ϵ_0 is the permittivity of free space in C^2/Nm^2 , Q is the flow rate in m^3/hr , γ is the liquid surface tension of water in N/m , and K is the electric conductivity in S/m . The droplet size was calculated to be 0.5 μm . For the extended calculation see Appendix C-5. The droplet size agrees with the values published in literature. A. Jaworek and A. Krupa [71] published an article where the mean size of the droplet that are produced in a cone-jet mode are about 30 micrometer up to fractions of 1 micrometer.

Charge of droplet

The maximum charge of the droplet is calculated according to the formula in Section 6.4.3, and was found to be $2.5 \cdot 10^{-15}$ C/droplet. The actual charge is 70% of Q_{\max} and was calculated to be $1.8 \cdot 10^{-15}$ C/droplet (See Appendix C-5).

Electric field strength

The field strengths that act on a droplet when it moves away from the needle were calculated by trial and error. Models published of the cone-jet spraying gave velocities differences between the droplet and the surrounding air between 12 and 17 m/s [56], and 10 to 15 m/s [61].

The velocity was used to balance the electrical attraction between the charge droplets and the electrode with the drag force of the droplets. The other forces were found to be negligible (see Section 6.6.1, assumptions). The electric force (left side of equation) and the drag force (right side) were balance and solved for E, the electric field.

$$q\vec{E} = C_D \frac{\pi}{8} \rho_{\text{air}} d_d^2 (\vec{v}_{\text{air}} - \vec{v}_i) |\vec{v}_{\text{air}} - \vec{v}_i|$$

where q is the charge of the droplet in Coulomb, C_D the drag coefficient, ρ_{air} the density of air in kg/m^3 , v_{air} the velocity air and v_i the velocity of the droplet in m/s. It was found that the electric field strength has to be between $4.9 \cdot 10^5$ and $8.3 \cdot 10^5$ V/m in order for the droplet to have a velocity between 10 and 17 m/s. The droplet is located in different field strengths when it moves toward the electrode, and that field strength is within the above mentioned values to get reasonable velocities. The extended calculations can be found in Appendix C-5.

Summary most important values

The values that were found by the model will be used by the design of the taxanes harvesting device. Below in Table 7, a list of the most important parameters is given.

Table 7 Most important values found by modelling

Parameter	Value
Droplet size	0.5 μm
Flow rate	0.01 ml/h
Charge on droplet	$1.8 \cdot 10^{-15}$ C
Electric field strength	Between $4.9 \cdot 10^5$ and $8.3 \cdot 10^5$ V/m
Velocity of droplet	Between 10 and 17 m/s
Electric attraction force between droplet and electrode	Between $8.6 \cdot 10^{-10}$ and $1.5 \cdot 10^{-9}$ N
Drag force	Between $8.6 \cdot 10^{-10}$ and $1.5 \cdot 10^{-9}$ N
Gravitational force	$6.4 \cdot 10^{-16}$ N
Electric repulsion force between droplets (distance 0.1 mm)	$2.8 \cdot 10^{-12}$ N

In Appendix C-6 the complete excel sheet of the calculations can be viewed.

7 Market

A successful product has to be well received by the market and it must satisfy consumer demands and demonstrate advantages over the competitor's product. For this purpose, the existing supply chain from biomass to the finished drug is investigated to identify important product characteristics or opportunities by which the product can be made more appealing to our potential consumers.

The market knowledge was obtained mainly through two channels; online articles and interviews with companies involved at various stages of the paclitaxel business. Questionnaires were made for different companies involved in the production of paclitaxel and from the results of these questionnaires the supply chain from biomass to drug was set up. Also, information was obtained about how the market for paclitaxel and its precursors works and what the marketing opportunities are for the product.

7.1 Companies involved in the current supply chain

Currently, the most used method to obtain taxanes is by extraction of the dried and grinded needles of the yew tree in form of dry powder. Cell culture is the other important source of biomass and it is adopted by one of largest paclitaxel producers, Bristol-Myer Squibb (BMS). In either case, the processing of biomass into drug is similar as explained in Chapter 4. The difference lies in starting biomass mass; either in form of dry powder or cell mass from fermentation [76].

There are mainly four parties along the supply chain¹ [77],[78]:

- Tree growing companies/ nursery garden companies/cell culture fermentation
- Companies that produce bulk paclitaxel or precursors by performing the extraction(s) and Preliminary purification
- Pharmaceutical companies which produce paclitaxel from its precursors and purifying it into drug ready for medical use
- Hospitals, who deliver the drug to patients, the end users of the product

On the next page, a picture of the supply chain can be viewed (Figure 24). The numbers in the picture correspond to the different steps in the supply chain that all are performed by one of the companies described above. The role of each party will be explained in greater details in the paragraphs following the picture.

¹ This list is established mainly based on the information provided by various companies interviewed

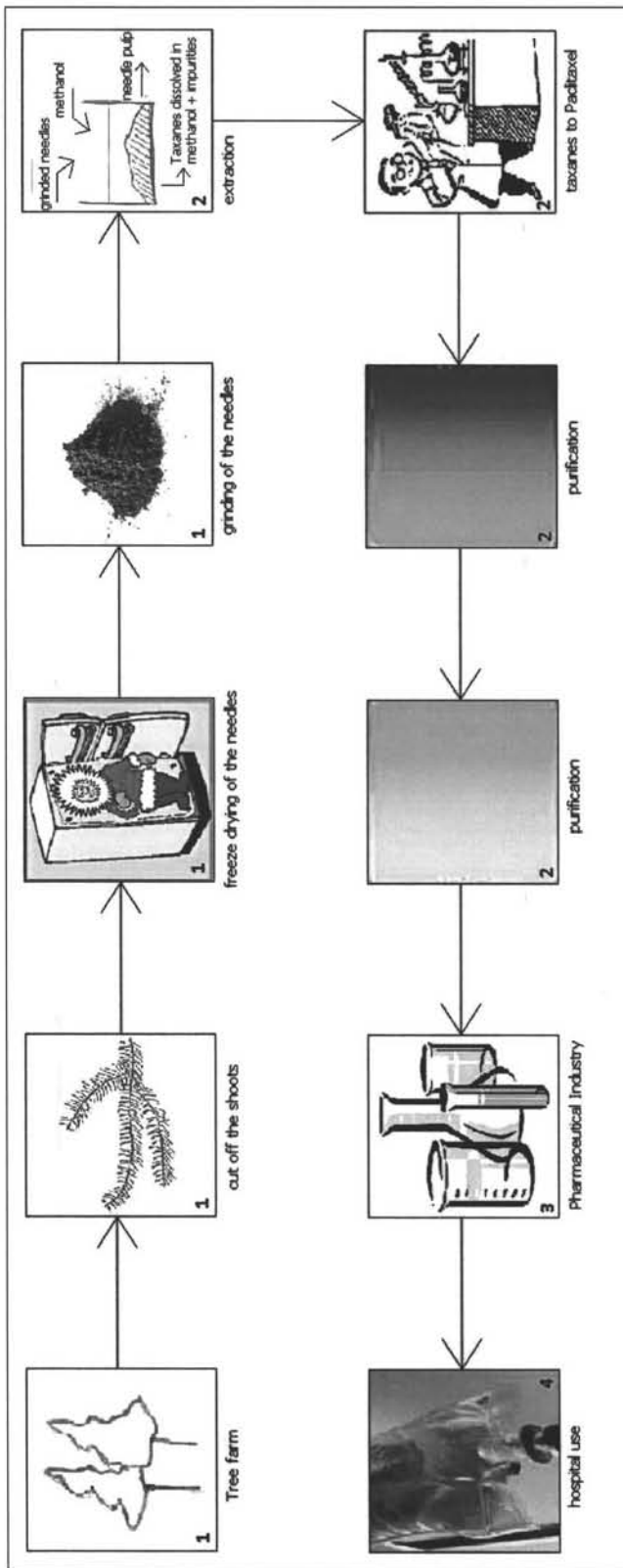


Figure 24 Steps involved in the production of Taxol

7.1.1 Growth of biomass

The production of paclitaxel by extraction takes several steps. It starts with the growth of biomass, either by fermentation of cell cultures or by the growth of yew trees. The Pacific yew, which is native to the USA, has been nearly extinct due to harvesting of bark in earlier days [79]. To preserve the natural environment and bio-diversity, killing of wild yews is forbidden [79]. Nowadays, once a year, mainly in late summer, the shoots of the yew trees are cut [80]. In Canada, needles are collected from wild *T. Canadensis*, which is present in large amounts across the country [77]. Europe has a rich source of *T. Baccata* from which cuttings are collected, there are for example plantations in the UK and in Poland [80],[81]. Furthermore, biomass is supplied by other parts of the world, including large plantations in China and India [82]. For the cell cultures, the biomass required by BMS is supplied by Phyton in Germany, a biotechnology company with expertise in plant cell cultures [76].

The obtained biomass contains taxanes, which can be extracted and purified. The taxane mixture consists of paclitaxel and its precursors, namely baccatin III, 10-DAB III. The extent of the different constituents in the taxane-mixture depends on the species used for the biomass and its location. After the extraction, the precursors are converted to paclitaxel by a synthetic process as described in Chapter 4.

7.1.2 Preparation for extraction

After the cutting of the shoots, the needles are dried by freeze drying. Hereafter the needles are grinded into a fine powder and can be transported for the extraction. This part is often done by the harvesting companies of the plantations themselves [77].

7.1.3 Production of bulk paclitaxel (extraction and preliminary purification)

Conventionally, the extraction is carried out with chemicals like methanol and chloromethane. The crude extract is purified by adsorption, precipitation and high performance liquid chromatography (HPLC), whereby paclitaxel and its precursors are obtained (for a detailed description see Chapter 4).

Bulk paclitaxel or purified precursors are obtained for two uses, namely [76],[83]:

1. small batches for research (<1kg)
2. large batches for pharmaceutical companies for drug production. (>1kg)

Numerous companies in Europe, Canada and Asia are involved in the production of bulk paclitaxel and most of the pharmaceutical companies have a stable supply from two or more companies of these companies [76],[82]. For example, Indena in Italy is purifying cell cultures for BMS. In Canada, part of the biomass is extracted and purified locally, while another part is done in China [77]. For example, most companies in Europe and the USA, which were interviewed, revealed that their bulk paclitaxel is either directly imported from China or made by their factories/contractors in China [76],[83],[84]. Therefore, nearly all the taxanes from *T. Baccata* of Europe are processed in China [82]. Furthermore, interviewee from UK companies claimed that they have not heard of any extraction and purification process in UK and Indena is the only company which was found involved in this business in Europe [81],[85]. The reasons given are mainly of economic purpose or availability of resources [85],[86].

7.1.4 Conversion to paclitaxel and downstream purification

Normally pharmaceutical companies purchase paclitaxel directly from suppliers or contractors and are mainly focussed on two steps of the supply chain. The first of those steps the conversion of the precursors to paclitaxel by a semi-synthetic route. As second step the purification of the naturally or synthesised obtained paclitaxel can be identified. The purification is done in various steps to reach the standards required by the Food and Drug Administration (FDA) for a deliverable drug. These two steps are often done by pharmaceutical companies like BMS and Bioxel [76].

Currently semi-synthetic paclitaxel is about 15-20% cheaper than naturally extracted paclitaxel [87] and the ratio of semi-synthetic paclitaxel to naturally extracted paclitaxel is 3:1 [77]. This is partly due to the availability of raw material; in general, baccatin III, one of the precursors for paclitaxel, is present in much larger amounts than paclitaxel itself (even 100 times higher for certain species) in plant cells [88]. The generic form of paclitaxel, produced by many companies all over the world, became available on the market in 2001 [89]. The production of docetaxel is still under patent protection, Sanofi-Aventis will have the monopoly until 2010 [90].

7.1.5 Hospital use and patients

The finished drug from BMS is a clear liquid, every 1 ml of which contains 6 mg of paclitaxel, 527 mg of Cremophor EL and 49.7%(v/v) dehydrated alcohol [91]. The hospitals deliver it to patients, the end users of the product. It must be diluted to 0.3-1.2 mg/ml with 0.9% sodium chloride or 5% dextrose before intravenous injection [91].

In the Netherlands, it is allowed to use all the anti-cancer medicines that are registered in the European Union and approved by the EU and Dutch commissions on approval of medicines. The medication can only be obtained via the hospitals. Although the dose of paclitaxel is low it is a large expense [92], but for the patients all the costs are insured.

7.2 Global market

In 1991, BMS was selected as the only partner for paclitaxel research and was granted exclusive marketing rights for the next five years. Because of this, BMS got a very large profit and the price of the drug was as high as 6.8 €/mg (6.8 million €/kg). In 1992 the drug was first launched and until 2001, when the patent has expired, Taxol brought BMS an average annual sale of € 1.34 billion with a yearly production of about 200 kg [97]. All the values in euro's which were given in dollars are converted whereby the currency changes are taken into account according to the different exchange rates of "De Nederlandsche Bank" [98].

7.2.1 Demand

With the expiration of the patent, companies all over the world started to produce the generic form of paclitaxel [93]. Generic drugs are imitations of the original drug and are in the same form as the original medicine. More and more companies are getting involved in the production of the generic drug on the American and European market [76], and because of the competition and improvement in the purification technology; the prices are gradually brought down. In Figure 25, the market growth of paclitaxel can be seen, the coloured areas represent the counties which already have, or are expected to get, approval for the manufacturing of paclitaxel [99]. The competition between companies is fierce and the technology is rather mature. Hence most companies use similar methods in extraction and purification.



Figure 25 The global paclitaxel market (2006) [99]

As a result, the world supply of 2003 increased to 350 kg, however the world demand is more than 500 kg of which 300 kg contributes to the USA [100], which indicates there is a vast shortage of the drug. In 2006, the world demand was expected to be more than 800 kg of which the American demand would already have been raised to 500 kg [101]. However, the amount produced in the USA is only 25-50 kg [100]. This is far from fulfilling the needs and therefore most of the paclitaxel is imported. In Figure 26 the expectation, published in 2003, of the global world demand for 2002 until 2008 can be seen [76]. This figure shows that the demand for derivatives like 10-DAB will rise very fast, where the demand for paclitaxel itself will rise with decreasing rate.

Furthermore, it is expected that the demand for paclitaxel will increase at a rate of 10-20% each year for the next 5-10 years after 2006 [102],[100]. However, the world demand expectation of 2008 is that paclitaxel will reach 1040 kg in 2012 of which the USA shares 91% [103]. This amount is lower than the previous forecasts.

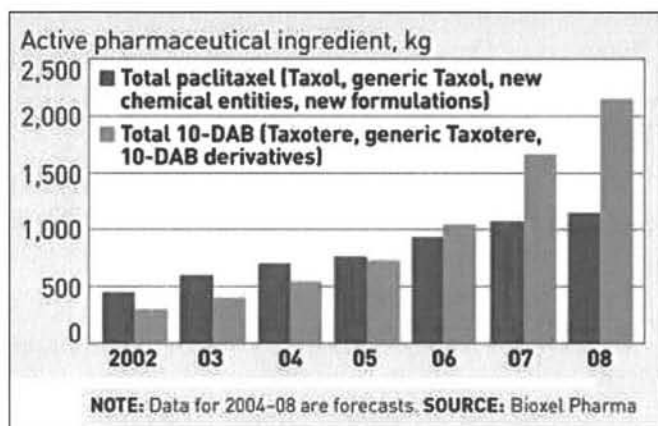


Figure 26 World demand of paclitaxel and its derivative 10-DAB (2003) [76]

7.2.2 Price development

Currently, the price of paclitaxel for Dutch hospitals is about € 135 for a 30 mg/5 ml flask [94]. This is equivalent to a price of 4.4 €/mg. One complete treatment in the USA nowadays costs about € 6800, this is about 3.4 €/mg [95],[104]. These prices indicate that it is still an expensive medicine [92], even though the price made a large drop compared with several years back [77]. This price drop will be even enlarged by the expiration of the patent for the production of generic docetaxel. This patent is owned by Sanofi-Aventis until 2010 and achieves more than € 0.8 billion of sales [95],[105],[98].

Since the patent has expired and more and more companies all over the world are involved in the production, it is unlikely that the high price is due to cartel formation. The main reason for the high price is due to the shortage, despite the growth of the amount of producers on the market. The demand is still larger than the supply; this could already be seen in Figure 26, with in mind the low annual production.

Furthermore, the price of paclitaxel is related to the production process, which is still complicated even though the technology is mature. The production of the drug consists of several steps and companies must still make profit. The harvested biomass from the yew trees or cell cultures is the starting material of the entire chain. After harvesting, the clippings are freeze dried, grinded and extracted, which can be done by various companies. Most of the smaller companies are only making small quantities whenever they receive an order, which mainly comes from the academic field for research purposes [78],[83],[87]. Further on in the chain the pharmaceutical companies can be found which get their bulk paclitaxel from multiple suppliers. It must however be noted that big pharmaceutical companies generally have an integrated supply chain starting from the biomass [76].

Besides the shortage and the complicated process, the price of the drug is related to its origin as well. The lowest price might be found in China, where the drug paclitaxel costs about 1.13 €/mg [84]. The price is about a third of the western price, which is based on prices quoted by Chinese local hospitals [106]. In general, products from Asian companies are cheaper than the European and American counterparts. However, to enter the European or American market certificates are needed to register the medicine. The requirements for these certificates are stringent and the certificate itself is expensive. For Asian companies it is hard to get such a certificate. Chinese manufacturers can seldom afford the costs to apply for approval of the drug and are therefore are not on the market for the drug, but only sell bulk paclitaxel [87]. Since there is nearly no domestic use of paclitaxel in China, practically all the produced bulk paclitaxel is exported to Europe, the USA and Japan. For 2004, the amount exported added up to 100 kg [100].

Bulk versus drug paclitaxel

There is a large price difference between the drug paclitaxel and bulk paclitaxel, even at a purity of more than 90% [96]. In 1992, the wholesale price of the drug paclitaxel is about 20 times higher than the price of the bulk paclitaxel [76]. It is investigated that most of the profit along the supply chain often goes to pharmaceutical companies [87].

For bulk paclitaxel, the starting material is provided either by cell cultures or by clippings of plantations located in Canada, Poland, UK and China. The most clippings are from wild *T. Canadensis* in Canada, and in Europe from the *T. Baccata* [79],[81].

For the bulk paclitaxel manufacturers a high taxane content in the needles is important and the clippings should be obtained in a sustainable way, since the importance of the certificate for sustainable harvest method [107]. In Canada a company improved the taxane content of the *T. Canadensis* and the dried clippings contain approximately 300 ppm of paclitaxel. In the past, the dried clippings used to cost nearly 5.5 €/kg [77],[102]. The Canadian prices for the clippings from 2002 till 2005 can be found in Table 8 [102]. It is expected, due to competition, the price will drop and stagnate around 2.80 €/kg [82].

Table 8 Prices for Canadian clippings [102]

Year	Price Wet (€/kg)	Price Dry (€/kg)
2002	1.93	5.50
2003	1.69	4.83
2004	1.39	3.97
2005	1.19	3.40

7.2.3 Future Forecast

Even though the competition is fierce at the different levels of the supply chain, the market prospect is still promising, according to the interviewed companies. The price per unit weight of the drug, bulk paclitaxel and clippings are all expected to drop, but the demand increases and expansion of the production scale will ultimately compensate the drop in price [77]. Various predictions have indicated an annual growth rate for the drug demand of 10-20% for the next coming years [102]. Involvement of more competitors will stimulate companies to improve their process and product quality, hence to reduce the cost.

After the price reduction of the drug paclitaxel, the medication is still expensive and therefore a certain proportion of the patients are not able to afford the drug. This is especially true in China and India where social health care is not good enough [106]. Many companies believe that once the drug becomes cheap enough, opening up the Asian market will bring another leap in sales volume [82],[84]. Consequently, lowering of the cost will increase the demand of the drug, which will propagate down the supply chain.

The forecast is also positive because docetaxel is becoming generic in 2010 and many pharmaceutical companies have already started working on the generic drug to get it ready for the market once Sanofi-Aventis' patent expires [84]. It is expected that this will induce another leap in the supply of precursors like baccatin III and 10-DAB. This has already been observed in 2001 when generic paclitaxel was launched [89]. Also, many pharmaceutical companies are currently improving their delivery methods, in the hope to reduce the side effects. If that is successful, dosage and duration of treatment can be increased. It is believed that paclitaxel and docetaxel will remain welcome anti-cancer drugs for two reasons. Firstly, oncologists are reluctant to change a drug once it has been proved to be effective on the patients [76]. Secondly, some pharmaceutical companies have suspended research on new taxane derivative drugs, due to unsatisfactory results [82]. Furthermore, compared with cell cultures, the use of yew trees as a raw material resource is believed to be

dominant [77]. When more of baccatin III and 10-DAB are needed, more trees will be needed and new plantations will be needed to satisfy this demand.

7.2.4 Key to Business

Most profits along the supply chain lie with the pharmaceutical companies, as shown by a huge difference in price between bulk paclitaxel and the finished drug. Pharmaceutical companies compete with each other over price. This can be seen from numerous lawsuits between companies regarding patents, especially when it concerned BMS [93]. The amount of competitors increases at each level of the supply chain, therefore the key to profit for bulk paclitaxel is to get involved into a globalized integrated supply chain [76], this will ensure a rather constant demand. Furthermore, it will be of great help if the efficiency of the process can be improved. One idea given by one of the interviewees is to have a starting material with higher taxane contents, or a starting material which requires less processing steps.

For the plantation owners, the best scenario is to grow yews with higher taxane content and higher growth rate [77],[82],[107]. The way to profit is to achieve larger quantities of taxanes from the same amount of land with minimal increase in cost [77],[82]. On top of all the financial considerations, sustainability is another concern. In fact, pharmaceutical companies must prove that their active ingredient is obtained from a sustainable source, so they can get approval to launch the product on the market.

The direct consumers of the milking device will be plantation owners and those companies who are doing harvesting. However, since each stage is closely related, it will be helpful if bulk paclitaxel manufacturers requirements are taken into account as well.

7.3 House of quality

The way for a product to enter the market is to beat the competition and to serve the customer as good as possible. As was explained in the design methodology chapter, the house of quality is a tool, which can help translate customer needs into product specifications. It can show what contradicting requirements must be solved in order to design a product that will beat the competitive products and has the properties customers like to see in a product. The house of quality consists of a series of matrices which are linked with each other [9]. In Figure 27 below the filled in house of quality can be seen. First the meaning of all the different parts of the house of quality is explained and then how it was filled in for the taxane milking device. Finally the contradicting requirements that resulted are explained.

First of all, the customer and product requirements have to be known. This can be seen in the left part of the HoQ. The product requirements are divided into technical details, performance measures and size of range. With these requirements, the new product can be compared to the existing products. Therefore the competitors with their products are considered in the right part of the house of quality. In our case the taxane milking machine is compared to the existing processes to get taxanes. The comparison is done by filling in the planned matrix to obtain the most important improvement factors. This can be seen in the last two columns.

When the right part has been filled in, the middle part (the bold square) can be filled in. In this square the customer requirements are linked with the product requirements; strong, moderate and weak relations between the customer requirements and the technical requirements of the product are filled in with three types of circles, as can be seen from Figure 27. If there is no relation, the cell is empty.

The 'roof' of the house of quality identifies the requirements that characterize the product. An improvement of one requirement can cause an improvement of another. In this case the interaction of the improvement is positive or supporting. The opposite can also be true; in that case the interaction is negative. From these interactions the contradicting requirements can also be found. Finally, the lower part of the house of quality can be filled in to find the product requirements that need most attention, the technical requirements. The technical priorities are calculated by the sum of the relationship weighing factor multiplied by the overall weighing.

7.3.1 Competitors

We compare our method with two competitors, namely harvesting the needles and subsequently applying extraction, and cell cultures, because these two methods are the two most used processes. The taxanes obtained with our product also needs to be purified, however we will skip some purification steps compared with the competitors. The comparison with competitors is thus on the processes they use, compared to our milking machine that uses the process of electrospray for harvesting.

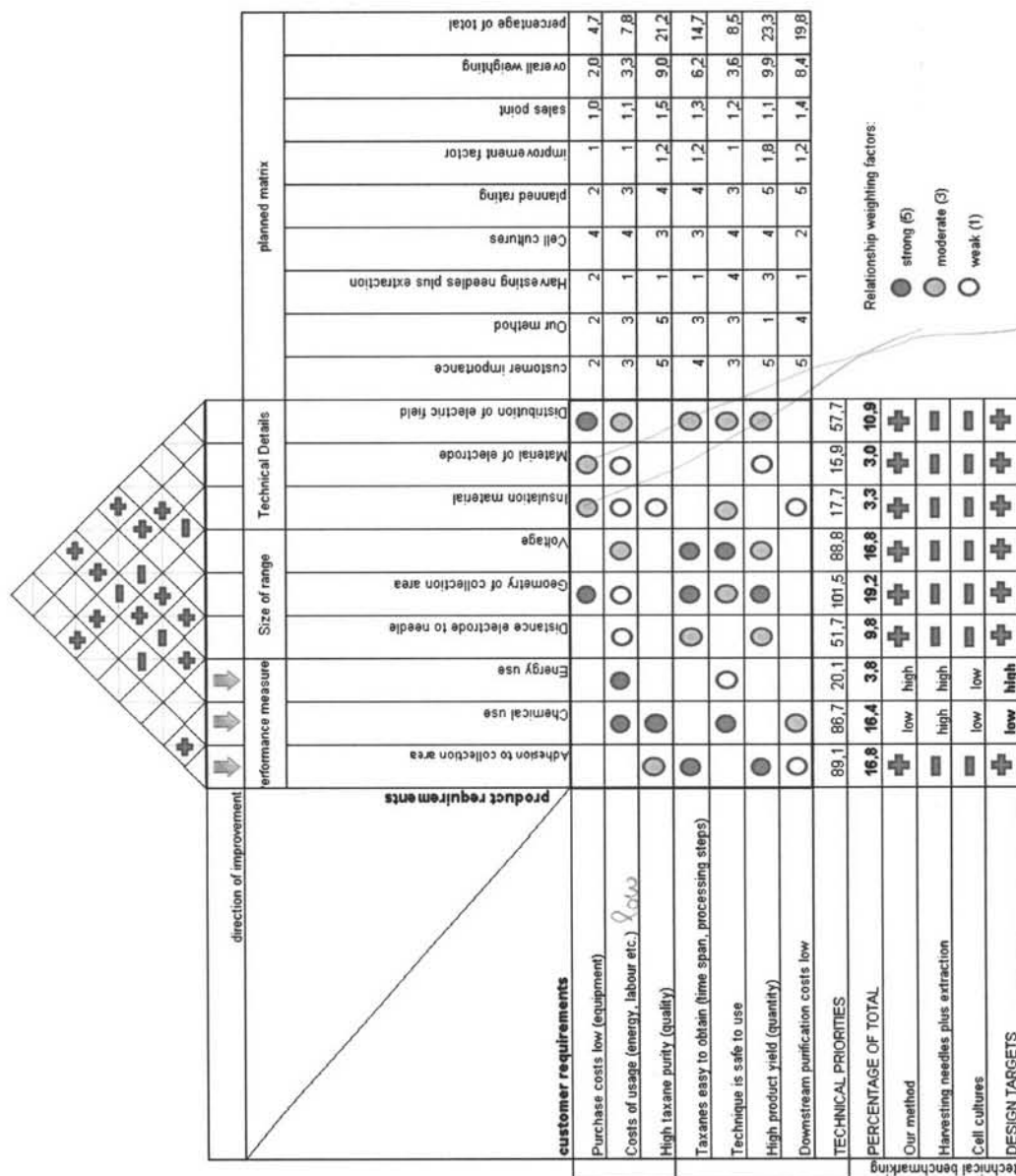


Figure 27 House of Quality for the taxane-milking device

7.3.2 Customer requirements

The customer of our product is someone who is producing precursors of Taxol, namely an owner of a tree plantation. Most of the customer requirements considered are direct customer requirements, but we also thought about the next customer in the product chain, because the demands of the customer of our customers are also relevant for us to consider. These are indirect customer requirements, related to the downstream purification difficulties and costs.

From the market research it appeared that the following customer requirements are the most important:

- **Purchase costs:** this refers to the purchase costs of the equipment needed for the harvesting of taxanes ("direct" purchase costs). In the case of electrospray this is the electrospray harvesting machine. We compare this with equipment necessary for cell cultures – i.e. a

vessel and extraction device – and equipment for cutting and extracting needles – cutting machines for the shootings and branches, equipment of grinding, freeze drying and extraction equipment

- **Costs of use:** this refers to all the energy and labour costs that are needed during the harvesting of the taxanes
- **High taxane purity (quality):** this refers to the purity of the taxanes that come out of the tree after the harvesting step, compared to the purity coming from the extraction (i.e. before purification), or directly from the cell culture. Thus after electrospray, after clipping, and after production in cell cultures
- **Taxanes easy to obtain:** this refers to the fact that it is favourable if obtaining taxanes doesn't take that long during the harvesting step, and that there are few, rather simple processing steps before purification to conduct
- **Technique is safe to use:** safety means that during the harvesting there is no danger to human health originating from toxic chemicals, high voltage, explosion danger, etcetera
- **High product yield:** a large quantity of product should be obtained per harvest (production rate)
- **Downstream purification costs low:** the purification costs after electrospray should be low, because this means that our customer can sell the paclitaxel precursors at a higher price because there are less impurities that pose a problem for downstream processing

7.3.3 *Planned matrix of customer requirements*

The three most important customer requirements are the taxane purity after harvesting, low downstream purification costs (because a low amount of impurities that are difficult to separate), and high product yield. How easy the taxanes are to obtain is also of importance to the customer, but slightly less than the purity and amount of product that are obtained.

The competitive advantage of our product lies in getting out the taxanes at a high purity; it is thought that the mixture that comes out of the needles contains much less substances than the dried clippings, of which mainly water (which will partially evaporate) and taxanes. This is in contrast with the other methods; extraction is really troubling because the whole needle is used as a starting point, and the needles contain many more compounds than only the sap from the tree. In the cell cultures method the main problem is to separate the products from the watery environment and the cells themselves. This is slightly better than for extraction, but still the purity will be low.

These competitive advantages also influence the "taxanes easy to obtain" in a positive way. The taxanes are quite easy to obtain with our method; although the harvesting takes a long time to only get out a little, this time span is not so long if you compare it with extraction. During the extraction method you first need to harvest the needles, grind them and freeze dry them before you can apply extraction. The process conditions [5] and the large amounts of solvents that are needed for extraction complicate obtaining the taxanes. Cell cultures perform similarly to electrospray; taxanes are easier to obtain than with extraction.

The easiness to obtain the taxanes and the purity will also influence the downstream purification costs. The downstream purification costs for extraction are high, because it is difficult to separate the chloroplasts from the taxanes [5]. The purer the taxanes, the less impurities are present. As said before the purity will be very high, for the electrospray harvesting of taxanes, and especially the chloroplasts are absent, which implies low downstream purification costs.

The yield of taxanes of our method on the other hand is low; the amount you get out per needle is most likely far less than for extraction and cell cultures. The focus of our product will therefore be on the quality instead of the yield.

7.3.4 The product requirements for the electrospray device

The performance measures, size of range, and technical details of the electrospray device are explained below.

Performance measures:

- **Adhesion of droplets to the collection area:** the droplets that are sprayed have to be collected in some sort of way. One of the problems that are currently faced with electrospraying taxanes is the fact that the droplets that are sprayed on the electrode have a very high adhesion and that a lot of solvent has to be used to get the taxanes from the surface [30]. As will be explained below, an insulator will be put on the electrode, and this will be at the same time the collection area. The insulator has the same problem as the electrode: sprayed taxanes will adhere to the insulator, either as water droplets with taxanes dispersed or as solid. The collection area will perform better if adhesion of droplets and taxanes to the insulator is small, because then it is easy to collect the taxanes that are inside the droplets
- **Chemical use:** the device will perform better if only a small amount of solvent is used to rinse the taxanes from the collection area
- **Energy use:** the energy use of the electrospray device should be low. This has to do with the distance of the needles to the field, the field strength (voltage), how optimal the field distribution is, and how well the insulator works.

The direction of improvement of these three performances is down: adhesion, chemical use, and energy use should all be minimised

Size of range – several ranges that are important for the design:

- **Distance of electrode to the needle:** the distance should be as small as possible, without creating short-circuiting. This distance should be optimised
- **Geometry:** this means the form and size of the collection area. The form could be round, curved, straight, etcetera
- **Voltage:** the voltage can be varied, and the optimum depends on the other parameters. There is a minimum voltage necessary at a certain distance of the needles to the plate. There is probably also a maximum voltage; above a certain voltage the insulator will become conducting

Technical Details

- **Insulation material:** a large percentage of the needles must be reached to enable milking; the electric field needs to be strong enough for these needles to spray. This means that the electrode should be close to the needles. However, short-circuiting must be prevented; when the needles are too close to the electrode, spark discharge will occur, burning the needles and leaving them unable to spray. Short-circuiting can be prevented by finding a good insulator that blocks current. The purpose of the insulator is to allow the needles to be close to the electrode, while not letting any electrodes pass for discharge. The structure and material of this insulator are also important because the electric field must not be blocked
- **Distribution of the electric field:** this should be optimised in the sense that all the needles sense a strong field, without using too much electrode material and energy

7.3.5 Correlation between product requirements and customer requirements

From Figure 27 the correlation between the product requirements and customer requirements is shown. The dark blue circles indicate direct correlations, while the light blue and white circles indicate indirect correlations. In the text below these correlations will be explained.

The purchase costs of the equipment are influenced by the geometry of the collection area; the more area and more complicated form of the collection area needed, the higher the price of the equipment. The distribution of the electric field also influences the purchase costs; for example if electrode wires are used that are immersed in the insulator it is more expensive to manufacture than a simple plate or foil. Other things that influence the purchase costs to a lesser extent are the (costs of the) materials of the insulator and electrode. Finally the device needs electronics, software, and sensors to be able to perform the electrospraying automatically. The electronics and software for the robot also influence the purchase costs of the device. The technology is available and it is not really high-tech, so it will not influence the price that much [108].

The costs of use are costs other than the equipment itself. The two factors that influence these variable costs most are the chemical use and the energy use. The energy use correlates to the voltage and the distribution of the electric field as was explained above, and other factors that influence the energy use a bit too are distance of the electrode to the needle, geometry of the collection area, and the kind of material that is used for both the insulator and the electrode.

The taxane purity is mostly influenced by the solvent use. The more chemicals are needed to get the taxanes from the collection area, the less the purity of the taxanes will be. The chemical use is mainly influenced by adhesion to the collection area. The kind of insulation material influences the taxane purity via the adhesion of the droplets.

How easy the taxanes can be obtained is mainly dependent upon two parts of the collection process, namely the step where the taxanes come out of the needle and are collected at the electrode insulator combination and how easy the taxanes can be obtained from the collection area. Whether the taxanes come out of the needle easily is mainly dependent upon the voltage and the distribution of the electric field, and indirectly on the distance of the electrode to the needle. Whether the taxanes can be easily obtained from the collection area depends mainly on the adhesion of the droplets to the collection area and therefore indirectly on the viscosity of the collected droplet. ?

The safety of the electrospray milking device depends on two main factors; the amount of chemicals that is used and how high the voltage is. The geometry of the collection area and the distribution of the electric field also contribute to the safety for the people working in the neighbourhood of the device. It depends on the insulation material that is applied and how well the insulation material is and whether the collection material can be touched. The energy use is indirectly correlated to the voltage, thus this has a weak relationship with safety.

How high the product yield is depends on the amount of taxanes that come out of the needles – thus there is a relation between the product yield and the voltage, distribution of the electric field and the distance between the needles and the electrode. The material of the electrode has an indirect influence via the voltage.

The geometry of the collection area and the adhesion of the taxanes to this area however have a larger influence on the product yield than the above factors. The viscosity of the droplets has a minor influence via the adhesion to the collection area as was stated before.

The downstream purification costs are moderately influenced by the chemical use and the viscosity of the collected droplets. There are minor relationships with adhesion to the collection area and insulation material because they influence the amount of chemicals that are needed for purification.

7.3.6 Correlation among product requirements

In this section the correlations between several product requirements are described. The order of explanation is from the left diagonal row in the roof of house of quality to the lower right. Double correlations were skipped: thus if a correlation between A and B is already explained, the correlation between B and A will not be mentioned again. The plus and minus signs behind the correlations indicate a positive or negative correlation, respectively.

The adhesion to the collection area has a correlation with:

- The chemical use; less adhesion means that less solvent is needed to get the taxanes from the collection area (+)
- The kind of insulation material that is used. The better the insulation material is designed, the less adhesion of the droplets to the insulation material will take place (+)

The chemical use has a correlation with:

- The geometry of the collection area; the best collection area is the one that reaches most of the needles; this *could* mean a large or complicated surface area. This can increase the needed use of chemicals (Which means that they can contradict each other, -)
- The insulation material; the more affinity the material of the insulator has with the droplets, the more chemicals are needed. Designing a better insulator means less chemical use (+)

The energy use of the electrospray device has a correlation with:

- The distance of the electrode to the needle; an optimised distance means minimal energy losses (+)
- The geometry; If the geometry of the collection area is not optimally designed, the energy use of the device will be higher (-)
- The voltage; a higher voltage means more energy losses and higher energy use (+)
- The insulation material; the less well designed the insulation material is, the higher the energy use (-)
- The material of the electrode; using a better suitable material (conductor) for the electrode means less energy use (+)
- The distribution of the electric field; an optimal distribution of the electric field means less energy use (+)

The distance of the electrode to the needle has a correlation with:

- The geometry of the collection area; if the geometry of the collection area is optimised, the distance of the electrode to all needles is optimal (+)
- The voltage; a higher distance between the needles and the electrode means that a higher voltage is needed to have the same field strength (+)
- The insulation material; a thicker insulation material means a higher distance between the electrode and the needles. A well-designed insulation material is as thin as possible without creating short-circuiting. Thus a less well designed insulation material increases the distance between the electrode and the needles (-)

The geometry of the collection area has a correlation with:

- The distribution of the electric field; the geometry of the collection influences the distribution of the electric field. The more the geometry of the electric field is optimised, the more optimal the distribution of the electric field will be(+)

- The material of the electrode; whether the material of the electrode can be made flexible also influences whether a flexible collection area is possible (+)

The voltage has a correlation with:

- The insulation material; more insulation means that a higher voltage needed. This means that a less well-designed insulator increases the (minimum) voltage that is needed for electrospray to happen (-)
- The material of electrode; if the resistance inside the electrode is high, the voltage that is needed is higher (+)

The technical priorities:

From the house of quality it follows that three areas of focus can be identified for the technical priorities:

- The geometry of the collection area (this has a priority of 16.8%)
- Related to the collection device; adhesion to collection device (16.8%), and chemical use (16.4%)
- Related to the electric field; voltage (16.8%), and distribution of the electric field (10.9%)

The second two areas are the most technically challenging ones. The geometry of the collection area is something important to take into account in the sense that it should be simple but effective in and supportive to the milking of the paclitaxel precursors.

Contradicting requirements

The three contradicting requirements that can be distinguished from the previous are the following:

- Reaching a large percentage of the needles, while short-circuiting is prevented. A too high distance means that fewer needles are reached for electrospray or a higher voltage will be needed on the electrode to maintain the right field strength; hence the yield per tree will be too low. Making the distance too short will mean that short-circuit will occur, burning the needles. The solution to this problem that was suggested in this section was putting an insulator on the electrode. In that case the needles can touch the electrospray device because they are not in direct contact with the electrode. The requirements, however, on the properties during operation of the insulator become much more important, because insulators will not be insulating under all conditions
- The second contradicting requirement is therefore that the insulator has good properties; it must let the electric field pass through, while on the other hand the taxanes and the solvent are not allowed to pass through and touch the electrode
- The third contradicting requirement is using a low amount of solvent to collect the taxanes, while rinsing as much off the collection area as possible. The use of chemical solvent will be necessary for the removal of the taxanes from the device, however, if too much is used the costs will rise significantly. Using too little will mean that too little of the taxanes are collected, which will decrease the amount of revenue from selling the taxanes

When the detailed design is conducted in the Chapters 8, 9 and 10 more detailed contradicting requirements will pop up, because these are related with certain choices regarding the design, e.g. the wetting properties of the insulator.

8 Design specifications

The main technical priorities to be focused on were explained in the previous chapter on the house of quality, whereby the technical priorities were translated into contradicting requirements. The contradicting requirements have to be solved in order to arrive at a good working milking machine of taxanes. There were three main contradicting requirements identified. The first one is reaching a large percentage of the needles and having a short distance between the electrode and the needles, while short-circuiting is prevented. The proposed solution in the previous chapter was to use an insulator to prevent this. The insulator has to have the right properties in order to prevent short-circuiting. In addition, there are two contradicting requirements for the insulator; letting the electric field pass through, while the taxanes and the solvents cannot pass through. The third contradicting requirement is using a low amount of solvent to collect taxanes, while rinsing the taxanes of the collection device is easy. In the detailed design sometimes some more contradicting requirements pop up when for example a choice for a certain type of insulator is made. These requirements will be discussed whenever needed in either this chapter or the chapters where the design decisions are made (Chapter 9 and 10).

In this chapter the main problems of contradicting requirements that have to be solved for each part of the milking device will be elaborated, by finding the parameters that influence the desired properties of the milking device.

The milking device can be divided into several parts, although they are all correlated to each other. Of course the device can be divided into the electrode and insulator, but first other factors that influence the design of the electrode and insulator will be elaborated, e.g. the shape of the tree, the distance between the needles and the insulator, and the collection mechanism.

8.1 Factors that influence the design of the electrode and insulator

The main influence on the electrode design is the shape of the tree. The trees of the yew species can be cut in many shapes [80]. These shapes will influence the design of the electrode and the taxane-harvesting machine in the following ways; either single branches are enveloped by the electrode-insulator combination, or a large surface of the electrode reaches more branches in one go. To reach more needles, one can even think of pushing the collection device into the tree. When choosing the shape of the tree, one must keep in mind the design parameters of the electrode that are given in Table 9 below. If a certain shape of the tree will eliminate the best option for an electrode, this tree shape may not be desirable.

The design of the insulator is influenced by two factors that are related to each other; the distance of the needles to the insulator, and the way in which the taxanes are collected.

Regarding the distance of the needles to the insulator a choice has to be made between the needles letting touch the insulator or not. This will heavily influence the collection mechanism and the kind of insulating material that should be used, because a few basic assumptions are based on this. For example, a decision has to be made if it is desired to facilitate spraying or not. If so, distance holders for branches are desired, although one would still want a minimum distance for spraying to take place. Then evaporation of the sprayed droplets should be taken into account, because really small droplets are sprayed, which will evaporate very quickly.

If the needles are allowed to touch the insulator or if this is even desired, a different mechanism instead of spraying should be taken as a basis. The mechanism is then the sliding of the droplet from the needle onto the insulator. In this case the wetting properties of the insulator become important, as will become clear in Chapter 10. If touching the insulator by the needles is desired, a mechanism

to push branches together might be needed. This might also be necessary if collection of the taxanes is performed at one side of the electrode-insulator combination, instead of two. So also a choice should be made whether collection of the taxanes will be conducted at both or one sides, related to the collection mechanism and the distance of the needles to the collector.

When this choice has been made, which also implies the choice of collecting a solid or a liquid on the insulator, the collection mechanism that is used to collect the taxanes can be chosen. Next to that, a discharge mechanism for the collected taxanes might be needed, as will be explained in Section 8.3 below. Finally the kind of solvent and the amount of solvent used has to be established, which will influence the easiness of downstream processing but also the materials that can be used as insulator due to chemical stability under solvent exposure conditions. Degradation of the taxanes under UV-light and heat will influence the frequency with which the taxanes have to be collected. The shape of the collection device comes forward from the choice of the collection mechanism and the amount of solvent used.

Finally, all the different parts of the taxane milking device have to be combined. For example, the way in which the electrode and insulator are combined has to be decided, as well as how the total machine will look like. This final combination will be discussed in Chapter 11.

8.2 Design parameters of the electrode

Previously, the electrospray mechanism was explained and that a positively charged electrode is needed for the design. There are several properties of the electrode that have to be designed; in Table 9 below an overview is given of the most important properties of the electrode, and the parameters that influence these properties. These parameters will be explained below per desired property. The four most important desirable properties of the electrode are a homogeneous field, appropriate field strength, low energy consumption, and low heating of the electrode. In general, these properties can be achieved by choosing the right electrode material. Furthermore, probably a system of a number of plates is needed to reach a large part of the needles for electrospray. With respect to this the geometry, spacing and positioning of the electrodes is important, which will influence the electric field strength and its distribution.

Table 9 Desired electrode properties with the parameters that influence these properties

Electrode property	Parameter of influence
Electric field configuration and distribution	Shape of electrode Roughness of surface and surface configuration Distance between electrodes
Field strength	Distance between electrodes Distance between needles and electrode
Energy consumption	Volume resistivity of the material used Current
Heating	Volume resistivity of the material used Current

8.2.1 Electric field configuration and distribution, and field strength

The shape of the field – its configuration and distribution – is influenced via the shape of the electrode, the distance between the different electrodes and the roughness of the surface (or the surface configuration). The direction of the field lines is mostly determined by the shape of the electrode and the distance between the electrodes. During electrospray, the droplets emerging from the tip of the cone jet will, after break up, follow the field lines to the region of lower potential. These field lines are directed perpendicular to the equipotentials [109]. Since all electrodes will be

charged positively, the field lines will not go from electrode to electrode. The 'neatness' of the field will be determined by the roughness of the surface. This is because the field lines start perpendicular to the surface of the electrode. If the surface is rough, the start of the field lines will be uneven as well. The electric field strength becomes higher when the curvature of the electrode increases. The charge accumulates at the largest curved locations; hence the electric field strength is higher [110]. This can be seen from Figure 28 where an example is given for a sharp peak and a blunt point [111]. The field strength is also influenced by the distance between the needles and the electrode. When the distance is too large a higher voltage is needed to maintain the field strength reasonably high at the needle tip, since the field strength will be lower after crossing larger distances.

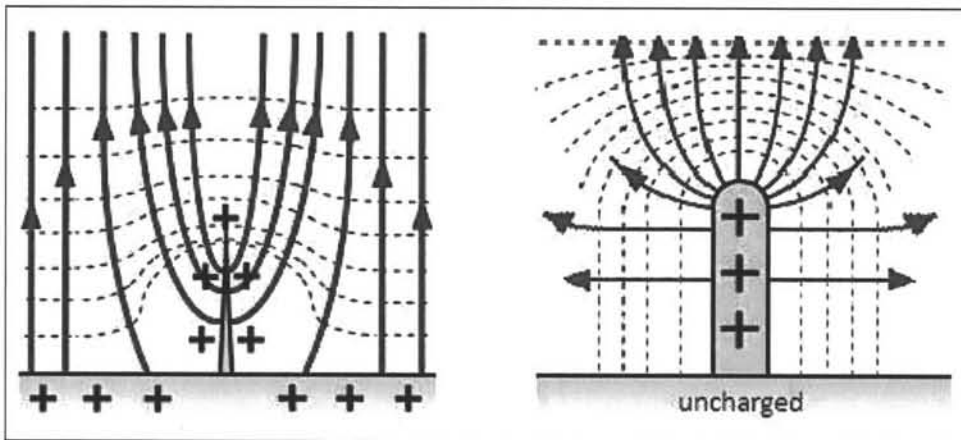


Figure 28 Electric field lines for a sharp peak (left) and a blunt point (right) [111]

8.2.2 Energy consumption and heating

Low energy consumption of the electrode is favourable, since it will affect the variable costs. The energy consumption of the electrode is influenced by the volume resistivity of the material that is used for the electrode, and the current that goes through. The current will finally be dissipated into heat that will result in energy losses, because only high voltage without current is desirable. As will be explained below, the so-called permittivity of the insulator will influence the percentage of the electric field that is let through. This will also influence energy consumption. The main energy losses of the electrode are thus due to current being transformed into heat, which is also related to the volume resistivity of the material of the electrode. When the resistance is very high, the material will heat up much more with increasing current, thus a low volume resistance is desirable.

Heating up of the electrode should also be low because of safety reasons. In order for the device to be safe in use the current should be very low, because this prevents heating. Therefore a high resistor is needed inside the unit that generates high voltage [112]. In fact, the current is already very low because the tree and the ground are included in the circuit and already have a high resistance. For the electrospray, the voltage on the electrode is required to be 4 kV. In order to close the circuit the needles spray charged droplets. When an insulator is put on top of the electrode to prevent short-circuiting, the field lines will still go through, but electrons are unable to pass. This means that there is no flow of electrical charge from the electrode to the needles, and hence there should not be an electrical current. In practice there will be a small leakage current, but the current will be very low [113].

8.3 Design parameters of the insulator

The electrode needs an insulator on top to overcome short-circuiting due to contact from the needles with the charged electrode. A large quantity of materials can be used as insulator, with all different kinds of properties. The different material properties can be compared with each other in order to make a concise choice for the final design. There are three main types of properties that can be identified for the application in a taxane milking machine: electric properties, (chemical) stability, and wettability of the surface. The electric properties can be subdivided into electric field shielding, current blocking, and discharge. The insulator shields the electric field, but the field lines produced by the electrode must be able to pass through the insulator. Otherwise, the needles of the tree will not encounter the electric field and will not spray. On the other hand, the insulator must block movement of charge, either electrons or ions. Water absorption, among others, strongly influences the electric properties.

Furthermore, in all circumstances the insulator must remain stable, especially under the conditions of electrospraying. The insulator must be fit for many cycles of use without degradation.

The wettability of the surface is important for the collection mechanism. The collection mechanism is also influenced by the mobility of the droplets or solvent, which is partly influenced by the wettability, but also by what kind of surface is used for the insulator.

The parameters that influence these basic properties can be seen from Table 10.

Table 10 Insulator properties with the parameters that influence these properties

Insulator property	Parameter of influence
Electric field shielding	Dielectric constant Water absorption Thickness of insulator Bulk configuration of insulator
Blocking movement of charge	Volume resistivity Thickness of insulator Dielectric strength Water absorption Bulk configuration of insulator
Energy dissipation	Dissipation factor Dielectric constant
Safe discharge	Relaxation time
Wettability	Contact angle (van der Waals forces)
Mobility of droplets and solvent	Contact angle Surface configuration insulator
Chemical and physical stability	UV resistance Resistance to chemicals Scratch resistance

8.3.1 Electric field shielding

The electrode with the needles can be seen as a capacitor, whereby the electrode will be positively charged (the + "plate") and the needles get a negative charge (the - "plate"). The insulator that is put in between, also called dielectric, can have several purposes; the plates (the electrode and needles) can be placed close together without touching, which increases the capacitance. All insulators will let the field pass through, to what extent depends among others on the dielectric permittivity of the

material and the thickness of the layer [114]. This results in a higher voltage that needs to be applied in order to let the charge pass through the dielectric layer [63]. The dielectric constant is the ratio of the permittivity of a substance divided by the permittivity of free space:

$$k = \frac{\epsilon}{\epsilon_0}$$

The capacitance can be related with the dielectric constant as follows:

$$C = k \cdot C_0 = k \cdot \epsilon_0 \frac{A}{d} = \epsilon \frac{A}{d}$$

Hereby, k [-] is the dielectric constant, C_0 [F] the capacitance if the space between the plates is vacuum, A [m²] the surface, d [m] distance between the plates, ϵ [C²/N·m²] the permittivity and ϵ_0 [C²/N·m²] the permittivity of vacuum. The formula holds as long as the electrode is a flat plate.

The structure or bulk configuration of the material that is used for the insulator – e.g. crystalline, amorphous, or with air channels or air bubbles in it – has influence on the dielectric constant, as well as the fillers and additives that are used in the case that a polymer is used as insulator [115]. Air presence implies that the dielectric constant is lower, which means lower shielding of the field. Fillers and additives can either increase or decrease the total dielectric constant of the insulator, depending on their own dielectric constant [115].

The electrospraying will imply that there is presence of water from the plant saps that are sprayed, and it can also be assumed that the insulator can get wet because of precipitation. Water absorption also has an influence on the dielectric constant and other electrical properties of an insulator [116]. If an insulator can take up a large amount of water, the water molecules can increase the blocking of the electric field, because they are dipoles, which will move under the influence of the field. Layers of dipoles will effectively shield the charge and thus the field of the electrode. The amount of the field that is let through is thus dependent on the dielectric constant, and how it is influenced during electrospraying, but is also linearly dependent on the thickness of the insulator. [117].

8.3.2 Blocking of charge movement

There are two main parameters that influence the movement of charge, either electrons or ions, inside solid materials. The first one is the volume resistivity (in $\Omega \cdot m$), which is a measure for the resistance a material provides against a flow of an electrical current. There are three types of materials in this respect; conductors like metals, semi-conductors, and electric insulators. Insulators do not allow electrons to flow because they have a large band gap; the valence band is full of electrons, and electrons can only move if they are excited in a higher energy level, called the conduction band. This costs energy, while in conductors the electrons can move freely in the same energy band, without need of exciting the electrons [118],[119].

The volume resistivity is related to the resistance in the following way [120]:

$$x = \frac{R \cdot A}{\rho}$$

Where;

x = thickness [m]

R = resistance [Ω]

A = cross-sectional area [m²]

ρ = volume resistivity [$\Omega \cdot m$]

Therefore, there is also a linear relationship between the resistance and the thickness of the insulator: increasing the thickness is similar to putting a larger resistor in the electric circuit [120].

The second main parameter of influence on blocking an electrical current is the dielectric strength. At a certain point of voltage supply to an insulator, there is enough energy supplied to excite the electrons to the conduction band from the valence band. Then the insulator is not insulating anymore [63]. The voltage at which this phenomenon occurs is called the breakdown voltage, and it will be an important parameter in the design of our product, because the insulator must be able to withstand the field strength at which electrospray occurs [119]. For air the dielectric strength is 3 kV/mm, but the insulator itself needs a much higher dielectric strength [121],[122].

Water absorption of an insulator can compromise the dielectric strength. Water is an electrolyte, and can thus create channels inside the material through which current can flow, which will lower the dielectric strength drastically. The bulk configuration of the insulator also influences the dielectric strength. Air bubbles or filler materials and additives inside an insulator can also create channels through which current can flow much more easily than in the rest of the insulator. Another example is impurities in insulating materials that can facilitate the flow of a current, e.g. in silicon solar cells that are doped with boron or phosphorous. The impurities disrupt the normal balance of electrons, from which surplus electrons or holes arise, that can jump from atom to atom in the silicon lattice, thus creating a current [123].

8.3.3 Energy dissipation

The amount of energy that is dissipated is dependent on the dissipation factor, which is the ratio of current dissipated into heat to the current transmitted. For applications like electrospray the energy dissipation is very low, because the current is also very low [115].

8.3.4 Safe discharge

Charged droplets are sprayed onto the insulator. Because the surface is insulating, the charge cannot flow away immediately. Thus the surface will be charged after spraying, and it needs to be discharged in a safe way. Not all insulators are completely insulating, which means that after a certain period of time, the charged droplets will lose their charge and become neutral. The relaxation time is the time it takes to discharge the droplets and can be calculated by the following formula [124]:

$$t_r = \frac{\varepsilon \cdot \varepsilon_0}{\sigma} = \frac{k \cdot \varepsilon_0^2}{\sigma}$$

If the discharge time is too long, i.e. longer than the time the electrospraying takes place, a safe discharging mechanism has to be devised.

8.3.5 Wettability and mobility

As said before, the wettability of the surface becomes important when the needles touch the insulator; if this is the case the surface should be more wetting than the needles because the liquid, consisting mainly of water with taxanes in it, is then drawn to the surface.

The wettability of a surface is defined by the contact angle a water droplet makes with the surface of a solid (see Figure 29 below). The underlying mechanisms of the contact angle are the van der Waals forces between the insulator and the water droplets containing the taxanes [125]. The van der Waals forces are short-range attraction forces between atoms and molecules. There are several types of short-range van der Waals forces between molecules and atoms: forces between permanent dipoles,

and instantaneous forces between fluctuating dipoles of non-polar atoms and molecules (also called London dispersion forces), or a mixture of these two types [125].

The London dispersion forces arise from the distribution of the electrons around the nuclei. The electrons density will fluctuate because of the movement of the electrons, from which a fluctuating dipole arises. The electron clouds of other molecules in the neighbourhood respond to the fluctuating dipole, and the net result is an attracting force between molecules [126],[127]. The London forces are mainly dependent on the polarisability of atoms or molecules; larger molecules can be polarised more easily, which results in higher attraction forces. A higher contact area will also increase the forces [128].

The contact angle can be calculated by Young's law; when a droplet of liquid is put on a solid surface there are three interfaces to be considered: the solid-liquid, solid-air, and liquid-air interface (see Figure 29 below). The interface between these three interfaces is being minimised because of the surface tension of the fluid. When we consider a water droplet we have to keep in mind that water is a polar fluid, and that all the water molecules prefer to be inside the water droplet in order to avoid having a hydrogen bridge less as is the case at the surface interface between the droplet and the air. The same is true for the interface between a droplet and hydrophobic surface [126],[129].

Because every interface tends to minimise its surface area, there are opposing forces. Young's law is actually the balance of these forces as can be seen from Figure 29.

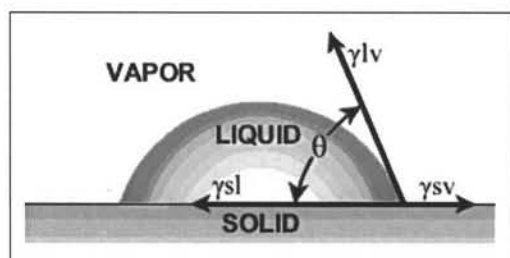


Figure 29 An illustration of Young's law [129]

The result is a certain contact angle between the droplet and the surface of the solid. The contact angle, θ , can be between zero degrees (complete wetting) and 180 degrees (no wetting) [126]. Young's law can be described with the following formula:

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos \theta$$

Where θ is the droplet contact angle, γ_{sv} , γ_{sl} and γ_{lv} are the surface free energies of the solid-vapour, the solid-liquid and the liquid-vapour respectively.

The wettability of the surface is also important for the mobility of the sprayed droplets or the solvent on the insulator. The flow of water droplets on a surface depends on the interfacial tension between the water and the surface, but also on gravitational forces, and the air resistance [130]. Certain types of surfaces facilitate the rolling off of liquids because they are very hydrophobic, other ones are hydrophilic and will slow down droplets because of the van der Waals interactions with the surface.

The mobility is, however, also influenced by the surface configuration of the insulator; an example is a lotus leaf that has very small – nano-range - pits on its surface, which will increase the apparent hydrophobicity and contact angle [131]. Larger scale surface configurations can also hamper or help the flow of liquids: a wave like pattern on the surface will let all the water or solvent flow to the lower gullies, where it is collected in streams of liquid going down. Liquid flow is on the other hand hampered when there are centimetre range cavities on the surface of the insulator, where puddles of liquid can form. In Chapter 10, many surface configurations are shown, and one surface configuration will be chosen.

8.3.6 Chemical and physical stability

The stability of the insulation layer is dependent on the material used and the way of use. To guarantee a long life, the material used should be chemically and physically inert to the influences during operation.

First of all, the insulator must be able to withstand UV irradiation, because the light of the sun consists of 2% of UV-light [132]. This is only a small part of the total solar spectrum, but the energy of UV-light is so high that it can break the molecular bonds of a polymeric material, resulting in cracking of the material [133].

Furthermore, the insulator should be able to withstand the solvent that is used to collect the taxanes; it can be assumed that a solvent is probably needed, because there will always be evaporation of the sprayed water droplets, leaving solid taxanes behind.

Finally, the surface must be resistant to the mechanical conditions: It must not be affected when scratched by needles that touch it, or sand that is lifted by the wind and blown through the plantation.

9 Design of electrode

In Chapter 8, the contradictive design parameters are considered and explained, which have to be solved in the design of the new milking device. In order to solve all the contradicting requirements, creativity sessions are held to devise alternatives, and morphological charts are made for each separate part of the design. In this chapter the design of the electrode will be explained. In Chapter 10 the design of the insulator will be discussed and in Chapter 11 the overall design of the milking device will be mentioned.

The design of the electrode depends on several factors; one among them is the shape of the trees. The shape of the trees largely determines what shape and size the electrode can take. Therefore, first the shape of the trees is discussed, after which enough information is gathered for the design of the electrode.

9.1 Shape of the tree

Since the tree is of influence for the milking device, it is important to make a concise choice for the shape of the tree. In order to do this, a morphological chart is made with all kind of possibilities for the tree shapes (see Figure 30).

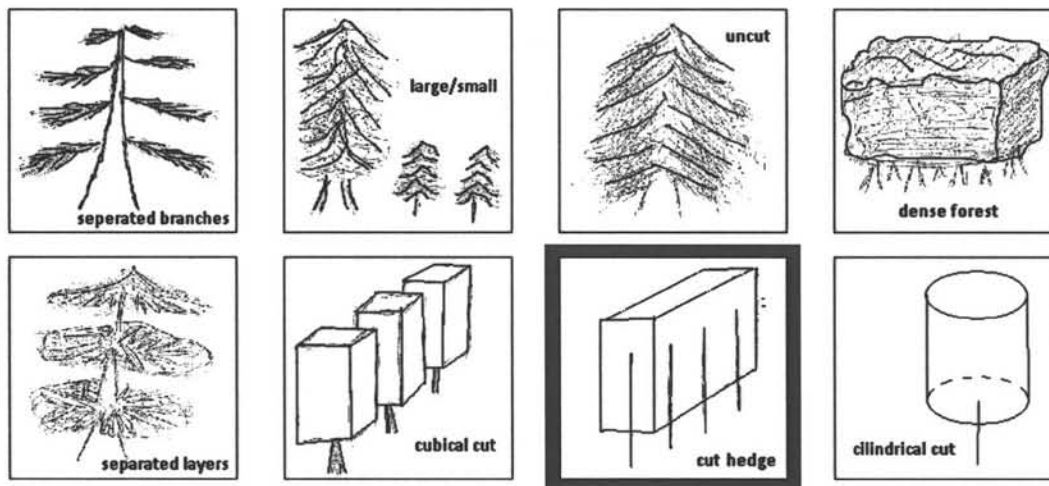


Figure 30 Morphological chart; shapes of trees

Although yew trees can be grown in many shapes, it is of importance to be realistic in the attainability of those shapes, compared with the easiness to harvest the taxanes. Ranking the drawings on easy access for electrospray, the separated branches and layers (drawing 1 and 5) will be most favourable. By these configurations the branches are clustered in horizontal planes. The size of the gaps can be optimized to the thickness and the electric field distribution of the electrode. However, the costs to maintain these complicated shapes are very high [80], and the yield per tree will be low. Since harvesting taxanes for an uncut tree (drawing 3) is rather complicated, for a dense forest (drawing 4) it is almost unfeasible. This is due to the unstructured branches and their unclear sizes. The cubical and cylindrical shaped trees (drawings 6 and 8), and the cut hedge (drawing 7) can be pruned by machine, which is cheaper. The cylindrical and the cubical shaped trees have the largest surfaces, but need more space and clipping than a hedge. Furthermore, space is needed between the trees, the amount of which depends on the size of the milking device and the amount

sunlight. It is assumed that this extra open space decreases the total surface area of the trees in such an amount that the hedge will eventually be the more effective one. Since a dense hedge is easy accomplishable and easy to clip, the cut hedge is chosen as shape of the trees. More information regarding the shape, species, and number of trees can be read in Chapter 12.

9.2 Design electrode

The design of the electrode depends on several parameters, among which the configuration of the apparatus, which is dependent on the shape of the trees. A small morphological chart shows the different configurations that can be applied (Figure 31).

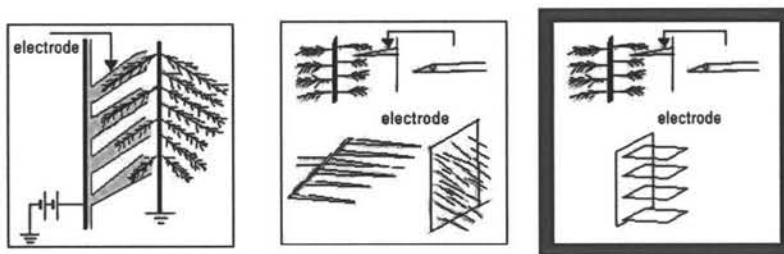


Figure 31 Different configurations for electrospaying of trees

The left most picture shows a configuration in which all the branches of the trees are surrounded by separate electrode tubes. Since the shape of the tree is determined to be a hedge, this will not be easily applicable. The two pictures at the right remain, in which horizontally orientated electrodes, supported by a vertical unit, are put between the branches. The middle picture shows spikes as horizontal electrodes and the right most picture shows plates. Since plates have a larger surface and are easier to apply, the horizontal plates will be chosen as configuration.

For the design of the electrode itself, a variety of shapes has been created and the outcome is visible in the morphological chart in Figure 32.

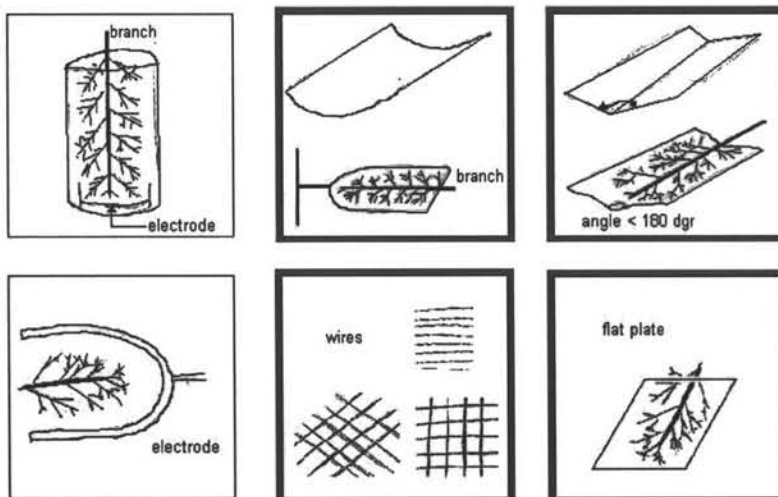


Figure 32 Morphological chart of electrode

The upper left picture surrounds the branch, which is hard to accomplish in a hedge, the lower left one is more like a spike and hence has not enough surface. The other four pictures might be feasible for the hedge; therefore they will be looked at more detail and the two pictures on the left are discarded.

For the electrode, a flat plate, a curved one or a V-shape with different angles is probable. Other possibilities are wires or crossed wires, namely a grid. All these shapes can be pushed in the hedge and if needed there is a possibility to surround the branches with electrode.

The different properties must be investigated and compared before the best shape can be chosen. In order to choose the best shape of the electrode, the electric field of these different shapes is modelled using the program Lorentz E [134]. Before the modelling, however, the electrode material is established, yielding a more realistic model. Furthermore, mobility issues and manufactural considerations are used to choose the best shape of the electrode.

9.3 Lorentz E - software

Lorentz is a program which uses the boundary element method to assess the electric field in the modelled area. The voltage that is stated for a volume is translated to charges on the boundary elements. To determine the trajectory of charged particles, Coulomb's law is used:

$$F = \frac{1}{4\pi\epsilon_0} \frac{q_1 q_2 (r_1 - r_2)}{|r_1 - r_2|^3} = \frac{1}{4\pi\epsilon_0} \frac{q_1 q_2}{r^2} \hat{r}_{21}$$

In which F is the force on a charge in N, q_1 [C] at position r_1 , due to the presence of another charge, q_2 [C] on position r_2 . In short form, the equation is the scalar form of Coulomb's law, in which r is the distance between the charges, and r_{12} the unit vector which gives direction to the force. The electric field can consequently be calculated by:

$$E = \frac{F}{q}$$

In which E is the electric field in V/m in which the particle is located, F [N] is the electric force on a particle with charge q [C].

Lorentz E performs these calculations for all boundary elements in the model. When the calculations are done, the program can show equipotential areas, field strengths, particle trajectories etc. in the model itself on a 2D plane. For needles to be able to spray, the electric field strength around the needles is most important, hence the electric field strengths of the different electrode shapes are compared with each other.

In order for Lorentz to calculate the required data, the material properties are of importance as well. The needles are modelled as water, since they exist of more than 60% of water [31]. The electrode material must be chosen as well before starting to model.

9.4 Electrode material

The energetic most favoured electrode materials lose as little energy as possible. Therefore the material needs a low resistivity, which means a high conductivity, since the resistivity is related to the conductivity as [63]:

$$\rho = \frac{1}{\sigma}$$

In which ρ is the resistivity of the material and σ the conductivity. The materials with the smallest resistivities are known to be silver and copper, with resistivities of respectively $1.47 \cdot 10^{-8} \Omega \cdot m$ and $1.5 \cdot 10^{-8} \Omega \cdot m$ [135]. Resistivities of more materials can be found in Appendix D-1.

Although copper has a higher resistivity than silver, copper will be the choice of material. This choice is mostly related to the price; copper is expensive, however silver is approximately 5 times more expensive (see Figure 33 [136]) and looking at price/quality ratio copper has the better one.

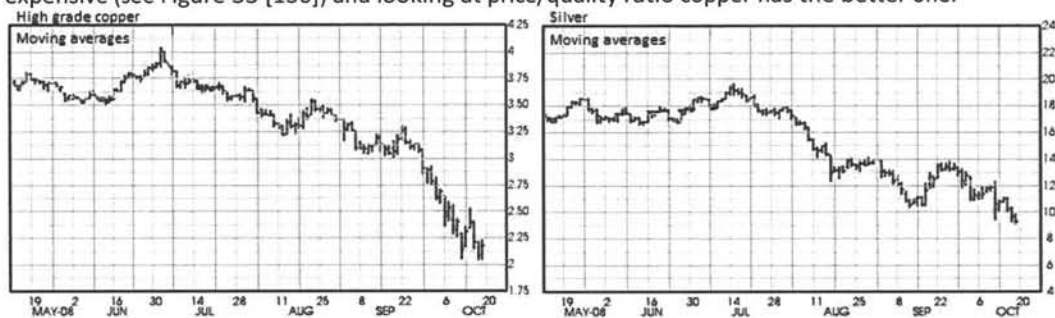


Figure 33 Prices of high grade copper and silver from May – October 2008 [136]

9.5 Model

Since the electrode material was chosen to be copper, the electrode is modelled as a copper plate (red plate in Figure 34). It is 60 mm X 60 mm wide, it is 2 mm thick and the top side has an altitude of 15 mm. The shape of the electrode will be changed, so different shapes can be compared. However, the size will remain approximately the same. The needles, modelled as cones of water (blue cones in Figure 34), hang at an altitude of 30 mm and with a unit height of 5 mm, resulting in the fact that the top of the cones are at 20 mm from the top surface of the electrode. The spacing of the needles differs from 5 mm to 10 mm in x and y directions, depending on the configuration. A symmetric situation (Figure 34(l)) and an asymmetric situation (Figure 34(r)) are modelled and compared. In the process of choosing an electrode shape, the insulator is neglected and only the interaction between plate and needles is investigated. All model input can be found in Appendix D-2.

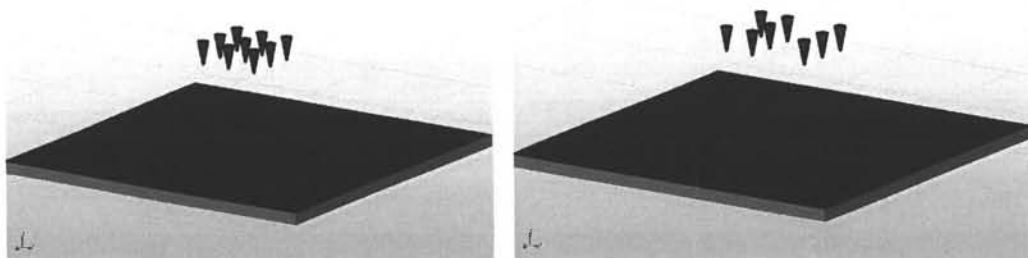


Figure 34 Model of plate electrode with symmetric (left) and asymmetric (right) needle arrangement

The electrode is charged with 4 kV in each model, and the needles are grounded. From the electric field plots it is visible that the electric field around the tips of the needles is largest; it is, in fact a lot larger than anywhere else; the red regions in Figure 35 have an electric field strength of approximately $2.8 \cdot 10^8$ V/m, whereas the light blue regions have an electric field strength of approximately $1 \cdot 10^8$ V/m and the dark blue 0 V/m.

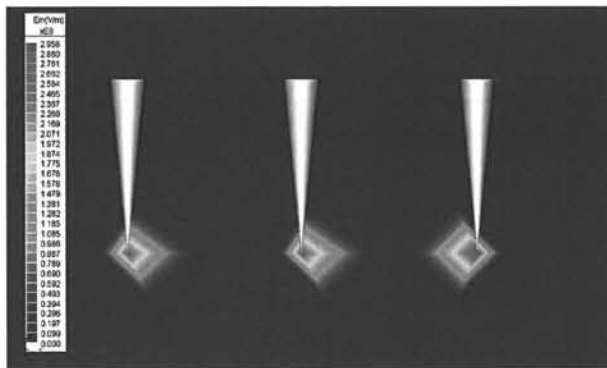


Figure 35 Electric field around grounded needles of water

The field strength right at the tip of the needles will not be used for the comparison between different shapes, because it will not be accurate enough. Therefore, for the symmetric case, at different heights between the electrode and the needles, the electric field strength will be investigated in order to make a fair comparison. The heights at which the electric field strength will be investigated are 20 mm, 25 mm and 28 mm. With the tips of the needles being at 30 mm high, the gradient between the tips and the plate will be better visible (Figure 36). Here it can be seen that the electric field strength only 2 mm away from the tips is much lower than directly around the tips. For the asymmetric case, only the electric field at 28 mm is assessed.

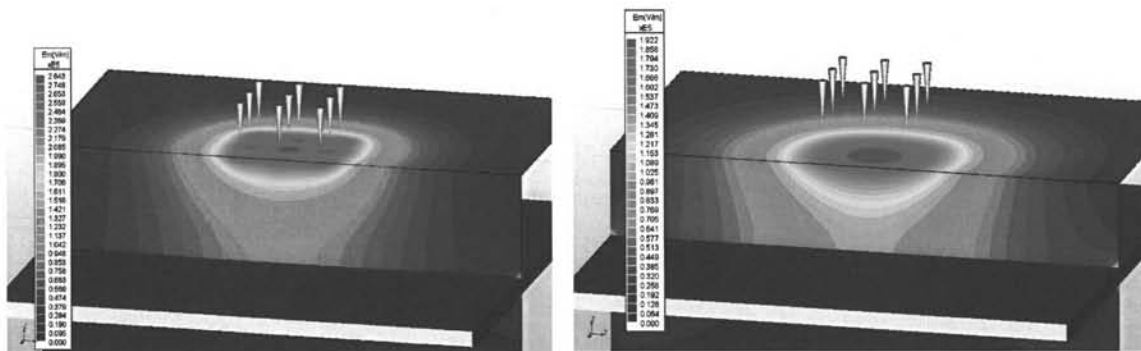


Figure 36 Field strength between plate electrode and needles at an altitude of 28 mm (left) and 25 mm (right)

9.6 Comparison

Different electrode configurations are modelled, among which two V-shaped electrodes with different angles (126 and 143 deg.), a curved plate, a flat plate, wires and a grid (see Figure 32). The shape of the electric field at a height of 28 mm of all these configurations can be found in Appendix D-3, the largest electric field strengths at heights 20, 25 and 28 mm are plotted in Figure 37. This is for the symmetric case only. Of these configurations the best are chosen for the apparatus, the best one being the one to achieve the highest fields at all, or most of the needles, hence a high, homogeneous field is required. However, practical considerations regarding shape and application weigh as well.

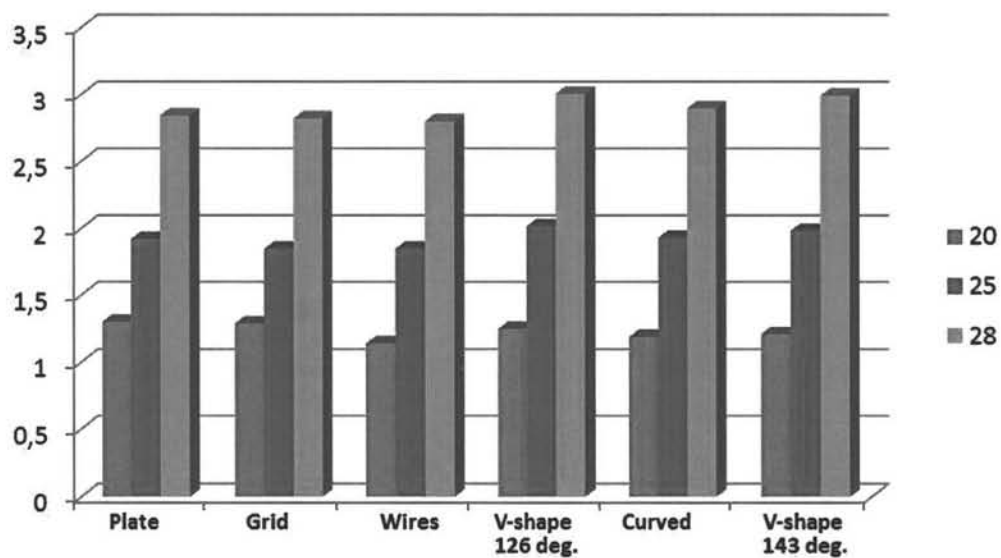


Figure 37 Electric field strengths at different heights for different electrode shapes

Considering the electric field strength at 28 mm for the different electrodes in the symmetric needle arrangement, the V-shaped electrodes achieve the best results. Looking at the maximum electric field strength in the asymmetric case, the difference between the V-shaped electrodes and the other configurations is even higher. However, in the asymmetrical case it can be seen that the maximum electric field is only reached by one needle, not all, and that the resulting electric field around the other needles is similar to that of the plate electrode. Further, it follows from Table 11 that the wires and the grid configurations have the lowest electric fields in any case.

Table 11 Electric field strength ($\cdot 10^5$ V/m) of different electrode configurations

Shape	symmetric	asymmetric
Plate	2,843	2,582
V-shape1	3,008	2,91
V-shape2	2,994	2,735
Curve	2,898	2,67
Wires	2,801	2,546
Grid	2,822	2,564

Since the wire and grid electrodes are eliminated, the choice remains between the V-shapes, the curved and flat plate electrode. Not only the electric field strength is important for the choice of shape, the application and the final shape of the apparatus counts as well. Since the application of the device includes the movement of the electrode/insulator configuration through the hedge (in the y direction of the model), the V-shapes and the curved plate electrodes will not achieve their goal. The goal of these shapes is to surround the branches with electrode, hence creating a better field. However, if the V-shape will be moved in the y direction, the branches that have the ideal height for this configuration will be forced to move underneath the electrode, instead of over it (see Figure 38). A way to solve this problem is creating an M shape instead of a V shape, so that the needles will be directed over the edge. However, this will increase the amount of material required per unit, hence the production costs, and it will induce other problems regarding the movement through the trees; the needles are supposed to be touching the insulator, which will be a layer on top of the electrode. It is rather questionable if the needles will touch the insulator in the middle as well as on the sides. When using a flat plate, these inconveniences are overcome.

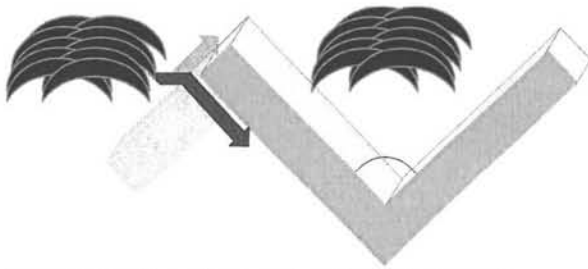


Figure 38 Needles being forced underneath electrode

Using a flat plate has more advantages over using other shapes than only the movement of the electrode through the hedge. A flat plate is easiest to make and easy to maintain, hence cheapest. Furthermore the electric field experienced by the needles has least variations for this configuration and has the most homogeneous electric field which is favourable.

10 Design of insulator

For the insulator design, several decisions have to be made regarding to the shape of the electrode, distance to the needles, the collection mechanism, the solvent used and the insulator material. In Chapter 9 the electrode design is already described, this chapter will therefore focus on the insulator design starting with the taxane collection mechanism. Hereafter the use of solvents will be described and the bulk and surface configuration of the insulation layer. Finally, the material for the insulator will be chosen, which will be based on the properties and parameters explained in Chapter 8. The results from this chapter will be combined with Chapter 9 in the total design in Chapter 11.

10.1 Collection of taxanes

The electrode design and its preferred shape is known, the kind of taxane collection mechanism can be chosen. Related to the collection mechanism, the shape of the collector as well as the way to reach the branches will be discussed. Since the harvesting device will be put into the hedge, the opportunity of collection on both sides of the device and the discharge mechanism will be explained as well in this section.

10.1.1 Collection on both sides versus one side

The taxane collection will take place on top of the milking device, but the collection can be done at the bottom as well. In the dense hedge are enough branches available between the different electrode/insulator plates. Therefore, a decision has to be made whether the collection is going to take place on one side or on both sides of the collection device.

Based on calculations in Appendix_C, the time taken for a water droplet of $30\text{ }\mu\text{m}$ to be fully evaporated with a velocity of 17 m/s is 0.4 ms . Hence, it is expected that sprayed sap containing taxanes is collected on the insulator as a solid. This is in agreement with the experiment observation as shown in Figure 39, where the product is visible as a white stain on the aluminium foil. A white stain appeared on the aluminium foil several minutes after charging the cathode. It is assumed that same form will appear on the cathode/insulator of the milking device.

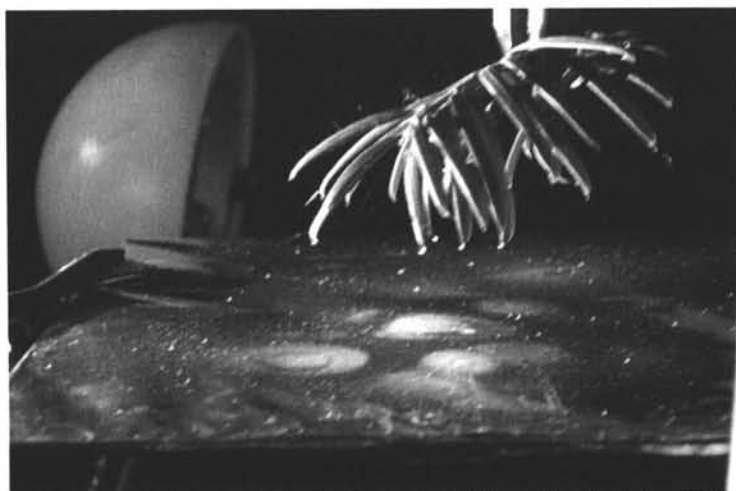


Figure 39 Product in the form of white stain visible at the aluminium foil during an electrospray experiment [60]

The insulator on the bottom side of the electrode can have the properties that the solid will stick onto the insulator; however the collection is going to be much harder, due to gravity. The solid has to be removed mechanically or rinsed with a solvent in order to collect the taxanes, yet the only way to do that will be to turn the electrode around. The spacing between two collecting devices is much smaller than the width of each electrode panel and makes the flipping complicated. Extra mechanical design features are required which may push up the cost of the machine. The flipping will also hamper the process from being continuous.

Hence, it was decided to collect only on one side of the electrode. The bottom will be coated with a good insulator to prevent interaction with the field strength of the adjacent electrode.

10.1.2 Collection mechanism

In order to get creative ideas, a morphological chart is made for the taxane collection mechanism (see Figure 40). In the following paragraphs, the advantages and disadvantages of the ideas are explained and the collection mechanism for the design will be determined by elimination.

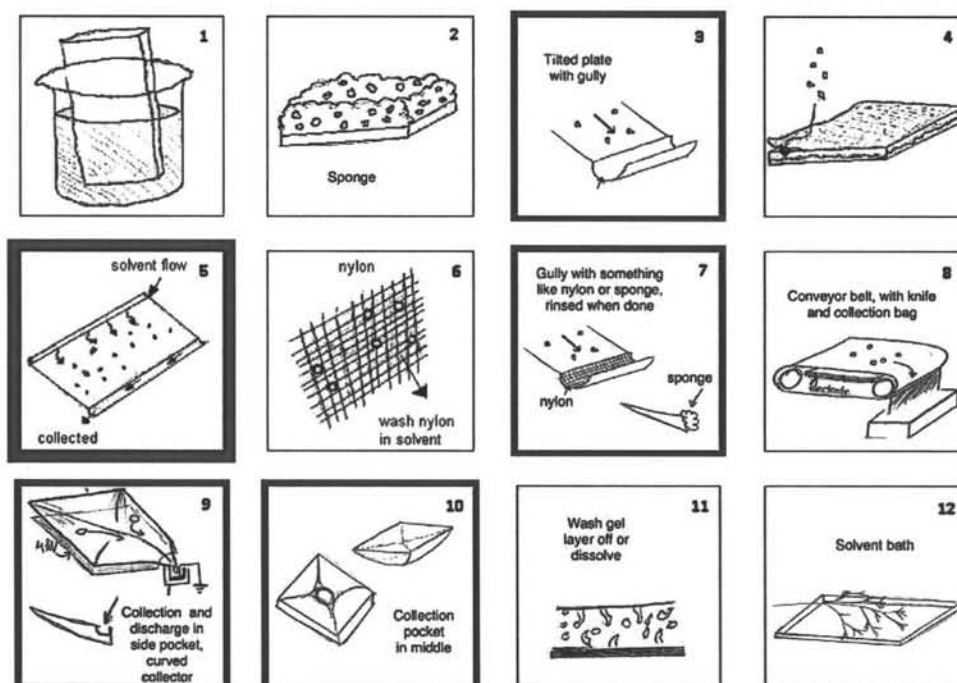


Figure 40 Various drawings of possibilities for collection mechanisms. The five feasible designs have a green frame. The finally chosen mechanism has a bigger green frame.

Taking a closer look at all the proposed new ideas, seven ideas for the collection mechanism can be rejected, which will be explained first. Hereafter, the remaining mechanisms will be discussed to come up with a final decision.

Refused alternatives

The first picture displays soaking the complete collection device with a solvent, after it is removed from the taxane milking machine. By this method, the design of machine itself is simpler. Rinsing crude extract while the plates are still mounted onto the machine will challenge the safety (short-circuiting) and mechanical design (in-built piping for solvent). However, operating in continuous mode is not feasible and the time required will be enormous as well as the organic solvent use. Considering sustainability and costs, this mechanism is refused.

quantify!

The second picture of the sponge as collector is refused as well. Sponges are good water absorbers in nature and have a higher affinity to liquid than the tip of the needle. However, the liquid absorbed is not supposed to touch the electrode to overcome short-circuiting. Furthermore, large amounts of solvent are needed for repeated washing before most of the extract can be recovered. This results in a downstream process with huge volumes, expenses and environmental concerns.

Picture four depicts the usage of a permeable material to cover the insulator, which allows movement of extraction in one direction. The extract is trapped in between the permeable material and the insulator. For harvesting of the extract, the permeable layer needs to be removed and the extract will stick to the permeable layer and the insulator. Hence, this system might be too complicated and is therefore rejected.

In picture six a nylon mesh, placed on top of the electrode, is used as collector. The extract can be collected by washing the mesh in organic solvent. Removing, washing and placing back of the mesh is troublesome. Furthermore, spark discharge can take place, which is unwanted. The insulation layer should be a closed layer, which excludes the mesh [112].

Picture eight represents a conveyor belt with knife and collection bag, whereby solid crude extract at the end of the belt will be scraped off and collected. There is no need for chemical solvents, hence no energy-intensive removal steps and adverse health and environmental effects. However, for the purification the use of solvent might be necessary. Further, this method works well with a thick solid cake layer, while the electrospray results in tiny droplets with low taxane content. The solid layer is expected to be very thin and it is likely that the knife is not touching the solid or either cuts and damages the insulation layer.

In picture eleven a gel layer as collector is shown. Gel is mainly composed of liquid, but has a solid like structure [137]. It can be easily made from many different materials like polymers and proteins. Gel has an inter-connected porous structure due to linkage between polymer chains. The liquid extract may seep through and get trapped inside the gel layer. Therefore the challenge lies in recovering of the taxanes, since the gel needs to be liquefied or solved. Heating degrades taxanes [51] and use of extra solvents cause environmental concerns and increase downstream purification costs.

The last refused mechanism is shown in picture twelve, namely a solvent bath as collector. The branches hang above a tray with solvent and the solvent will be touched by the needles. Since the needles are covered with a waxy cuticle layer, the needle surface is not wetting and the extracted liquid moves into the solvent rather than staying on the needles [73]. The trays are integrated with the electrode and insulator and large amounts of solvents are needed. Movement of the device through the hedge expands the risk of spoiling solvent and damaging the tree, another difficulty is evaporation of the solvent. Furthermore, moving a tray through the hedge is complicated, since the branches are unevenly arranged.

Feasible alternatives

The five pictures not mentioned show some similarities and might be feasible as collection mechanism. All of them consist of a plate where the extract is removed by gravity and collected at a temporary storage unit. The differences between the designs are the discharge mechanism, shape of the collector device, and the need of solvents to collect the taxanes. In order to make a choice, the shape of the collector and its pros and cons will be considered.

Shape of the collector

In picture nine and ten, the collector surface is curved and the extract can be collected and immediately discharged respectively at a corner or in the middle of the plate. A curved surface is

more expensive to make. Other disadvantages are that a curved plate might have influence on the electrode and its electric field and that it is relatively thick and therefore harder to insert into the hedge.

Instead of using a curved surface, a flat plate can be tilted slightly as shown in picture three, five and seven. In picture three and seven, gully or gully lined with an absorbing material like nylon or a sponge are used respectively. This will keep the droplets from rolling off, however extra steps are needed to remove, rinse and replace the gully.

The methods mentioned above are based on collecting taxanes when only gravity and in some degrees electric attraction forces are acting in the direction of the collector. However, the extract present on the plate will be in solid and liquid phase in very small quantities. Not all the taxanes will be collected in this way. This problem can be avoided with a solvent flow. Therefore, the most promising design is shown in picture five, the tilted plate with solvent flow.

Chosen alternative: tilted plate with solvent flow

The plate is tilted and flushed periodically with an organic solvent. The solvent dissolves the solid extract and washes away the liquid droplets. Periodic continuous operation will largely reduce the amount of solvent needed and it removes both solid and liquid extract from the device. On top of that, the continuous removal of the extract solves the problem with the degradation of taxanes and it also saves trouble of washing either the insulator or the absorber one by one. Therefore, it is chosen to use this collection mechanism in the design of the taxanes harvesting machine.

10.1.3 Distance to the branches

The branches can either touch the milking device or can be separated from it, which depends on the collection mechanism and the use of an insulator. A morphological chart is made to show how the branches can be separated from the milking device, which can be seen in Figure 41.

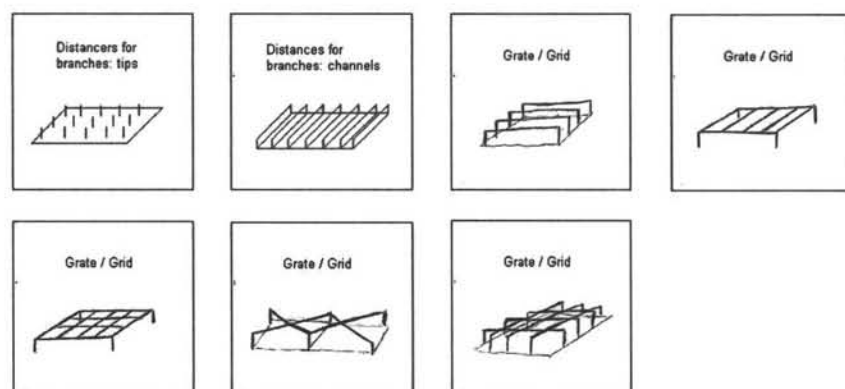


Figure 41 Distance holders to separate the branches from the device

Since the collection mechanism will be a tilted plate with an insulator, there is no need to have a distance between the needles and the plate. However, physical contact between the needle and the well-insulated electrode may even facilitate the transfer of extract, depending on the wetting property of the insulator. More explanation about the desired properties of the insulator can be found in Section 10.4.

Without the use of distance holder, a few needles will touch the insulator, while the majority of the needles will still be separated from the insulator. As discussed previously in Chapter 6, the field strength increases as the distance between the electrode and the needles decreases. Therefore, pushing the needles closer to cathode increases the field strength and hence a lower voltage can be

set on the electrode to get the same electric force required to pull cell sap out of the stomata. Therefore, the best design has no gap between the needles and the electrode. This can either be realised by pushing the branches to the electrode mechanically or by the collection device configuration.

Pushing branches together

Mechanically pushing the branches to the electrode will make the device unnecessary complicated. Therefore, pushing the branches to the electrode due to the device configuration has more preference, if it is sufficient enough. This can be elaborated with a morphological chart, which can be seen in Figure 42. Various configurations of the collection device are shown, which will decrease the distance between the needles and the electrode.

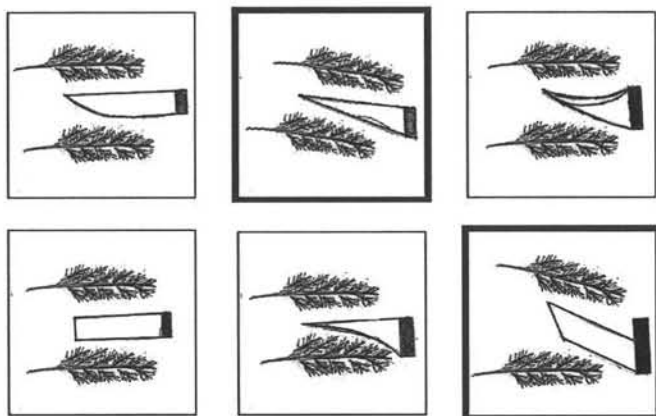


Figure 42 Collection device configurations

The collection device consists of a thin insulator layer on top and the electrode right under it. At the bottom another layer is applied that will be used to push the branches down to the electrode below. The part below electrode can be made from an insulating material, accordingly it will not only push down the branches below, but it also shields the electric field interference between two adjacent electrodes. The best geometry depends on the pushing effectiveness (shape of bottom part) and the way the collection is going to take place (shape of top part).

Since it is conceivable that a part of the extract will be in the solid phase and a solvent might be used, a flat top layer is not usable, which excludes three pictures. The right upper picture shows a curved plate, which is already discarded because of its expenses during the manufacturing and influence on the electric field. The two remaining pictures, shown with a small green frame, can be combined in order to get the most favourable configuration. The exact choice will be made and explained during description of the integrated design.

10.1.4 Discharge mechanism

Since the extract is negatively charged, they repel each other. For a successful collection, a discharge mechanism is required, since working with charged material brings safety issues with it. Two discharge options are considered: discharge by grounding or by the air.

When the collection place is grounded and the material of the collector is a conductor, the extract will be attracted toward this place due to the potential difference. This gives an extra pull although it is expected to be very small. Once the extract touches the collector, its charge will directly be removed. In case of discharge by the air, the charge of the extract is lost through the air. When the extract is collected at an insulator and stays there for a while, a certain time (relaxation time) is required before it is discharged. This process is a lot slower than discharge by grounding. Therefore, discharge by grounding is preferred since the charge is removed very fast.

10.2 Solvent use

From Section 10.1.2 it has become clear that the favourable collection mechanism of the taxanes includes the use of a solvent. There are several organic solvents in which taxanes can be dissolved, and which are also used during extraction of taxanes from dried and grinded needles [138],[139]. These solvents can be seen from Table 12 below.

Table 12 List of solvents and their properties. Based on [140]

Solvent	NFPA Blue (health)	NFPA Red (flammability)	Boiling point (°C)
Chloroform	2	0	61.2
acetonitrile	2	3	82
t-butyl methyl ether	1	3	55.2
Methylene chloride	2	0	40
Dimethyl sulfoxide	1	2	189
Alcohols			
Methanol	3	3	64.7
Ethanol	2	3	78.4
Propanol	1	3	97.1
Butanol	1	3	117.7
Acetates			
Methyl acetate	1	3	56.9
Ethyl acetate	1	4	77.1
Propyl acetate	2	3	102

Three properties of organic solvents are important to consider when choosing the best solvent for the taxanes. First of all the solvent should be effective; i.e. the solubility of taxanes in the solvent should be good. The only solubility data that could be found is the solubility of paclitaxel, which is about 10 mg/ml for methanol, 40 mg/ml for ethanol, and 200 mg/ml for dimethyl sulfoxide [51], [139]. The solubility of precursors to paclitaxel is probably similar [51]. Unfortunately, no solubility data of taxanes for the other solvents could be found. This makes selecting a suitable solvent more difficult.

The solubility, however, is not the only important property of a solvent. Because of known adverse health and environmental effects of many organic solvents [142], the solvent should be as harmless as possible for humans and the environment during use. Therefore some criteria were set to evaluate the adverse health and environmental effects of organic solvents.

According to the NFPA (National Fire Protection Agency) values listed in Table 12, a flammability number of 4 is considered as unacceptable, because a substance will then vaporise easily, and will burn readily after ignition by a spark. Furthermore, a NFPA number of 3 or 4 on the health scale is considered as unacceptable, because a short exposure to the substance could cause residual injury or instant death.

The NFPA scale, however, was only made as an indication for hazards occurring during emergencies, and therefore no chronic exposure data on humans and the environment is included in the NFPA scales. For that purpose the material safety datasheets (MSDS) were developed. From these sheets the maximum work level exposure levels in air and possible long-term health effects can be found. Compounds with adverse effects on humans or the growth of the Yew trees were considered as unfit for the use during electrospray. These effects are only considered for individual organic solvents after

a rough separation between fit and unfit solvents, by eliminating unacceptable NFPA numbers and unacceptable boiling points as will be explained below.

Shortly, the use of a harmless vegetable oil or something alike as a solvent was considered because of all the negative health effects of (volatile) organic solvents. But then the third important property of a solvent is compromised, which is related to easy downstream processing and separation of taxanes. This means that the boil-off of the solvent should be easy, as concentration of the solution is probably needed for downstream processing [34]. A solvent with a low boiling point is therefore preferable. The upper limit for the boiling point that was chosen is 90°C, because of the heat resistance of taxanes, and the energy costs during concentration of the solvent; above 40°C there is accelerated degradation of taxanes [34]. This will result in many impurities in the solution, which will then have to be removed with high cost techniques like HPLC [143]. Therefore vacuum distillation is preferred, in order to lower the boiling point of the solvent to 40°C or less. If the solvent has a boiling point above 90°C, a too high vacuum is necessary to lower the operating temperature below 40°C, which will increase energy use of the downstream processing.

Another criterion is to eliminate all solvents with a boiling point below 60 °C in atmospheric pressure, because a low boiling point implies high volatility, which results in too much solvent loss during operation of the electrospray. Furthermore, there will be increased health and environmental risks because of vapours of the organic solvents present. The MSDS sheets on the solvents all warn about conditions where vapours of the solvents arise [144]. If there is no wind on the plantation, local build-up of vapours can occur.

Combining the NFPA data and boiling points from Table 12 and eliminating the unacceptable ones, only three viable options are left; chloroform, acetonitrile, and ethanol.

The maximum workplace exposure levels of ethanol are much higher than those for acetonitrile and chloroform [144]; 1880 mg/m³ for ethanol as compared to 49 mg/m³ (10 ppm) and 67 mg/m³ (40 ppm) for chloroform and acetonitrile, respectively. Furthermore, there are no signs of intoxication from ethanol vapours for concentrations below 1000 ppm.

Furthermore, a major risk of acetonitrile is that it is also readily absorbed through the skin [144]. Chloroform is probably a carcinogen, and can also cause damage to plants, e.g. brittleness of roots and chromosomal damage [144]. Ethanol in low concentrations, on the other hand, is rapidly broken down without causing damage. At higher concentration it can cause microbial death because it works as a disinfectant [144].

Another advantage of ethanol is that the final formulation of paclitaxel for hospital use is dissolved in ethanol [93]. Thus traces of ethanol are not disastrous in the medicine. Attention should be paid, however, to the purity of the ethanol used for rinsing off the taxanes from the collection device; industrial ethanol (96%) with 4% of water and only traces of other impurities [145] is of course acceptable in this case.

Therefore, considering health, environmental and practical impacts, ethanol was finally chosen as solvent.

10.3 Bulk configuration

The bulk configuration of the insulator refers to the inside structure of the insulator. As stated before, the electric field must be able to penetrate through the bulk configuration, while the solvent and the sprayed droplets cannot seep through.

Furthermore, the insulator should facilitate the collection of the taxanes. From Figure 43 below four different options for the bulk configuration of the insulator are shown. In the previous sections a tilted plate was chosen as collection mechanism. The micro porous gel layer and mesh were already eliminated, but still it is interesting to look from the insulator point of view which of the four options below will be the best.

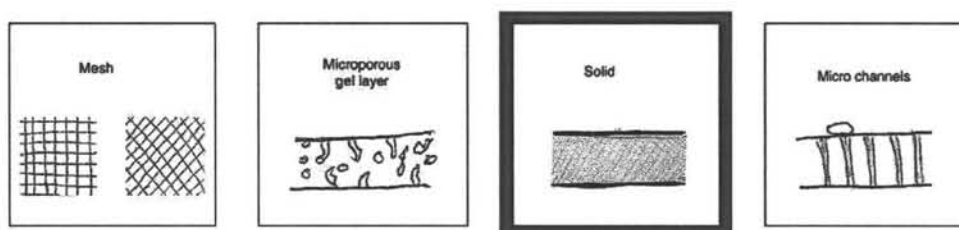


Figure 43 Possibilities for the bulk configuration of the insulator

The mesh design of the insulator was the initial idea (the most left picture), because it will allow maximal penetration of the electric field due to presence of many open spaces. However, the sprayed droplets are in the micrometer range. To stop these droplets from reaching the electrode, the spacing between the mesh should be of the same dimension. As was said before, the collection of taxanes must be done by taking the insulator off the machine and wash it in a solvent, which costs a lot of time. Secondly, a mesh will not be able to be an insulator, because electrons can still go through the open spaces of the mesh and reach the other side of the insulator. At the surface spark discharge will then take place [112].

For the same reason, the most right picture with the micro channels will not work: the structure is open thus electrons can get through and spark discharge will occur at the surface. The gel layer shown in the second picture can be made via polymerization[141]. The gel is firstly cast and then laid onto the electrode, and after spraying the gel is dissolved to get the taxanes. Not only the collection is laborious as was explained before, but also the insulating properties of a gel are doubted, because the gel contains a large amount of water or otherwise conducting liquids.

Therefore, it is believed that the third box shows the best option for the insulator design, namely a solid bulk configuration. It covers the electrode completely, which eliminates the concern that the collected extract may seep through and touch the electrode, and create short-circuiting in that way. Also the charge at the electrode will be confined to its surface, and cannot go through channels to the surface and create spark discharge.

10.4 Surface configuration

Not only the bulk configuration of the insulator is important; the surface configuration can either help or hamper the efficient collection of the taxanes. The morphological chart, which can be seen in Figure 44, shows several possible surface configurations.

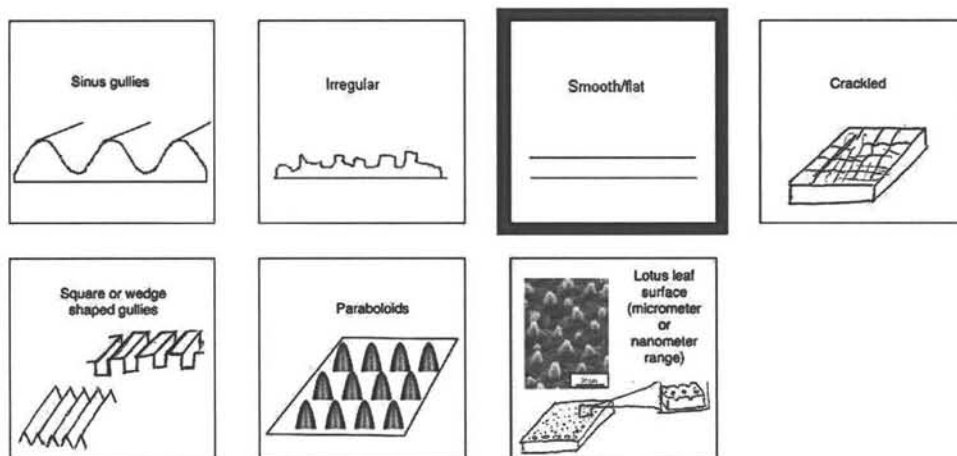


Figure 44 Drawings of different surface configurations of the insulator

In the previous sections the choice was made to let the needles touch the insulator, to facilitate collection, and collect partly liquid droplets. Collection will only be facilitated if the surface of the insulator is more wetting than the surface of the needles, because a wetting surface will draw the liquid from the needles. Therefore the desired contact angle of the insulation surface was determined. For young needles of the pine tree – a needle tree comparable to the yew tree – of the age of 6 months or less, the contact angle is approximately 100 degrees [73]. If there is pollution on the needles, however, the contact angle will decrease to 80-90 degrees [73]. With this information, the assumption is made that the desired contact angle was maximally 80 degrees. Furthermore, it should also be noted that if the wetting property of the surface is very good, it may become difficult to collect the extract; therefore a moderately wetting surface is preferred.

A preferred wetting surface excludes the lower right picture of the lotus leaf surface. A lotus leaf-like surface will only increase the contact angle with the droplets that are on the needles [131]. Thus the droplets will stick preferentially on the needles and will not come off when in contact with a lotus like surface.

Furthermore, there is no apparent advantage of using a cracked or irregular surface or a surface with paraboloids. This is because the mobility of the droplets will not be increased by the surface configuration, and also the transfer of droplets from the needles to the surface will not be facilitated. So it is of no use to apply such a surface configuration.

Finally a surface with square or sinus like gullies in the centimetre range could be used, to collect the droplets in gullies. The advantage will probably be minimal, because a solvent will still be used to collect all the taxanes. Dissolving and rinsing of the solid will be the easiest on a flat surface since the liquid will evenly be spread across the surface. Therefore the flat surface of the insulator will be used in the final design.

10.5 Insulator material

There are several possibilities for the material of the insulator. The insulator must have good electrical properties, good weather and chemical resistance, low water absorption, and favourable weather conditions. The parameters that govern these properties were explained in Chapter 8. The electric properties are considered as most important, so they will be elaborated first.

10.5.1 Electric properties

A table of electric properties of several materials – i.e. thermoplast polymers, thermoset polymers, and glass materials – can be found in Appendix E-1. The electric properties that are listed in this appendix are the dielectric constant, volume resistivity, dielectric strength, and dissipation factor.

Mainly polymer materials are listed because it turned out that the dielectric constant of inorganic solids is often too high [146]. The only inorganic materials that have a reasonable low dielectric constant are glass materials like quartz and silica glass [147], which are also listed in Appendix E-1. The tables contain around 50 polymer and glass materials, where polymers with a too high dielectric constant were also pre-excluded. The dielectric constant of air at 20°C is equal to 1.00059, while the dielectric constant of vacuum is equal to 1 [148]. An insulator with a dielectric constant close to 1 is thus desired, and therefore it was assumed that a dielectric constant up to 5 is reasonable, while a constant higher than 5 is too high.

A quick scanning of Appendix E-1 learns that the volume resistivity of the listed materials is often high enough (in the order of $10^{10} \Omega \cdot \text{cm}$), while the volume resistivity of air is $10^{11} \Omega \cdot \text{cm}$ [115]. Only a few glass types have a resistivity that is much lower than air (lower than $10^9 \Omega \cdot \text{cm}$); in the appendix these numbers have been marked with red.

Furthermore, the dielectric strength is only for a part of the materials high enough; the applied field is 4 kV/cm, or 0.4 kV/mm. All materials can withstand this field strength, but the field around the needles is much bigger. From the simulations that were done, it was found that the field strength very close to the needles could be as high as 15 kV/mm. Because a part of the needles will touch the insulator, all insulators with a dielectric strength lower than 19 kV/mm were marked with red. Thus an extra safety factor was taken into account to make sure that the performance of the material is good enough during electrospraying, while needles are touching the insulator.

Finally, the dissipation factors of the materials are listed, which is a measure for the power losses, as was explained in Chapter 8. Because of the high voltage and low current, the dissipation factor is less important than the other electric properties. Furthermore, the maximum dissipation factor listed in the tables of Appendix E-1, is 0.04. This means a maximal loss of only 4%, which is acceptable.

10.5.2 Chemical stability

Besides favourable electric properties, the chemical stability of the material is important. The insulator must withstand regular contact with ethanol, since ethanol is chosen as solvent for the collection of the taxanes, as explained in Section 10.2.

Furthermore, the insulator must be able to withstand UV irradiation, because the light of the sun consists of 2% of UV-light [149]. This is only a small part of the total solar spectrum, but the energy of UV-light is so high that it can break the molecular bonds of a polymeric material, resulting in cracking of the material [150].

As a result, in Appendix E-2 the ethanol and UV resistance of thermoplasts, thermosets, and glass types is given. Four different letters are used to indicate the performance of the materials.

For ethanol the letters in the table have the following meaning [151]:

- U = Unacceptable: effect of contact varies from catastrophic failure (dissolution) to severe degradation (cracking)
- M = Marginal: only short exposures at low temperatures is allowed and for applications where significant loss of mechanical properties is not critical
- A = Acceptable: acceptable performance in ordinary exposure. For long term exposure at low temperatures there might be some minor loss of properties
- Ex = Excellent: the polymer is unaffected by the chemical reagent, with respect to time, temperature, and stress applied

The polymers that perform marginal or unacceptable in contact with ethanol are marked with red, because such materials will deteriorate immediately or within the period of use of the milking machine. All glass types are highly chemically stable and will thus not deteriorate when in contact with ethanol.

For UV-resistance the letters in the tables mean the following [151]:

- U = Unacceptable performance: cannot withstand UV-light, polymer bonds will be broken as soon as the material is exposed to UV-light and the material will get brittle very soon
- M = Marginal performance: can only be exposed to small doses of UV-light, otherwise the material will get brittle after a few months. Additives like UV-stabilisers or UV-absorbers are necessary
- A = Acceptable performance: will not degrade under UV-light, only after a long period of constant exposure. Use of UV-stabilising additives recommended
- Ex = Excellent performance: high to very high stability under UV-light. No UV-stabilising additives are necessary

All polymers that have an unacceptable performance under UV-irradiation are marked with red, because even UV stabilisers cannot slow down the breakdown of the material. There are many UV absorbing or stabilising additives, however, that can improve the performance of marginal performing plastics. It must be noted that additives are no complete cure to UV instability, and that a large amount of UV stabilisers is needed. Furthermore, additives can influence the electrical properties of polymers in a negative way [147].

10.5.3 Water absorption

Water absorption by polymers will lower the dielectric strength and affect the mechanical properties in a negative way [152] and in Appendix E-3 the water absorption of polymers is listed. Two types of water absorption are given: the percentage of absorption after 24 hour immersion in water and the equilibrium water absorption, which is often reached after a very long time of immersion in water. From several sources it appears that a concentration of more than 0.30-0.50% of water could already have a large influence on electric properties like the dielectric constant and resistivity [153],[154]. Therefore a 24-hour water uptake of maximally 0.50% was chosen as a constraint, and for equilibrium absorption a maximum of 1.0%. It is assumed that the equilibrium absorption of water is never reached, because the spraying is only conducted when it is not raining. The 24-hour absorption of water is considered as more important; the plant saps that are sprayed onto the insulator consist mainly of water. Furthermore the air on the plantation could become humid during (a part) of the days that electrospaying is conducted. A number of polymers could again be marked red because of too high water uptake, especially nylon plastics are known for their high water uptake.

All the electric, chemical stability and water absorption figures that were chosen from Appendices E-1, E-2 and E-3 were combined, in order to eliminate all the materials that have insufficient properties for the application in the taxane milking machine. Only seven thermoplastic polymeric materials, subdivided into several types, have the desired properties; acetal (POM, polyoxymethylene), PAI (polyamide-imide), PEEK (polyether etherketone), PPO (polyphenylene oxide), PTFE (polytetrafluoroethene), PFA (polyfluor alkoxy), and PS (polystyrene).

From these seven polymers, PTFE and PFA (Teflon polymers) could also be eliminated: in the first place, the contact angle of Teflon and Teflon-PFA is very large; almost 180° [125]. Furthermore, the contact angle of Teflon like polymers cannot be modified by surface reactions, because it is a very inert polymer, having only tightly bound fluorine atoms at the surface of the polymer [115]. On the other hand, the surfaces of the other polymers can easily be modified to become more hydrophilic, even if the contact angle with water is too high [158],[162],[164],[165]. For example the contact angles that could be found were 78° for polystyrene, while PAI has a contact angle of 87° , and both surfaces can be modified to lower the contact angle [155]. In Chapter 11 the ways in which surfaces can be modified will be elaborated.

10.5.4 Harris profile – final choice of insulator material

The remaining five types of polymeric materials have favourable properties to be used as insulator of the electrospray machine. For these materials, seven requirements concerning the usability and durability were formulated and below the performance of the five polymers regarding these requirements are elaborated. Finally the information on performance is combined into a Harris profile, to make the differences between the different polymers more clear. The chosen requirements are the following:

- Low price
- Easy to manufacture
- Not scratch sensitive
- Low use of additives
- Short relaxation time
- Small layer thickness
- Durability (weather resistance)

POM

POM, polyoxymethylene or Acetal, is a by-product of the two step reaction between an alcohol and an aldehyde and forms an oxymethylene chain by the polymerization of anhydrous formaldehyde. POM is a cheap polymer, the price lies between the 0.14–0.18 €/kg [156],[157],[104]. The manufacturing is relatively easy [158],[159], but it shows scratch sensitivity, namely 10-20 kJ/m² [158]. Additives can be used to enlarge the durability, since the degradation by UV-light can be overcome by 1-5% of additives. Furthermore, additives can protect degradation by high temperature, moisture and bacteria. Without additives, the weather resistance will be marginal or acceptable [115]. The relaxation time is calculated to be $2.9 \cdot 10^{-8}$ s [124],[160].

PAI

PAI, polyamide-imide or Torlon, is a thermoplastic amorphous polymer with good mechanical, chemical and thermal performances [147],[150],[161]. However, it can easily be blended with other polymers to enhance its performance [148]. It can be manufactured under high temperatures and in a wide variety of forms, for example as a thin film or a coating [148],[149]. The production is very costly, but PAI has excellent characteristics relating to the scratch resistance [147],[149] and is highly wear and weather resistant [146],[161]. Furthermore, the relaxation time is calculated to be $7.18 \cdot 10^{-8}$ s [124],[160].

PEEK

PEEK, polyether ether ketone, is a thermoplastic crystalline polymer and has an excellent performance at very high temperatures [152],[162]. The disadvantage of PEEK is its extreme high cost; therefore the application is limited for uses where the extreme heat resistant properties are very important [162]. Furthermore, PEEK shows some scratch sensitiveness, namely 7.5 kJ/m^2 [162] and additives can be used to improve its stiffness, strength and/or stability [151]. However, without additives it has already an excellent chemical resistance, it is not sensitive for moisture adsorption and is not affected by water [151]. Because of its resistance to water, the weather has slightly impact on its durability [163]. Furthermore, the manufacturing of PEEK is not difficult; however a high temperature is required [162]. The relaxation time calculated is $2.65 \cdot 10^{-6} \text{ s}$ [124],[160].

PPO

PPO, polyphenylene oxide, has a good heat resistance, high strength and low water absorption, but has a high price compared with ordinary plastics. Therefore only small amounts are manufactured. For the manufacturing, a machine with a slow feed and low speed is required, since the polymer is difficult to cut, but the circumstances are rather standard [164]. The durability is excellent and there is no need for additives, since degradation is slightly affected by the weather and environmental factors as temperature and moisture [115]. In fact, PPO can be coated on metals to protect them from corrosion [153]. Furthermore, the scratch impact strength is $10\text{-}20 \text{ kJ/m}^2$ [164] and the calculated relaxation time is $9.13 \cdot 10^{-8} \text{ s}$ [124],[160].

PS

PS, polystyrene, is a brittle polymer that is easy and cheap to manufacture and is widely used for packaging, disposables and household goods. The outdoor performance is poor; it degrades rapidly under UV-light [165], although this can be improved with additives [115]. Furthermore, PS can be used as coating [73] but it is very sensitive for scratching. The scratch strength is $< 3 \text{ kJ/m}^2$ [165]. For the relaxation time $1.7 \cdot 10^{-12} \text{ s}$ is calculated [124],[160].

Harris profile

The requirements for the five different polymeric materials are compared and performance values are given, namely -2 (bad score), -1 (moderate), +1 (good), and +2 (very good) with corresponding colours. This can be seen in the Harris profile in Table 13.

Table 13 Harris profile with five polymeric materials with desired properties

	POM				PAI				PEEK				PPO				PS			
Requirements	--	-	+	++	--	-	+	++	--	-	+	++	--	-	+	++	--	-	+	++
Low price				[16]	[5]				[11]				[12]							[13]
Easy to manufacture			[14]	[15]			[3]				[11]			[10]						[13]
Not scratch sensitive		[14]						[1]	[11]					[12]			[13]			
Low use of additives		[4]				[5]	[6]				[7]				[4]		[4]			
Short relaxation time			[20]	[21]			[20]	[21]			[20]	[21]		[20]	[21]					[20]
Small layer thickness				[18]				[1]	[23]			[8]			[9]					[19]
Durability (weather)		[14]						[2]	[25]			[22]			[4]	[13]				

All the requirements have got their value for the five different alternatives and a quick overview of the strong and weak points can be seen in the Harris-profile. It must be noted that not all the requirements have the same importance. For example, PPO has the longest relaxation time, while a short relaxation time is favourable. However the relaxation time of PPO is short and good enough for

the insulator design. The relaxation time is therefore of minor importance for the final decision. The same is true for the layer thickness, since all the alternatives can be manufactured as a coating, and the dielectric strengths of all the materials allows a thin layer [124].

Important requirements are the low price and the durability. Three alternatives (PAI, PEEK, PPO) are expensive but have an excellent durability, while two alternatives (POM, PS) are cheap but degrade easily. Since the harvesting device should have a lifespan of at least several years, replacing the insulator within a short period of time is unfavourable. Therefore POM and PS are left and a choice must be made between PAI, PEEK and PPO with three remaining requirements, namely the manufacturing easiness, the scratch sensitivity and the use of additives.

By comparing the values, PAI is of most advantage. PAI is it not scratch sensitive and is relatively easy to manufacture, although the use of additives might be necessary, because of UV-light sensitivity. However, additives like other polymers can be blended without any difficulty. In contrast PPO has no need for additives, but shows some scratch sensitivity and it is more difficult to manufacture. PEEK is highly scratch sensitive and shows moderate behaviour for the manufacturing process and the need for additives. All in all, the best alternative for the insulator, which solves the contradicting requirements the best, will be PAI, polyamide-imide.

11 Total design and manufacturing

In this chapter, a complete picture of our product will be painted by summarizing the design process performed in the previous chapters. The material, configuration, dimension, and operating conditions of all the essential parts and the integration scheme of these parts will be presented. Clippings will stay as the main source of profit for plantations, therefore avoiding modification of the plantation itself has been taking into account by the total design of the product.

11.1 Design

To facilitate easy harvesting of both clippings and milked extract, the trees are grown in the simplest form: hedges, which also sets limitations on the configuration, shape and dimension of the electrode. The electrode is a flat plate, since analysis by Lorentz E showed that a copper electrode in shape of a smooth plate is able to provide sufficiently strong field strength. The voltage of the electrode is 4 kV, and the thickness of the electrode is 2 mm since this is a standard size that is deliverable by most electrode manufacturers.

Around the electrode an insulator layer of polyamide-imide (PAI) is applied. At the top a flat layer of about 0.16-0.32 cm PAI is applied which is thick enough to prevent ionization by high voltage and is thin enough to maintain the field strength felt by needle tip [115]. At the lower side, the insulator thickness is much bigger and the surface is profiled like a horn shape. The sharp edge helps the assembly to push its way through the layered hedge. On top of that, it shields the field strength of the electrode and prevents interference with the field from below. Also, it pushes the branches down towards the next electrode so that more taxanes can be collected. The overall thickness of the assembly is such that the gap in between two assemblies is about 3-4 cm.

11.1.1 Size of plates

Yew hedges grow in a layered pattern with most needles locating within 20 cm from the exterior. To access as many needles as possible, plate has a width of 20 cm. The length of electrodes determines how much time is required by one machine to harvest a fixed area. The longer the plate, the less the time is required. However, longer plates will encounter problems like the ease of movement between hedges and the balancing of the plates on the support. Hence, our proposal is to tailor the length (in range of 1 to 5 meters) based on the consumers requirements. More vertical supports will be required for longer plates.

Multiple plates parallel to each other are mounted onto a vertical support. The spacing affects the easiness of the movement of the machine through the branches, and the amount of harvesting. This latter is due to the change in field strength and number of needles that spray. We will provide our consumers with a product range between 5 to 10 cm. Number of plates required will be in range of 14 to 28 assuming the hedge is 1.4 m tall (see Appendix F-1).

11.1.2 Surface modification

For needle tips that are in physical contact with the insulator, the insulator surface has to be more wetting than the surface of the needles to enhance the transport of sap from the needles to insulator. Young needles have a contact angle around 100° at the solid-liquid (water) interface. Therefore the desired contact angle of the PAI was set at 80° , however the contact angle of an unmodified PAI surface with water is 87° (see Section 10.5). To decrease this angle, the surface will be treated with UV light (see Appendix F-2) [168]. This treatment is known as a robust and environmentally friendly process. No VOC are used or produced; the by-products are only small molecules such as CO_2 and water [168]. Consequently, the method is very useful to modify the surface of the insulator to fulfil the requirements that were set.

11.1.3 Solvent flow

Ethanol is used as solvent. It will be stored in a reservoir that is placed by the vertical stand. It is transported to the collection plate by in-built piping system. The collection plates will be on movable arms (Figure 45(l)) so that it can be tilted during rinsing and collection. In that way the solvent moves down by gravity. The piping leads to the top of the plate from where it flows down the plate by gravity (Figure 45(r)). While it streams down, solid and liquid extract dissolve and are flushed away with the ethanol. The solution is collected at a gully from where it is transported to a second reservoir, a reservoir where the taxane solution is stored (Figure 45 (l)). The storage reservoir is cooled (below 4°C) and dark to prevent degradation of active pharmaceutical ingredients. This is also the reason why the rinsing is done periodically, with a minimum frequency of once every 50 minutes.

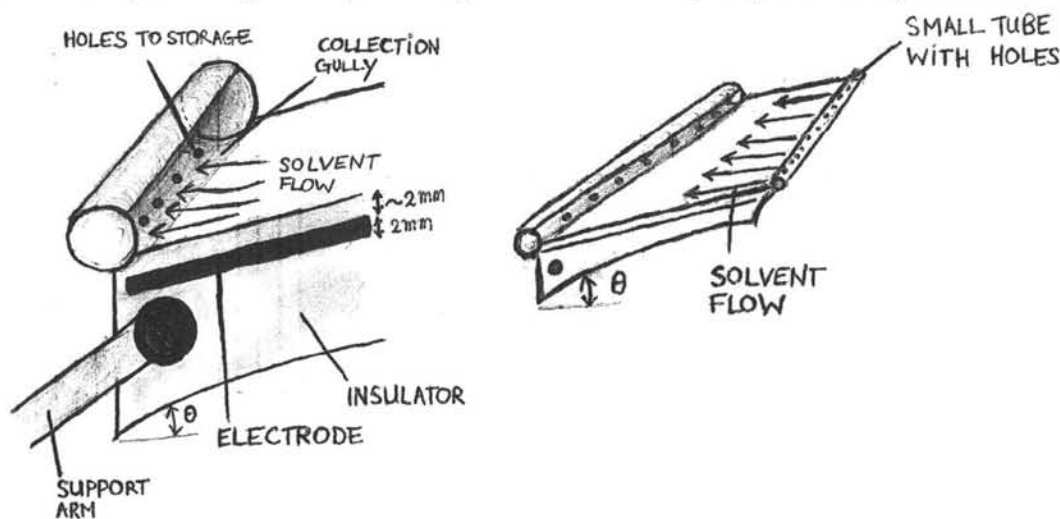


Figure 45 Solvent flow towards collection gully

11.1.4 Energy source

In Figure 46 the total milking system can be seen. The electrode/insulator assemblies are mounted onto a vertical support and moved along the hedges by automatic controlled robotics. The energy required will not be supplied through electrical cables as our product demands high mobility along the hedges. A combustion engine is not a good choice either, as heat is produced instead of electricity. Two possible solutions are a diesel aggregate or fuel cells. They both allow conversion of chemical energy to electricity which is needed to charge the electrode and as energy source for the robotic motions. Disadvantages are that fuel cells are rather expensive and a diesel aggregate tends to be bulky. In the short run, a diesel aggregate is the best choice. However in the long run, fuel cells will be dominant, as it is more sustainable, compact and energy-efficient.

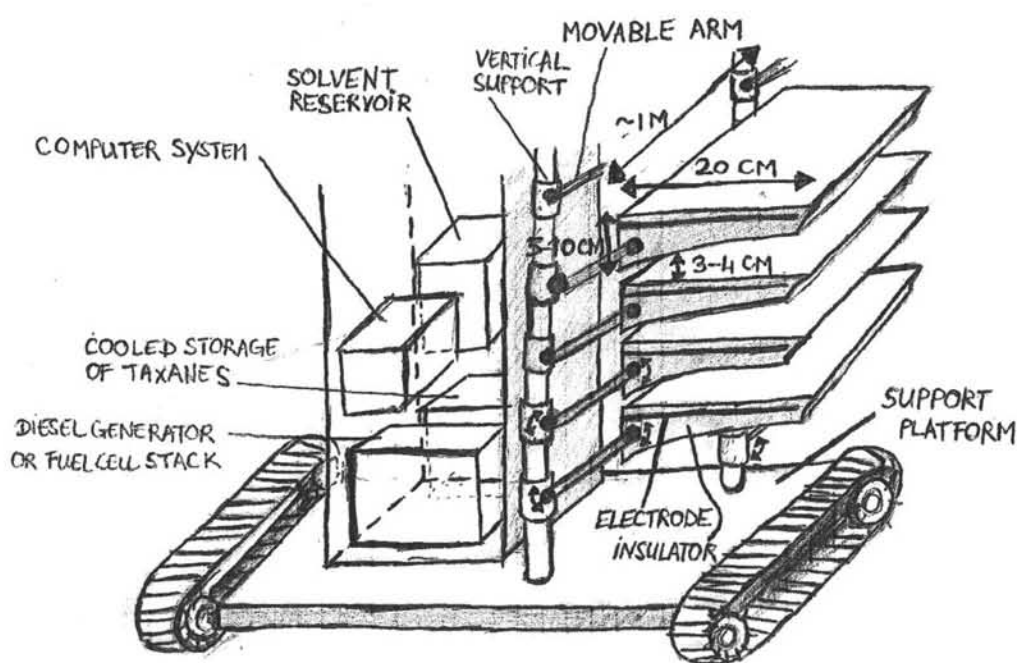


Figure 46 Total taxane milking system

11.2 Manufacturing process

11.2.1 Insulator

Polyamide-imide (PAI) is chosen as the material for the insulator due to its unique combination of advantages [169]. The manufacturing and moulding process of amide-imide copolymer is a rather mature technology. Patents about the fabrication of copolymers are described in articles that can be traced back to 1974, if not earlier [170].

Injection moulding

Injection moulding is the most commonly used manufacturing technique for making plastic objects [171]. A mould, in general, is still a comparably expensive equipment piece, but the cost normalized by increasing numbers of duplicates will be cheap [171]. It can be made from different materials like steel, aluminium and a beryllium/copper alloy [172]. Steel is more resistant to wear and can be used to make large amount of castings over a longer duration, however, it is also expensive. Aluminium is

a much cheaper alternative, although the lifespan may be shorter. Aluminium, it is the better choice in our situation as one mould can still be used for up to hundreds of thousands duplicates with the help of computer-controlled machineries [172].

Plastic PAI pellets or granules are heated to a melt, which is injected into a mould under pressure. After the melt is cooled, the parts can be removed by opening the mould [171]. Figure 47 below shows the typical configuration of an injection-moulding-machine.

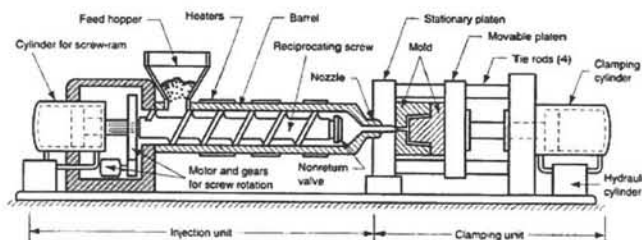


Figure 47 Injection-molding machine

One of the most important properties of thermoplastics, including PAI, is ability to withstand repeated melting and freezing cycles. This qualifies them as a sustainable raw material. Hence our insulator can be removed and reshaped by heating and re-moulding [173]. In case of worn insulators, our customers can bring the insulator back for sustainable replacement.

UV stabilizers

There are two types of UV-stabilisers that are widely used nowadays; the most used type is UV-absorber like benzophenones, benzotriazoles and hydroxyphenyl triazines. The other type is called HALS, Hindered Amine Light Stabilisers. UV-absorbers absorb the UV-light and dissipate it into thermal energy, while HALS works by catching free radical intermediates that are produced because of bond breaking by the UV-light [174].

HALS are extremely efficient stabilisers. A major advantage is that there is no lower layer thickness or concentration limit to ensure good radical scavenging properties, in contrast to UV-absorbers [175]. Furthermore, HALS have a very high longevity because they are regenerated in a cyclic process under UV radiation [175]. Therefore we chose to use this type of UV-stabiliser in the insulator. Figure 48 below shows the general chemical structure of HALS UV-blockers.

The amounts of additives put into the plastic are based on the situation, and the form in which the stabilisers are supplied. If the UV stabiliser is supplied in the form of pellets or beads 1-3% needs to be added to the PAI blend, while only 0.25%-1% needs to be added for liquid UV-stabilisers [174]. For HALS only extremely small percentages are needed [175]. As was said in Chapter 10, the additives can easily be blended with the PAI thermoplast.

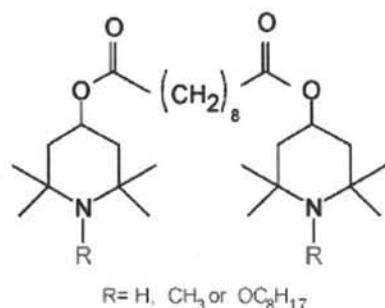


Figure 48 General chemical structure of HALS UV-block

11.2.2 Electrode

Based on the discussion in Chapter 9, our electrode will be made of a smooth, flat copper plate of 2mm. Such copper electrode is now commercially available in the market. Many manufacturers and suppliers can be found online [176]. Considering the fact that our electrode is one of the simplest geometry, it will be rather easy to obtain an electrode.

11.2.3 Integration of Insulator and Electrode

Torlon is the name for a PAI copolymer made by Amoco Performance Products Inc [177]. It can easily be made into many different shapes by injection-moulding onto fibers, films and adhesives on screw-injection molding machine [149],[178]. Furthermore, due to its low thermal expansion coefficient, it allows integration of metals. According to the molding-guide of Torlon by Solvay Advanced Polymer [169], steel, brass, aluminium and some other metals can be moulded into PAI to make it an integrated unit. By inserting the copper electrode into the PAI melt during moulding, the collection configuration described previously can be easily achieved: at the lower side of the electrode a thick, well insulating layer will be applied which will push the branches down towards the next electrode, and at the top a thin layer will be made to prevent shortcutting.

According to our design, the collection device will be flushed periodically to remove the collected extracts. The adaptations needed for the piping can be made through mould-design, and if necessary by conventional cutting and drilling of metal. The different pieces can be mounted together to completed the electrode/insulator/collection device (plate assembly).

11.2.4 The Complete Product

As described earlier in this chapter, 15 to 20 plate assemblies will be mounted onto a vertical support. Through the vertical support can electricity, solvent, and dissolved extract also be supplied and transported. The whole apparatus will be placed on a moving platform which will be operated and navigated automatically. These controlling systems are deliverable by many companies producing robotic applications all over the world, for example by Siemens [179].

12 Economic evaluation

In this chapter the market value of our taxanes harvesting product is estimated to see if our product is worthwhile. The predictions for the taxanes market are very positive, therefore it is expected that there is room for new companies to develop. Together with the already existing yew tree plantations who make money from yew clippings, they are our potential clients. Since it was not possible to get information from the existing yew tree plantation companies about the profit they make, a plantation set-up that prospective yew tree plantation owners can use was designed. From here estimations about the profit were made.

The economic evaluation is split in two parts. First, a taxanes harvesting plantation is designed, and secondly a model is of the plantation. Lastly, an economical evaluation is based on this model to find out what profit a customer would make when our device would be used, and from there for what price our device could be sold.

12.1 Design of plantation

In order to make an economical evaluation about our harvesting machine, a base case plantation was designed. Basic decisions about the species, location, geometry etc. of the plantation are made to be able to make an estimation about the profit that a nursery company would make if our harvesting device is used. From this information an investigation can be made of what our device may cost maximally. Below, the choices made for the design of the plantation are explained.

12.1.1 Species

Different species have different yields on the various taxane contents. For the decision about the kind of species to use the plantation, the best option would be to make first a test field with the species of interest, and find out from which one the most taxanes can be harvested by electro spraying. However, the decision about the yew species had to be made to continue the calculations, and therefore one species was chosen based on a short analysis that was done among the different yew species.

An article published by Rozendaal [14] shows the different taxanes concentrations per yew species. The taxanes that are of most interest are paclitaxel, 10-deacetyl baccatin III and baccatin III. 10-deacetyl baccatin III is the starting material for the semi-syntheses, which can be converted by semi-synthesis to baccatin III and in the second step to paclitaxel. These important taxanes are multiplied with their market price and summed up. The results can be viewed in Table 14. The market prices and an extended table can be viewed in Appendix G-1.

Table 14 Yield of different taxanes in different yew species where 1 is paclitaxel, 4 is baccatin III, and 5 is 10-DAB

		In µg/g dried needles			
		1	4	5	total
<i>Taxus Baccata</i>	European yew	41	14	762	1,20
<i>Taxus Baccata</i> cv.		63	10	468	0,78
<i>Taxus Brevifolia</i>	Pacific yew	130	296	41	0,44
<i>Taxus Canadensis</i>	Canadian yew	285	224	2665	4,49
<i>Taxus Celebia</i>	Chinese yew	26	0	70	0,13
<i>Taxus Cuspidata</i>	Japanese yew	105	15	120	0,31
<i>Taxus cuspidata</i> cv.		136	18	116	0,34
<i>Taxus Floridana</i>	Florida yew	516	0	1689	3,10
<i>Taxus Globosa</i>	Mexican yew	433	168	1395	2,70
<i>Taxus x hunnewelliana</i>		41	0	63	0,14
<i>Taxus X Media</i> cv.	<i>Baccata x Cuspidata</i>	211	36	230	0,61
<i>Taxus Wallichiana</i>	Himalayan yew	272	0	1092	1,94

The data in the column 'total' can be used to compare the value of the three main taxanes in the needles among the different species. It can be seen that the needles of three yew species, the Canadian, the Florida, and the Mexican yew are the most valuable. Assuming that all species have the same growth rate, based on the outcome of the calculations, the Canadian yew is the most promising species. The *Taxus Canadensis*, also known under the name Ground Hemlock or Canada yew [180], was studied in more detail.

Taxus Canadensis costs about USD 10 for a 2-4 feet tree for small purchases [181]. This is comparable with other yew species [182],[183],[184]. Although it is not proven that electrospraying can be applied to *Taxus Canadensis*, it is found to be justified that it this application could also be applied for the *Canadensis* since in the botanical garden of the TU Delft it was found that electrospray harvesting works for all four yew species present.

A difficulty about this species is that the Canadian yew is not worldwide available. From origin the yew is from Canada and the north of the USA and it is not allowed to export the tree to areas outside North-America [185], which restricts the location of the plantation. However, the other two yew species with high taxane concentrations, *T. Globosa* and *T. Floridana*, have even more severe problems with cultivating because for their presence on the list of endangered species [186],[187].

The Chatham Biotec Ltd. company is at this time breeding *Taxus Canadensis* species with high taxane concentrations and growth rates [188]. This information is very useful for the future plantation. Whether it is possible to take an elite *Taxus Canadensis* species from the firm above or not, it is unlikely that the amount of trees that is needed for our plantation is readily available. The order needs be placed long (about 3-4 years) before the trees should be delivered, which should be taken into account by the planning of the plantation. However, this is the same for all selected species and the concern about the delivery time of the *Canadensis* is thought to be a minor issue, since the planning of implementing electrospraying at large scale will anyway not be a technique that will be realized in less than 3-4 years.

12.1.2 Location

The best place to grow the *Taxus Canadensis* trees is a humid, continental climate. Preferably, the ground should be moist, well-drained, and consist of silt loams [180]. The *Taxus Canadensis* is only present in North-America. In Figure 49, the distribution can be viewed.

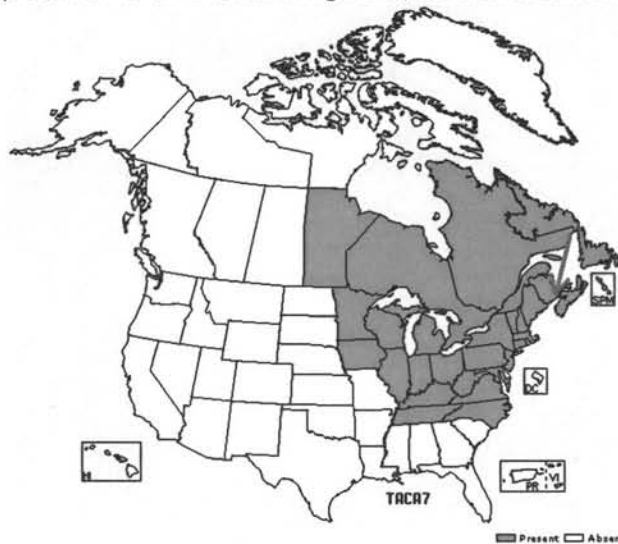


Figure 49 Distribution of the *Taxus Canadensis* with the red arrow pointing to the Youngs Cove, adapted from [189]

There are strict export regulations for the *Taxus Canadensis* [188], therefore it is not possible to locate the plantation at another region than North-America.

Currently, the Chatham Biotech Ltd. is setting up a plantation in the west of Canada in the Youngs Cove to breed elite *Taxus Canadensis* species. This location was selected because of the presence of a microclimate due to the nearby lakes. Here, there is late frost and a sandy clay loam layer on top of bedrock which is favourable for the drainage [188],[190]. The choice of this company, which is specialized in breeding *Taxus Canadensis* trees, to pick this particular location was the main reason for us to base the design on this location. The place of the plantation is indicated in Figure 49 by the red arrow.

12.1.3 Shape of tree

The trees will be planted in long rows so that after a few years hedges are formed. By planting 4 yearling trees per meter [191] in 6 years a hedge can be formed [192]. The shape of the hedge will be rectangular. The reason for this shape is because this form is the easiest to handle by cutting (and most likely milking) the trees, and therefore the cheapest. When the trees can be cut straight, this job can be done automatically by robots or by a machine that is pulled by a tracker. Due to the high number of trees on the plantation, it is very costly to do the cutting by hand, therefore no other shapes are considered [192].

12.1.4 Spatial distribution

Determining the distance between the rows, the main parameter that has to be taken into account is the shadow of the adjacent row. When the rows are too close together, the bottom part of the tree might not get enough light. Also, the machinery has to be able to drive between the rows. Over the years, even when the trees are periodically cut, the hedge expands. In other words, the distance

between the rows becomes narrower. When the distance has become too small, the hedge can be cut in a way to slim it, however that year no harvesting is possible.

To minimize the effect of the shadow on the growth of adjacent hedges, the hedges are aligned in the north/south direction (see Figure 50).

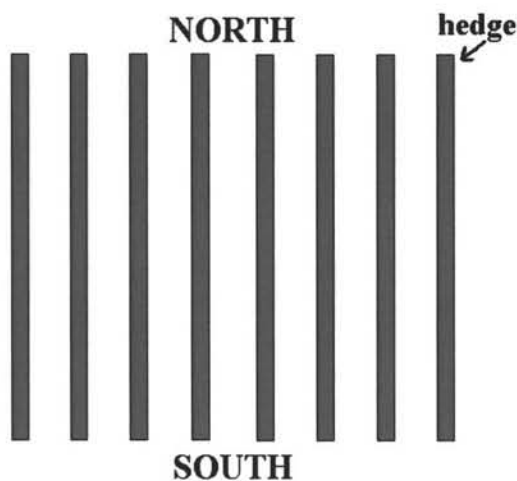


Figure 50 Spatial distribution of the hedges; hedges pointing towards the south

A model is made to find the maximum amount of effective surface area (area that can be sprayed) by varying the height of the hedges. This model will be discussed in Section 12.2.

12.1.5 Cutting and milking

To win taxanes via the extraction process, or by milking the trees, it is important that the amount of needles per volume is high. A higher density of needles means more taxanes. To achieve a high density of needles in a hedge it has to be cut regularly. If the trees in the hedge are not cut, the trees will become similar shaped as Christmas trees (see Figure 51). By cutting the tree, more branches will be formed which causes the increase in density [192].

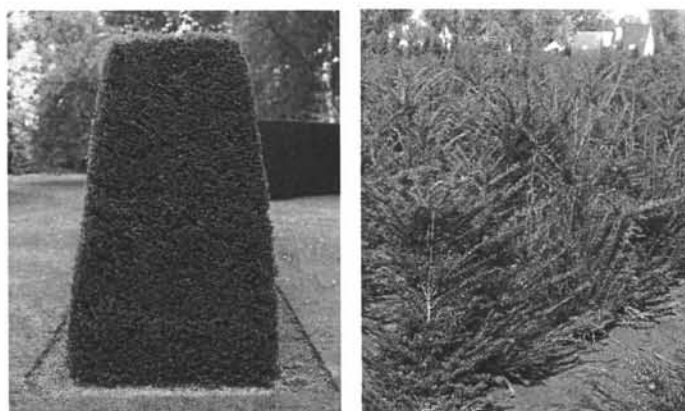


Figure 51 Difference between needles density by cutting (r) and not cutting (l) [193]

Yew trees grow from spring to fall. When a yew hedge is kept without the aim to harvest taxanes, it is normally cut once a year, before the longest day is reached [192]. By the extraction method, the trees are cut in the spring and sometimes in the fall as well, since the concentrations at these periods are the highest. In the summer (June-August) the concentration of taxanes in the *Taxus Canadensis* drops dramatically [194], and also in the winter the concentration is very low since the tree is not active [30].

It is the best clip the trees at the same time as is done for the extraction method, because at that time period the cuttings are worth the most. It is known that young shoots give higher yields than older needles [30]. Also, the milking should be done in the period before the cutting, however enough time has to be between the milking and cutting, so the trees have time to replenish the taxanes. The following planning is proposed (see Figure 52).

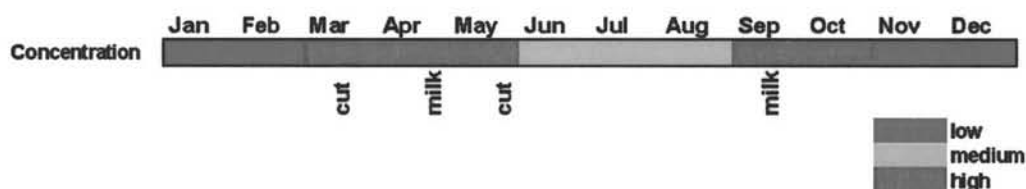


Figure 52 Time line with concentration taxanes in the needles per year and the best time to cut and milk the yew trees

When the hedges are cut in mid-March, at the end of April the young shoots can be milked. If again the hedges are cut at the end of May, the young shoots growing during the summer can be milked in September. The tree grows about 20 cm per year, so for the calculation it can be assumed that every time about 10 cm of young shoots are present at the hedges, ready for milking.

12.2 Modelling of plantation

The goal of the following model was to design a plantation that can harvest as much taxanes as possible. The main parameter in the design of this plantation was the height of the hedges. The challenge was to find an optimum between the height of the hedges and the effective surface area of the hedges.

12.2.1 Assumptions

To model the plantation, several assumptions had to be made. Below, the assumptions are listed. Some assumptions are graphically represented in Figure 53, and in Table 15 complex terms that will be used in this report are defined. The explanation of each assumption can be found in Appendix G-2.

- Height of trees planted is 0.5 m
- Four trees are planted per meter in hedge lanes; the distance between the trees is 0.25 m
- The width of the hedge is 0.8 m
- Growth rate is 0.2 m/yr in all directions
- The minimum angle that sun makes with respect to the horizon is 44°C
- Milking takes place from first year that the trees are planted
- The effective surface area of the trees increases linearly with height
- The maximum cumulative profit period is set at 10 years

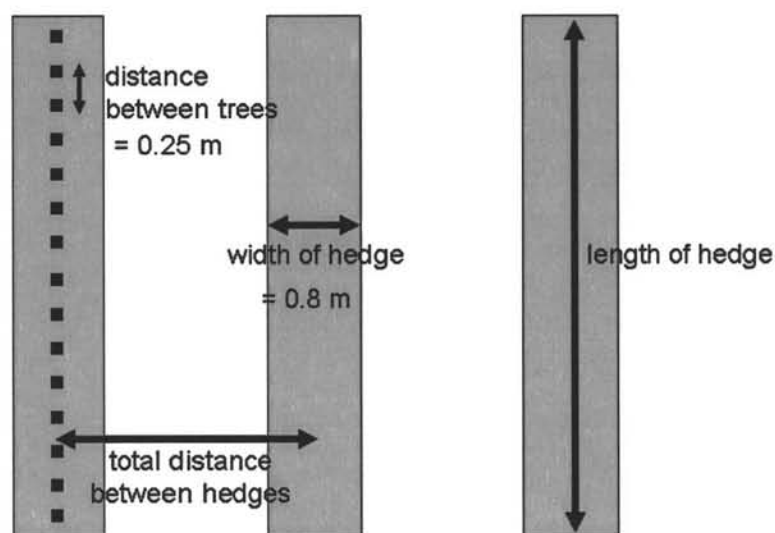


Figure 53 Graphical representation of terms used by assumptions

Table 15 Definitions of complex terms

Effective surface area	the surface area of the hedges where electro spraying can be applied; i.e. the surface of sides of the hedges
Maximum cumulative profit period	the period over which the maximum amount of profit is made. i.e. if the maximum cumulative profit period is 10 year, in the period from 0 to 10 years the maximum amount of profit is made.
Payback time	the time in which the investment costs is earned back

12.2.2 Model

The following model, in which the above assumptions written above are included, was used to find the height at which the production of taxanes is the highest. The model is made for a plantation of one hectare; however this can easily be scale up.

Total effective surface area

A list of tree heights between 1 and 2 meters was made and the total effective surface area per hectare was calculated. This was done in the following way (for the results see Appendix G-3); The corresponding total distance between the hedges (from stem to stem) was calculated by taking the height of the tree and divide it by $\tan(\text{angle_of_sun})$. 0.8 m was added to take the width of the hedges into account.

$$\text{total distance between hedges} = \frac{\text{height of tree}}{\tan(\text{angle of sun})} + 0.8\text{m}$$

The area per tree is the surface area that one tree occupies. This was calculated by multiplying the distance from stem to stem between the different hedge lanes by the distance between the hedges in one row (4 trees per 1 meter = 0.25 m).

$$\text{area per tree} = \text{total distance between hedges} * 0.25\text{m}$$

From the area per tree, the number of trees per hectare was found.

$$\text{number of trees per hectare} = \frac{10000m^2}{\text{area per tree in } m^2}$$

The effective surface area per tree (the surface area of the hedges where electro spraying can be applied) was calculated by multiplying the height of the tree with the distance between the trees in one hedge lane (0.25 m) and by 2. The factor 2 is included since the hedges can be sprayed on two sides.

$$\text{effective surface area per tree} = \text{height of tree} * 0.25m * 2$$

The total effective surface area is the effective surface area per tree multiplied by the number of trees per hectare.

$$\text{total effect. surf. area per hectare} = \text{effect. surf. area per tree} * \text{nr. of trees per hectare}$$

Surface area before the tree is full grown

The total effective surface per hectare calculated above gives the surface area per hectare for a hedge of a certain height. However, the plantation starts with trees of 0.5 m high. Therefore a table was made (see Appendix G-4) where the cumulative effective surface per tree up to a certain year (based on the desired height of the tree) was calculated without cutting the height of it. By this calculation, the growth and therefore the increase of the surface area was taken into account. Below the steps that were taken for this calculation can be viewed.

Per quarter of a year the height of the trees were calculated. The initial height was 0.5 m and the growth rate 0.2 m/yr.

$$\text{obtained height} = 0.2m * \text{years of tree on plantation} + 0.5m$$

The effective surface area at that year per tree was calculated in the same way as was done before.

$$\text{effective surface area at that year per tree} = \text{height of tree at that year} * 0.25m * 2$$

Then, the cumulative effective surface area up to a certain year per tree was calculated. This was done by summing up the surface areas per quarter and multiplying is by $\frac{1}{4}$ to take into account the time periods for which the effective surface area counted (quarters).

$$\text{cumulative eff. surf. area up to that year per tree} = \sum \text{eff. surf. area at a certain year (per quarter)} * \frac{1}{4}$$

Finding the optimum height

To calculate the maximal surface area and therefore the maximal amount of taxanes that can be obtained from the trees, the height of the trees is the main parameter that has to be fixed.

Assume that a plantation is started with full-grown trees of a certain height, where the trees are kept at a certain height with minimal spacing between the hedges (dependent on the height). When the height of the trees that are planted increases, the total surface area of the trees per hectare increases, although the number of trees per hectare decreases (see Figure 54).

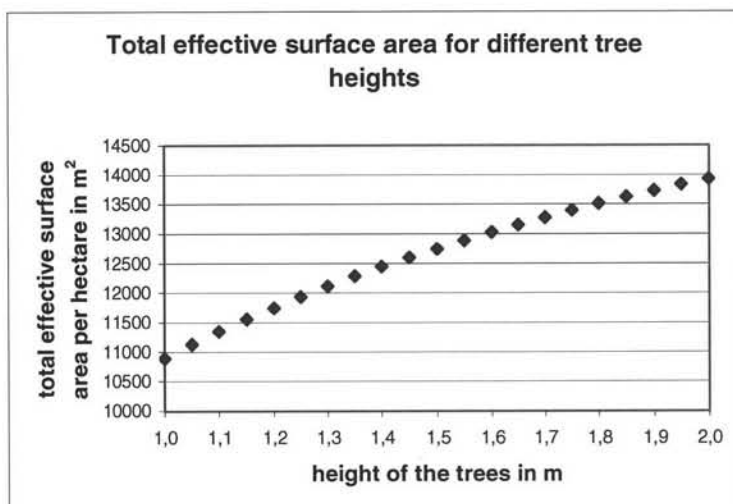


Figure 54 Relationship between the heights of the trees versus the total effective surface area per hectare

However, the higher the tree has to become, the longer it takes to reach that height. For shorter target heights, the increase in total effective area is higher in first few years before the target height is reached. This is because, the shorter the target, the smaller the spacing between the hedges, and therefore the more trees per unit area. The advantage of having tall trees manifests after short tree reaches their target height because the effective area then stops increasing as the trees are constantly cut. In other words, with smaller tree more profit can be made a short term, and at the long term more profit can be made with higher trees (see Figure 55).

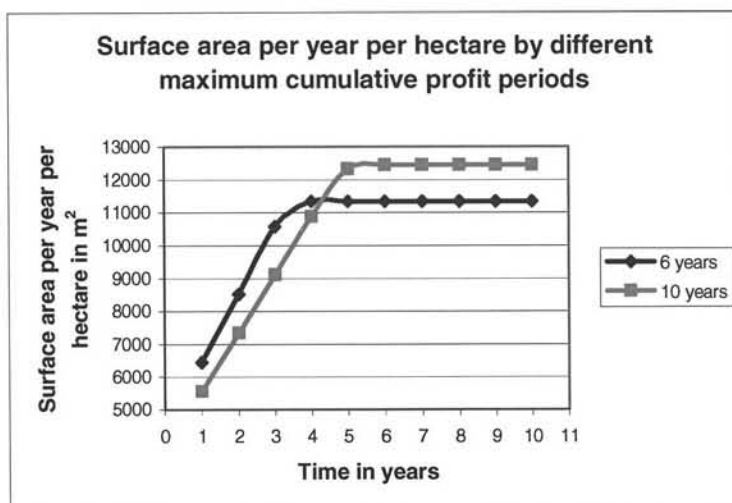


Figure 55 Surface area per year per hectare by different maximum cumulative profit periods

The two contradicting conditions described above can be defined as following:

1. The higher the hedges the more taxanes can be obtained later on.
2. The higher the hedges, the longer it takes before the plantation is optimally used.

To find the optimum between these two points, it was needed to set a time period in which the plantation has to obtain the highest amount of taxanes. This term was decided to be 10 years. So, the calculations are based on the assumption to obtain as much taxanes as possible in one decade. The

reason why 10 year was chosen is that this was thought to be the minimum time that our plantation would exist. From the information about the market it is predicted that in 10 years there is still a high demand for taxanes and that there is therefore still a market.

Cumulative surface area over ten year

To calculate which height of the trees will give the highest amount of taxanes over a time span of ten year, i.e. has the highest cumulative surface area, the calculation is split in two parts:

- > the cumulative surface area up to the point that the tree reaches that certain height
- > the surface area when the tree is full-grown

For tree heights between 1 and 2 meters, first is had to be determined how long it would take to reach a certain height.

$$\text{number of years to obtain the height} = \frac{\text{height of tree} - \text{initial height of tree}}{\text{growth rate}}$$

Then, from zero up to the year that it takes to reach the desired height the cumulative surface area before it is full-grown is used (see Section 12.3.2). For the time period that the tree is full-grown the total effective surface area is used (see Section 12.3.1).

$$\begin{aligned} \text{cum. eff. surf. area over 10 year} &= \text{cum. eff. surf. area up to that year per tree} * \text{nr. of trees} \\ &+ (10 - \text{years to reach that height}) * \text{total eff. surf. area per hectare for that height} \end{aligned}$$

The results of the cumulative effective surface area over a time span of 10 year for the different heights can be seen in Figure 56.

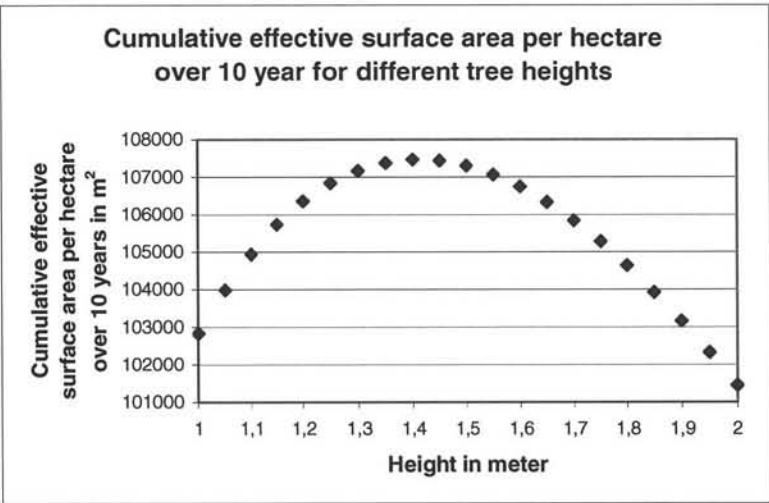


Figure 56 Cumulative effective surface area per hectare over a time span of 10 years for different heights

The results show clearly that there is an optimum in the height of the tree. This optimum is at 1.4 m. This value is closely to the height that Mauritz estimated (1.5 m) when his nursery was visited [192].

12.3 Economical evaluation of plantation

The aim of this economical evaluation of the plantation is to see what the extra profit would be for a company that would use our harvesting machine and based on this information what the price will be that can be asked for our device. Milking with our device will be an extra way to earn money for the yew tree plantation owners on top of the earnings from selling the clippings. Therefore, the earnings from milking are compared with the earnings from clipping. The costs of the milking device are discussed, and from there a conclusion about the price is made followed by some comments based on a proposed scenario.

12.3.1 Assumptions

To do an economical evaluation of the plantation, several assumptions had to be made. Below, the assumptions are listed. The explanation for each of them can be found in Appendix G-5.

- best case scenario; 10% of the total amount of taxanes in the needle can be harvested (milk frequency is 10 times a year)
- worst case scenario; 2% of the total amount of taxanes in the needle can be harvested (milk frequency is 2 times a year)
- the percentage of needles are been sprayed is 60%
- the price of dried clippings is 0.00275. €/g
- the price of the dry extracted material obtained from electrospraying is 1 €/g
- 20 cm of the branches will be cut per year and sell as clippings
- the needles at the outer 20 cm of the hedge will be milked

12.3.2 Revenue

There are two sources of income for the plantation owner; selling the clippings and selling the product from electrospraying. Since all trials to get information from companies that sell yew clippings failed, variables such as the number of needle per volume, and weight of needles had to be calculated in order to get an estimate of the earnings. These numbers were found by small experiments done by the authors of this report. The results and calculations to obtain these basic numbers can be viewed in Appendix G-6. Below, the results of the earnings from milking and clippings are discussed and compared. The corresponding calculations can be found in Appendix G-7 and Appendix G-8.

Earnings from milking

For the income from milking the trees, for each of the two scenarios the earnings are calculated. By the worst case scenario 1.2% of the total amount of taxanes in the needles can be obtained per year (2% yield · 60% efficiency), and for the best case scenario this percentage is 6% (10% yield · 60% efficiency). For the worst case scenario in the first year € 255 can be gained, and after 5 years € 572, while for the best case scenario the earning will rise from € 1.277 to almost € 2.900 per hectare per year (see Figure 57).

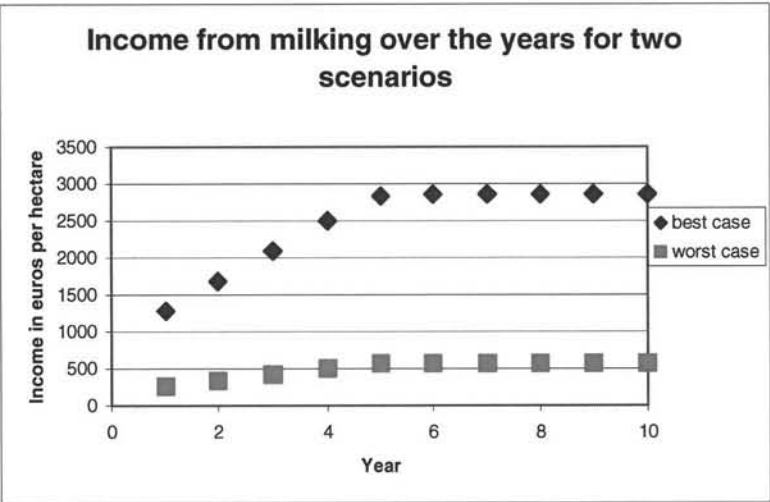


Figure 57 Income from milking over the years for the best and worst case scenario

Earnings from milking compared with earnings from clippings

The earning from clippings is estimated to increase from almost € 17.000 per hectare at the first year to € 37.500 per hectare when the hedges are full-grown (see Figure 58). These numbers are much higher than for milking, and therefore selling the clipping is going to be the main income for the plantation holder.

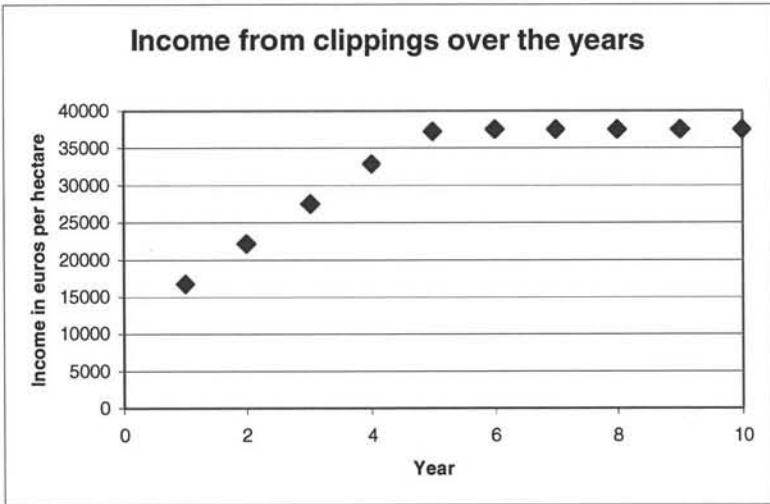


Figure 58 Income from clippings over the years

When the two incomes are compared, it shows that for the best case scenario this means that 8.0% extra profit can be made by inducing electro spraying, however for the worst case scenario this percentage is 5 time less.

12.3.3 Costs

The extra costs that have to be incurred when our device is implemented in the plantation are compared with the extra income from the milking. Whether a yew tree plantation owner will buy our taxanes harvesting device depends on the investment costs that have to be done and the expected payback time.

Investment costs

The advantage of our device is that no large adjustments have to be made in order to use our machine at a plantation, and that therefore the investment cost will only consists of the purchase of the machine. Besides the fixed, investment costs, there are the variable costs, such as the energy costs, solvent costs, taxes, and storage of taxanes. It is believed that all those costs do not give weight against the purchase costs of the machine, and that there are for now negligible. Our device uses high voltage (4 kV), however the current is very low (0.096 mA) [195]. The power used over 30 min. is 0.4 W which indicated that not much energy is used. The current solvent price ranges from € 811 to € 1027 per metric ton for 96% industrial grade ethanol [196]. It is expected that less than one metric ton per hectare per year is used. That would mean that the costs for the solvent are more than a factor 10 smaller than the purchase costs of the taxanes harvesting device and that it therefore is justified to neglect it.

Milking range of one machine

To find the value of the machine, it has to be known how many hectares can be milked by one milking machine. This is dependent on how much taxanes can be harvested in a certain time period per machine, which is in turn dependent on the size and the speed of the machine. For an extended version of the calculations done below, see Appendix G-9.

From small scale electrospray experiments (see Section 5.4) it was found that the estimated time to get the maximum amount of taxanes out the needles without damaging it is about 30 min. The maximum size (length) of the electrode is set at 5 m (see Chapter 11), since when it is designed to be longer problems with the mobility will be encountered. With the number of trees per hectare, the distance that the taxanes harvesting machine has to complete is calculated. It was found that, using the information written above, it takes 37 full days to electrospray one hectare, and that the best case scenario (spraying 10 times a year) will be hard to execute.

The profit that can be made with the device determines the price of the device. If the electrospraying of one hectare takes 74 days (37 full days), the worst case scenario, electrospraying twice a year, should be doable although the planning of milking will become very tight. The earning from milking in the worst case scenario will only be € 572 per year at the highest. With this income, the buyer of the machine will never be able to pay it back, since the estimated value of the machine is between € 50.000 and € 100.000. Also, with such a low income, nothing will be left over if the energy and solvent costs would be taken into account. It seems that no realistic price could be given to our harvesting device since no profit would be made with it.

Required speed of machine

Without improving the device, the taxanes harvesting device will never be introduced into the market, since no profit can be made out of it. In the following paragraph a scenario is given where it is assumed that the best case scenario works. The idea is to show how much the technique of milking trees has to improve to turn it into a valuable product. From a proposed selling price of the device, the speed necessary to make the machine market valuable is determined and compared with the current speed.

The purchase costs of the machine plus the interest is estimated at € 50.000. Assuming that the customer wants a payback time of 10 years, the device needs to harvest per year an amount of taxanes worth € 5.000. In the best case scenario, per hectare € 2860 will be earned. This means that one device needs to be able to harvest the taxanes from 1.75 hectare every year. In Figure 59 the relation between the spraying time per tree and the number of days needed to spray 1.75 hectare 10 times a year (best case scenario) is shown. The initially calculated number of days is multiplied by two, since it is assumed spraying can only take place at 12 of the 24 hours per day due to weather conditions.

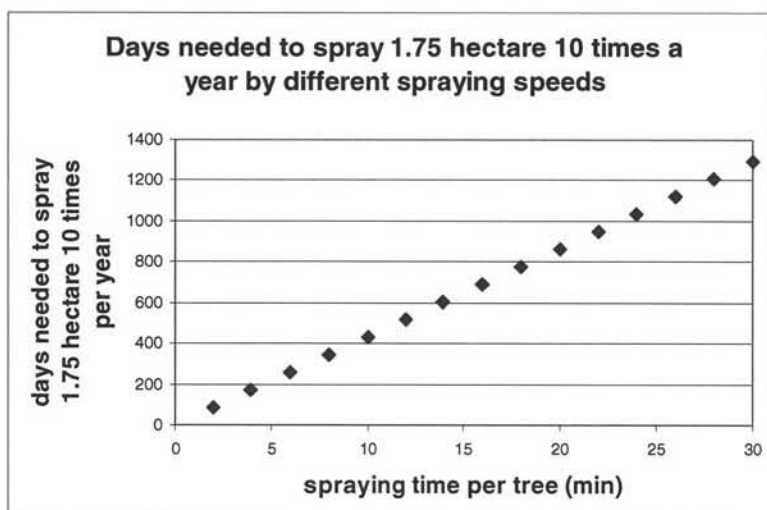


Figure 59 The number of days needed to spray 1.75 hectare according to best case scenario by different spraying speeds

The graph shows that the upper limit of the spraying time per tree is around 8 min. (346 days) because by lower speeds it takes more than 365 days to spray all the trees per year. Also, the milking has to be performed at certain times of the year (see Figure 52). In the winter, for example, it is not favourable to spray and also during the other seasons there is most likely much fluctuation in the amount of taxanes that can be harvested.

To keep some flexibility, the spraying time per tree has to be lower than or equal to 2 min. (86 days). This is an immense difference with the 30 min. spraying time that it takes by the experiments. This gap cannot be overcome by spending more time on the design and making small adjustments. The only way to solve this problem is by improving the mechanism. The taxanes have to get out quicker. To increase the speed, it needs to know how the mechanism of mobility of taxanes exactly works. With this knowledge, the key question of how to increase the flow rate of sap together with taxanes in getting out the needle might be found. Once the answer is found and the mechanism is optimized, another try could be given to scale the system up and put it onto the market.

Once a quick-moving device is developed, the economic evaluation could be extended with writing a proposal for a lease contract, or renting the device to plantation owners. When one machine can be used for multiple customers, the renting price can go down drastically. However, for our situation it is not of interest to discuss options like these.

13 Conclusion and Recommendations

13.1 Conclusion

The global market demand for paclitaxel and its precursors is much higher than the worldwide production. Forecasts show that the demand will increase rapidly with an annually rate of 10 to 20% per year up to 2011-2016, and that the market will be solid for at least the next 10 years. This prediction is promising for the success of bringing a new technique to obtain taxanes onto the market. Based on our objectives, from the many techniques that could potentially be used to harvesting taxanes one technique was chosen. Electro spraying was selected due to the hopeful information that was received from a research group at the TU Delft regarding this subject. The principle behind the electrospraying was investigated and a proposed transport mechanism of the taxanes during the electro spray process was set up.

The results of the house of quality were used to determine the focus of our design, to identify the contradicting requirements, and to define our product. Our product will be a device that can be sold to yew tree plantation owners who make money from selling the clippings, as a way to produce extra taxanes. The geometry of the collection area, issues related with the electric field (voltage and distribution), and the design of the collection device (adhesion and chemical use) were given priority. The contradicting requirements were used to specify the design specifications. From the design specification a list with desired properties for the electrode and insulator was composed. These properties were in turn used to come up with a design that satisfied all these characteristics.

The result is a taxanes milking machine with horizontal plates at a distance of 10 cm from each other. By putting a voltage of about 4 kV on the electrode, an electric field will be generated around the needles which will cause the movement of needle sap toward the electrode. The electrode is made of copper and is moulded in the thermoplastic, poly-imide-amide (PAI) which will act as an insulator. At the top a 0.16-0.32 mm thin insulating layer will be put to allow the electric field to bypass it without much loss in its strength but avoiding short-circuiting.

The bottom of the electrode will be consisting of a much thicker PAI layer to prevent interaction of the electric field of the different electrode plates. At the same time it will help to push the branches closer to the surface of the collection plate beneath it. The advantage of this is that the electric field strength is higher closer to the electrode, and that therefore a lower voltage can be used to obtain the same electric force.

The extract released by the needles of the yew will be collected in solid (when the needles do not touch the insulator) and liquid form (when the needles are in contact with the insulator). Another advantage of needles touching the insulator is that when they are in contact with the insulator, the release of needle sap can be enhanced by increasing the wetting properties of the insulator due to the affinity of the sap with the two surfaces. The surface of PAI is modified with UV treatment in order to make the contact angle of the sap with the needle greater than the contact angle of the sap with the insulator and increase the attraction toward the insulator.

Since the extract is in solid and liquid phase on the insulator plate, a design was made where the extract is flushed away periodically with ethanol. By gravity, the solvent is collected from the slightly tilted plates to a place where the solution is stored under dark, cold conditions to slow down the degradation process. The entire device will be placed on a moving platform that will be operated automatically.

From the economical evaluation, it was found by using the same conditions as by the small scale experiments that potential users will make no profit with our product. The amount of hectare that can be covered by one machine is too small to get enough income from selling the taxanes extract from it to balance out the costs. The time needed to get the maximum amount of taxanes out the needles by electro spray has to decrease from 30 min to less than 2 min. in order for our clients to make profit with our harvesting device. Without this huge improvement the future of our product is very sober. There will be reason to enter the market since success will most likely fail to occur. However, more research about the transport mechanism of taxanes under influence of electric field might give a solution to speed the process up. Right now, no clear explanation is available. In the future, when this subject might get deeper investigation, ways to increase the movement of taxanes might be found. Therefore, we strongly recommend more research regarding the transport mechanism of taxanes inside the needle before more steps toward commercialization of this product are considered, which is expanded in more detail in the following paragraph.

13.2 Recommendations

The design of a device to harvest taxanes with electro spraying was hindered by the limited amount of information available about the subject. Therefore, the following recommendations for future research regarding this subject are done:

More research about the basic mechanisms:

- The biological background of where taxanes are produced, how it is transported, and where and how it is stored
- The composition of the extracellular sap and the sap that comes under influence of electro spraying out of the needles
- More reliable data about the composition of the extract after it is sprayed, and the composition change over time (degradation of taxanes, water content/evaporation rate)
- The mobility of taxanes; in which form it is transported (dissolved, in colloids, or another form), and how the mobility of taxanes is influence by an electric field
- The electricity loop through the earth, tree and electrode; how ions and electrodes are involved in the electricity loop

With this information it is hoped to answer the unanswered questions in our report and verify our proposed theories. The information is crucial to improve the efficiency of the machine. The only way to improve the design of the machine is to get a better understanding about the background information. Once the rate limiting step of the mobility of taxanes is found and the mechanism behind it, it is easier to tune this step to increase the production. Besides that, correct information is therefore very important to have a sound basis for the design. In this report a lot of values are based on assumptions, which decreases the accuracy of the results.

14 Evaluation of the project

This report is the result of five master students' intensive work over a period of three months. While in the beginning the cooperation between the members of the team was far from optimal, after a few weeks the teamwork already had improved significantly. The group was quite diverse; the members have different backgrounds with respect to study and origin. Two have done their bachelor abroad, in Cincinnati (USA) and in Singapore respectively. The remaining three did their bachelor at the TU Delft, two did the bachelor Chemical Engineering and one the bachelor Sustainable Molecular Science & Technology. This diversity was very useful, since it enabled us to approach the problems from different point of views. The specific knowledge that was available was used if problems in that particular field came across. The latter aspect, the difference in the origin of the group members, caused some trouble in the beginning of the project, but further along the road the miscommunication reduced a lot. It was a very valuable experience to work in a bicultural group; it is an experience that will definitely be helpful in the future, whether it is for a next project at the university, at a company, or in daily life.

It was rather disappointing to reach the conclusion that our product is not ready for the market. This could have been avoided by doing more market research and getting more data about the small scale experiments before the topic was chosen. Each of us had the impression during start-up of the project that the technique was much more mature and the yield would be much higher. Afterwards, the electrospraying of yew trees seems to be a technology that is too early in its development to be made into an intended product.

Nevertheless, we are still proud of our work and happy with the final result. We ventured ourselves into a very challenging topic and went through the entire product design process. We have done extensive research and have gained a lot of knowledge in many different fields. This combined with our chemical engineering knowledge was integrated in the complete design of our product.

The design is a result of some very fruitful and interesting brain-storming sessions, which helped us to generate numerous creative ideas. Team-building events, such as the Belbin personality test (see Appendix H), the peer review, and even the dinners together have helped us to understand each other, improve the communication and work together better. Therefore, we believe that our product design is a good demonstration of our creativity and teamwork.

Furthermore, the design methodology that we devised – a combination of design tools and rules from chemical engineering, industrial design and cradle to cradle – worked pretty well. Applying the design method gave the project structure and direction. The industrial design tools of the morphological chart and the Harris profile were a very handy addition to the chemical engineering tools we used. We have also become even more familiar with the house of quality and we think that it is a useful tool to identify technical areas of focus for product design. It is a pity, on the other hand, that we could not implement all the tools and rules of the cradle-to-cradle design method. One reason is the fact that we are still inexperienced in the area of product design; this is only the second course where we had to design a product. This means that we had our hands full with learning to work together and applying the chemical engineering tools and rules. The second reason is that applying sustainability gives a lot more work: making lists of all the possible materials and finding all information on their environmental advantages and disadvantages takes a lot of time. A final reason could be that we put three types of design methodologies together, and that it is difficult to integrate them and apply them consistently. We found that cradle-to-cradle is more an independent method that is different from the chemical engineering method for product design we used, while the

industrial design tools could be integrated easily in the four design steps. We even think that they made our life simpler by giving even more focus and direction to the design process.

Despite encountering some difficulties in the project, we believe we succeeded in fulfilling the requirements for the course Conceptual Product Design, to design a technically challenging chemical product.

List of symbols

A	[m ²]	Surface area
C ₀	[F]	Capacitance in vacuum
C	[F]	Capacitance of the material
C _D	[–]	Drag coefficient
D	[m]	Distance between the plates
d	[m]	Droplet size
E	[V/m]	Electric field strength
F	[N]	Force on a charged particle
g	[m/s ²]	Gravitational acceleration
h	[m]	Column height due to capillary force
k	[–]	Dielectric constant
K	[S/m]	Electric conductivity
q	[C]	Droplet charge
q _{max}	[C]	Maximum droplet charge
Q	[m ³ /h]	Flow rate
R	[Ω]	Resistance
r	[m]	Radius of a sphere
t	[s]	Time
t _r	[s]	Dielectric relaxation time
V	[V]	Voltage
v _{air}	[m/s]	Velocity of air
v _l	[m/s]	Velocity of the droplet
x	[m]	Thickness of insulator
γ	[N/m] = [J/m ²]	Liquid surface tension of water
γ _{lv}	[N/m] = [J/m ²]	Surface tension of the liquid-vapour interface
γ _{sl}	[N/m] = [J/m ²]	Surface tension of the solid-liquid interface
γ _{sv}	[N/m] = [J/m ²]	Surface tension of the solid-vapour interface
ε ₀	[C ² /N·m ²]	Permittivity of vacuum
ε	[C ² /N·m ²]	Permittivity of the material
θ	[degrees]	Droplet contact angle
ρ	[Ω·m]	Resistivity
ρ _{air}	[kg/m ³]	Density of air
ρ _l	[kg/m ³]	Density of water
σ	[Ω ⁻¹ m ⁻¹]	Conductivity

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