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Methods to Study Foliar Penetration of Agrochemical Active and the Effect of Solvents Used

Master of Science Thesis

KAJSA LUNDBERG

Department of Chemical and Biological Engineering
Division of Applied Surface Chemistry

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Kajsa Lundberg

Examiner: Prof. Krister Holmberg
Supervisor: Dr. Peter Westbye

This work was performed at AkzoNobel Surface Chemistry, Stenungsund, Sweden

Department of Chemical and Biological Engineering
CHALMERS UNIVERSITY OF TECHNOLOGY
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Abstract

With an increasing population and need for food together with a decrease in arable land the need of a more effective farming is high. The use of crop protecting agents has improved the framing efficiency. However, problems to deliver the agents to their site of action have led to overuse. To reduce this overuse and the environmental concerns that they create a more efficient delivery system of the crop protecting agents needs to be developed.

There are many different factors affecting the delivery of crop protecting agents to the crop; drift of drops, size of drops, droplet dry down and uptake ability for example. In order to further develop high performing formulations understanding of how these different parameters affect each other is essential. In this thesis the focus has been on foliar uptake from a solvent system and the method that has been used is a Franz cell methodology, where diffusion over membranes has been studied over time.

Another concern in the farming industry is the fact that many solvents today originate from a petroleum based feedstock and work is done in order to find more environmentally friendly alternatives. N,N-dimethyldecanamide and N-decyl-N-methyl formamide are two newly developed alternatives and their uptake ability has been investigated in this thesis.

The results show that foliar uptake can be measured on both formulated system in the form of an emulsifiable concentrate and in its diluted form (i.e. emulsion) using Franz cell methodology. In order to approach a method as close to reality as possible measurements on dried down drops were also done and a method for doing this has been developed. The Franz cell results showed that N,N-dimethyldecanamide and N-decyl-N-methyl formamide have similar uptake ability and indicate that they both are good solvents for the purpose.

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1. Introduction

In order to satisfy the world's populations need for food, 10 billion MT of crop has to be produced annually [1]. Since the population is steady increasing it is believed that the production of crop needs to double to the year 2030 [2]. To meet these demands there are two possible solutions, either forest and woodland areas can be ploughed to make room for farming or the efficiency of the existing farming can be increased [2]. Since there is a decrease in arable land due to spread of urban/industrial areas and deserts, the first option is not sustainable.

One way to increase the yield of crop is to use crop protection agents to reduce the competition from unwanted weeds, fungus and insects. Since the use of crop protection agents started the efficiency of farming has improved by as much as 50% [1]. However, since there is a problem to deliver the crop protection agent to the site of action they have been extremely overdosed, leading to environmental concerns. To stop using crop protection agents is not an option since the efficiency will decrease dramatically and entire harvests can go lost [1]. Instead the focus today lies on optimizing the formulations, in order to eliminate the overuse and thereby the environmental impact [3].

One way to decrease the amounts of formulation that are sprayed onto the fields is to increase the biological efficiency of the system. This can be achieved by adding adjuvants to the formulation [3]. Adjuvants are often surfactants or polymers that help stabilize the formulation, controlling droplet size distribution upon spraying (i.e. to reduce drift), increase the adhesion of droplets to the leaf surface or to make the spray droplets resistant to rain [3]. Adjuvants can also increase the uptake to weeds/ plants by helping the crop protection agent penetrate the outer wax layer, which is the main barrier in order to get inside the plant [4].

In some formulations, solvents are used to improve the solubility of the agrochemical actives. An increase in solubility leads to another possibility to decrease the amount of crop protection agents sprayed onto the fields and thereby minimizing the environmental impact. However, many solvents today originate from a petroleum based feedstock and work is done in order to find more environmentally friendly alternatives.

To be able to further develop high preforming formulations, understanding of how the different parameters like spray volume, formulation type and droplet dry down affects bio efficacy needs to be evaluated. In this study the focus will be on uptake and how to develop a method to better show how the uptake from a liquid system occurs out in the fields.

1.1 Aim

The aim of this study is to establish a method to measure the penetration of agrochemical actives from a solvent system through leaf cuticulum. In order to approach a model as close to reality as possible the solution will be studied both as a solution and in the form of a droplet. The method used was a Franz cell methodology, where diffusion over membranes can be studied over time.

The method was developed using two solvents, N,N-dimethyldecanamide and N-decyl-N-methyl formamide, which are both newly developed solvents to replace petroleum based ones. As a model active ingredient (AI) a fungicide called tebuconazole will be used.

2. Theory

In this chapter the theory behind the diffusion mechanism across and the composition of the outermost layer of the leaf, namely the cuticular layer, is described. This chapter will also cover the general principals on how stable solvent formulations, in this case emulsions, are created. First however, the application of how one use crop protection agents and the major problems connected to them will be discussed.

2.1 Application

In agriculture the crop is protected by spraying the plants with a formulation containing crop protection agents. The formulation consists of a concentrate, containing the AI in a solvent that is mixed with water in the farmers mixing tanks and then sprayed on the fields. The formulation type depends on the state of the AI. If the AI is in liquid state solubilized in the solvent an emulsion will form upon mixing with water and if the AI is in solid state dispersed in the solvent a suspension will be formed. Soluble liquids are another formulation type where the AI is dissolved in solvent creating a homogenous phase upon dilution in water; no emulsion or suspension is formed.

In this study emulsions are chosen to look at and the two different solvent used, N,N-dimethyldecanamide and N-decyl-N-methyl formamide can be seen in figure 2.1. N,N-dimethyldecanamide is today commercially available. However, N-decyl-N-methyl formamide has showed to have improved properties regarding water solubility. N-decyl-N-methyl formamides water solubility is lower than for N,N-dimethyldecanamide [19] and which is advantages due to that a solvent with high water solubility can cause precipitation of AI upon mixing with water. This will cause the AI to end up on the bottom of the farmers spray tank and the effectivity of the formulation is decreased.

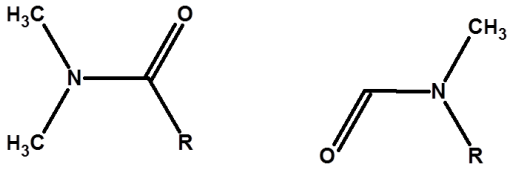

Figure 2.1. The structural structure of N,N-dimethyldecanamide to the left and N-decyl-N-methyl formamide to the right.

Apart from AI-precipitation there are other factors affecting the efficiency of the formulation. The spraying of crops is a complex system with many factors affecting the final amount of AI reaching its site of action; once the spray leaves the nozzle the drops should find their way to the plant and adhere to the leaf surface. Depending on if the AI has a systemic or contact action the formulation also needs to be designed to enhance the penetration into the leaf or increase the wetting of the leaf surface; contact AI:s need good wetting in order to work while systemic AI:s needs to penetrate the leaf in order to have an effect.

Drift of the droplets caused by wind makes it difficult to make sure that the right plants are sprayed, which may cause the formulation to pollute nearby streams/land and to damage

sensitive adjacent crops. This phenomenon is called *spray drift* and is more pronounced for small and light drops [4]. Large drops, however, have a tendency to splash or bounce off the leaf surface upon collision, due to that larger drops have higher kinetic energy and produces a more energetic smash onto the leaf [4]. Therefore an optimum in drop size exists, where both the spray drifts and splash effect is kept as low as possible. The drop size can be altered by changing the nozzle used or the formulation of the sprayed solute; different formulations may create different droplet sizes in the same nozzle [4].

In Table 2.1 the efficiency of the different steps from drift of drops to foliar uptake by the plant is listed. It can be seen that large losses occur from the different steps. By the addition of adjuvants the drop size, deposition properties and uptake can be enhanced. Adding polymers to the formulation has showed to improve the adhesion to the leaf surface; the polymers increase the elongation viscosity of the spray and thereby minimize the rebound from the leaf surface [5].

Table 2.1. A summarizing table of the losses from the different delivering-steps drift, deposition, uptake and translocation, to get agrichemicals onto the fields. [3]

Representative spray application efficiency, system efficiency and off-target loads for an herbicide application

Spray efficacy processes	Process efficiency (%)	System efficiency (%)	Off target component (%)
Deposition[a]	80–95	80–95	20–5
Retention[b]	10–100	8–95	92–5
Uptake[c]	30–80	2.4–76	97.6–24
Translocation[d]	10–50	0.24–38	99.6–62

[a]Amount deposited within target area.
[b]Amount captured by plant.
[c]Amount of retained material taken up into plant foliage.
[d]Amount of absorbed material translocated from absorption site.

However, for a systemic active the uptake is very important; if there is no/or very little uptake into the leaf an efficient delivery system cannot be utilized. Obviously environmental factors like humidity, wind and rain affect the uptake, but the main barrier for uptake is the leaf itself, more specific the outermost cuticular layer [3].

The plant has developed the cuticle in order to protect itself from unwanted water loss and to block infiltration from foreign substances [6]. The cuticle mainly consists of a polymer called cutin impregnated with wax, creating a strong and flexible material that can stand against wind and heavy rain without breaking. The structure is however not a simple homogenous structure; it is constituted of several layers with different properties [7]. First a layer of epicuticular waxes facing the surroundings, underneath a cuticle proper followed by the cuticle layer facing the epidermal cells [7]. Both cuticle proper and cuticle layer consists of a polymer matrix of cutin and in some species also cutan, with embedded intracuticular waxes. Due to the crystallinity of the intracuticular waxes no agrochemical can penetrate through it and a tortuous diffusion path around the IW becomes the reason for the limiting transport.

Cuticle penetration can however be enhanced by the use of adjuvants [8]. Addition of different surfactants/adjuvants has showed to improve the solute mobility in the cuticle membrane (CM) by fluidize intra-cuticular lipids and thereby increase the space in which the AI can diffuse [6]. For this to work the adjuvant and AI needs to penetrate the CM simultaneously. Besides facilitating solute mobility adjuvants may also remain on the leaf

surface and increase the concentration of AI and thereby the driving force to diffuse trough the membrane [6].

It is also believed that foliar uptake depends on the dry down process of the sprayed droplet [3]. For a systemic AI a high coverage of the leaf is not necessary, in fact studies by Faers M A, Pontzen R [5] has indicated that a lower coverage result in a higher uptake for adjuvant containing formulations. This is believed to be a result of a higher concentration of AI and adjuvant, due to the smaller covered area.

2.2 Diffusion across membranes

The uptake through the leaf cuticle is made possible by diffusion. The diffusion process of liquids and gases can be described by Fick`s first law, see equation 2.1.

$$J_i = D_i \frac{dc_i}{dz} \qquad (2.1)$$

The diffusion flux, J_i, is a measurement on how many molecules that will pass through a defined area during a specific time. The driving force for diffusion is the concentration gradient, $\frac{dc_i}{dz}$, where c_i is the concentration and z is the position. This states that molecules travel from an area with high concentration to another area with lower concentration. The diffusion coefficient, D_i, is the molecular mobility of the diffusing substance. [9]

However, when looking at diffusion from a liquid phase over a membrane equation 1 becomes inadequate. When dealing with diffusion between different phases, for example liquid to solid, simply looking at the concentration gradient as the driving force is not enough. When the saturation level of the liquid inside the solid phase is reached, the concentration in the solid material can still differ from the liquid phase, due to a difference in chemical potential. Equation 1 then suggests that even at equilibrium there should be a driving force of liquid into the solid phase. [9]

To solve this problem one needs to change concentration to chemical potential, since the chemical potential in two phases is equal at equilibrium. The driving force for diffusion could then be redefined as the gradient in chemical potential; since there is a gradient in chemical potential if and only if there is a gradient in concentration [9]. Rewriting equation 1 with the chemical potential as the driving force gives the generalized form of Fick`s first law, see equation 2.2.

$$J_i = -\frac{D_i}{RT}\, c_i \frac{d\mu_i}{dz} \qquad (2.2)$$

μ_i is the chemical potential, c_i is the concentration inside the membrane, R is the universal gas constant and T is the absolute temperature. The chemical potential for a neutral substance over a membrane can be defined as in equation 2.3. [10]

$$\mu_i = \mu^* + RT * lna_i \qquad (2.3)$$

Combining equation 2.2 and 2.3 into equation 2.4, it's visible that there are different components affecting the diffusion over a membrane. First it`s the gradient of thermodynamic activity of the solute over the membrane ($\frac{da_i}{dz}$) and secondly the effects within the membrane, the diffusion coefficient and concentration ($D_i\, c_i$). [8]

$$J_i = -\frac{D_i}{RT}\, c_i \frac{d\mu_i}{dz} = -D_i c_i \frac{dlna_i}{dz} = -D_i c_i \frac{da_i}{a_i dz} \qquad (2.4)$$

The thermodynamic activity (a_i) of an AI in a solution has been defined by Fagerström et al [8] as the actual concentration of the AI divided by the solubility limit for the AI in that solution.

Looking at diffusion over an inert membrane, the main component affecting the flux is the thermodynamic activity. A responding membrane, like the leaf of plants, can interact with the solute which causes the flux to depend on the diffusion coefficient. Fagerström et al [8] looked at the influence of adjuvant on both non-responding (silicon) and responding membranes (the cuticulum of leafs). They could show that adding adjuvant strongly enhanced the diffusion, but only for the responding membrane. This concludes that adding adjuvant affect the diffusion by increasing the diffusion coefficient of the diffusing substance.

The cuticular membrane (CM) is a lipophilic membrane that mainly consists of the polymer cutin and wax. To characterize the solubility of a substance in the CM the lipophilic/hydrophilic balance of the solute can be determined. The difference in solubility between two phases, one lipophilic and one hydrophilic phase, is called the partition coefficient and is a measurement on a substance lipophilic/hydrophilic balance. The definition of the partition coefficient can be seen in equation 2.6.

$$K = \frac{concentration\ in\ lipophilc\ phase}{concentration\ in\ hydrphilic\ phse} \qquad (2.6)$$

If $K = 1$ the solubility is the same in each phase, if $K > 1$ the solubility is higher in the lipohilic phase and if $K < 1$ the solubility is higher in the hydrophilic phase. The CM represents the lipophilic phase and the water solution containing the AI the hydrophilic phase, hence a substance with a K >1 dissolves easier in CM. [10]

For practical reasons the octanol/water partition coefficient, $K_{o/w}$ can be used instead of the cuticle/ water partition coefficient ($K_{c/w}$), since measurements of the $K_{c/w}$ can be hard to obtain. Good relationships between $K_{c/w}$ and $K_{o/w}$ have been found for lipophilic substances [11].

The concentration profile of a lipophilic substance over a membrane is seen in figure 2.2. The concentration gradient will not be linear over the membrane if the solute has a chemical preference for the membrane. The activity gradient is however always linear over the membrane, which is also visible in figure 2.2 [8].

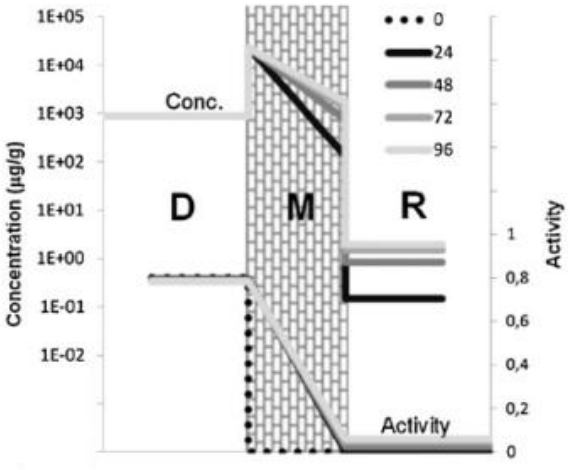

Figure 2.2. A schemtic picture of the concentration profile over acuticulum membrane. [8]

2.2.1 Diffusion experiments across plant cuticles

To study in vivo diffusion of an AI across a membrane the outermost layer of the leaf, where the main penetration barrier exist, is used as membrane. Three main ways of studying the penetration of CM exists; using a horizontal membrane, using a vertical membrane with a flow of solution through the membrane or using a vertical membrane with a statically solution [8]. UDOS (Unilateral desorption of the outer surface) is a method using a horizontal membrane where desorption of solute is measured by applying the formulation to the morphological inner surface of the CM. One drawback with this method is that the effect of accelerators or other added compounds on solute permeability is lost [6].

In this study a method with vertical membrane and statically solution will be used, called Franz cell diffusion. The formulation is applied to the morphological outer surface of the CM and the penetration of the outer limiting layer is measured. SOFP (Simulation of foliar uptake) is a similar method but with the difference that the solution is allowed to dry on the surface, while in Franz diffusion the boundary conditions are kept fixed by having an infinite dose of solution [8]. In figure 2.3 a schematic picture of the Franz cell equipment is showed.

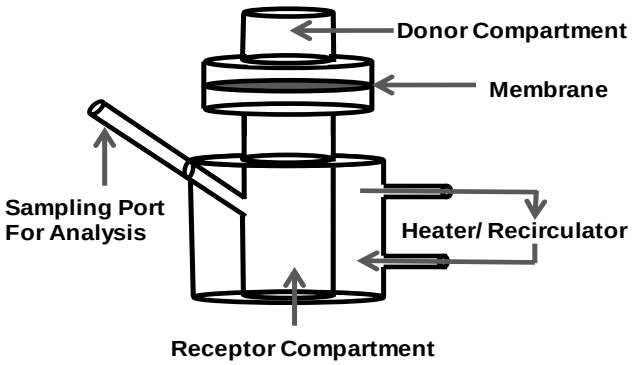

Figure 2.3. Franz cell consisting of a donor compartment where the studied formulation is added, a receptor compartment from where samples are taken and a membrane over which diffusion take place. [19]

2.3 Leaf cuticulum

As mentioned before the outermost layer of the leaf, the cuticle represents the main barrier for substances to penetrate the surface. All terrestrial plants have developed a cuticle to protect them from uncontrolled water loss and to act as a shield against foreign substances

to enter the plant [10]. This makes the cuticulum the barrier that must be penetrated by agrochemicals in order to enter the plant [6].

In this study the cuticulum from a plant called Clivia Miniata Regel was used as a membrane for the diffusion studies. Different ways of removing the CM from the leaf exists, either enzymatic isolation or isolation by using a dermatome. Fagerström et al [8] showed that the different isolating techniques did not significantly affect the permeability of the AI. However, it was observed that the maturation of the leaf had a strong impact on the penetration, comparing tip to base. At the tip a stronger barrier had time to build up than at the base, therefore in this study all membranes are retrieved from the middle of the leafs.

Plant cuticle mainly consists of a lipid membrane that is built up by a polymer called cutin and in some species, including Clivia Miniata Regel also a polymer called cutan, waxes and polysaccharides [12].The membrane is a heterogeneous membrane and varies both in chemical composition and structure [10].The outer part, facing the surrounding, is believed to consist of cutin and waxes and no polar polysaccharides. The underlying layers have an increasing amount of polar substances like polysaccharides, cellulose and pectin [12]. A schematic picture of the structure of the cuticular membrane can be seen in figure 2.4.

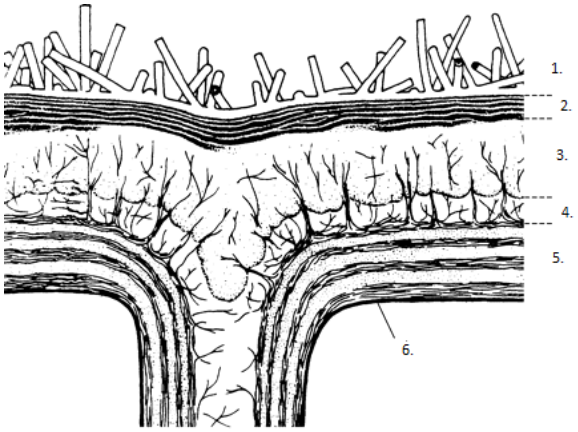

Figure 2.4. Schematic picture over cuticle membrane; 1.Epicuticular waxes; 2. Cuticular proper; 3. Inner layer with increasing amounts of polysaccharides, cellulose and pectin; 4. Pectin layer; 5. Cell wall; 6. Plasmalemma. [6]

The cutin polymer acts as a matrix with the waxes imbedded and the whole system behaves like a semi-crystalline material, with both crystalline and amorphous phases. The crystalline parts are held together by amorphous chain segments that give the material flexibility. The cuticular membrane act as a viscoelastic polymer with elastic/amorphous parts that makes the material less brittle and plastic/crystalline parts that increase strength in the material. Flexibility and strength is of importance for cuticular membrane, since it needs to withstand for example wind stress without breaking. [6]

The waxes have been identified as the main barrier for penetration. The composition of the waxes is complex and often contains a mix of aliphatic alkenes, alcohols, fatty acids and esters, triterpenes and other cyclic lipophilic substances are often present as well [12].The composition varies with different species but often contain different phases. The highly ordered crystalline phases are in principle inaccessible to penetration and penetrating agents must take a longer tortuous path around these areas. Diffusion experiments on CM that had the waxes extracted showed that penetration happens much faster, since the penetrating agent could take a more straightforward path through the membrane [6].

The cutin polymer consists of cross-linked hydroxyl fatty acids where the monomers are mainly constructed of derivatives of saturated fatty acids in the chain length of C16-C18, with the hydroxyl groups mainly situated in the middle or the end of the chains. The monomers are crosslinked by ester, peroxide and ether bridges. [10]

2.3.1 Droplet dry down

The final stage before the AI penetrates the leaf surface is the dry down of the droplet. It is known that different formulation types and the surfactants/adjuvant content highly affect the deposit pattern on the leaf [13]. The solubility of the different compounds and the affinity for the leaf surface are other factors that highly influence the dry down [14]. The appearance of the pattern is believed to affect the uptake of the AI, therefore it is of highest interest to understand the connection between dry down and uptake.

Faers et al [13] has studied the dry down of suspoemulsions; a combination of an emulsion and a suspension where the AI is in crystalline form and an emulsified oil is used as adjuvant. The result showed that reducing the deposit size/drop size increases the uptake. This could be explained by that a high coverage dilute the formulation and thereby decrease the association between the AI and adjuvant. Although to prove that this is valid for all systems further studies need to be done.

During dry down of the droplets it was also observed by Faers et al [13] that the deposit on the leaf wasn't uniform. Due to that the AI-particles were "pinning" at the edges and thereby prevented the drop from contracting, faster evaporating occured at the edges. This creates a capillary flow outwards from the center, transporting the AI and adjuvants to the edges of the drop, forming a ring. This pattern is often referred to as "coffee ring". Since the AI and adjuvants are concentrated to the edges, the possibility for enhanced uptake exists there.

However, it was also observed that addition of surfactants/adjuvants introduces a surface tension gradient across the drop (Marangoni stresses). The outgoing capillary flow creates higher surfactant concentration at the edges, lowering the surface tension. The Marangoni stresses create a flow towards higher surface tension areas; producing a flow of surfactants inwards towards the center of the drop. This mechanism results in a separation between the AI particles that are accumulated at the edges and adjuvants in the center. These phenomena could reduce the uptake of AI, due to the low association between AI and adjuvants. At high surfactant concentrations, fluctuations in Marangoni stresses have been observed by Truskett et al [15], leading to the formation of Bénard cells, due to convection flow.

Faers et al [13] also observed that the surface tension of the adjuvants affected the deposit pattern; a high surface tension leads to a large contact angel and a tall drop. The lower density of the oil adjuvants causes them to collect in the center. The capillary force is not sufficient to pull the oil drops out to the edges together with the AI-particles, causing separation between AI and adjuvants.

The "coffee ring" pattern has shown to be more common when the AI is in solid state [14]. However, the dry down using the AI in liquid form in a soluble liquids-system has been studied by Hunsche et al [14]. The AI was the common herbicide glyphosate, mixed with two different ethoxylated rapeseed oil adjuvants. The formulation was placed on an aluminum surface and without adjuvants the formulation is evenly spread over the deposit area. When

adjuvants are added the deposit pattern changes and small residue islands are formed mainly at the edges of the drop, consisting of AI and adjuvants. However, when adding an adjuvant which showed to have lower association to the AI (higher ethoxylated degree) separation is observed; AI forms a deposit in the center while the adjuvants form a “coffee ring” at the edges of the drop.

The influence of humidity was also evaluated on the droplet dry down. At low relative humidity (<10%) lower association between AI and adjuvants was observed due to faster evaporation. The droplet volume had influence on the deposit pattern but it did not show any effects on the AI-adjuvant association.

2.3.2 Foliar uptake

Once the drop is dried on the leaf surface the diffusion through the cuticulum starts. The heterogeneity of CM indicates that hydrophilic and lipophilic substances diffuse through the CM in separate pathways and through separate mechanisms. The first evidence of two separate pathways was first presented by Popp et al [11]. It was suggested that the cuticular permeance for lipophilic compounds could be predicted by the octanol/water partition coefficient (see equation 6). However, when applying this approach to a hydrophilic compound it seems highly unlikely that this substance can penetrate across the CM, due to a very low $K_{o/w}$. It was then suggested that hydrophilic compounds diffuse through aqueous polar pores in the membrane.

Most AI are lipophilic substances and it is likely to believe that they will diffuse trough the lipophilic parts of the CM; cutin/cutan and waxes. In order to penetrate the surface the substance needs to sorb to the cuticular lipids, diffuse through the CM and finally desorb into the plant. Diffusion experiments have showed that the largest penetration resistances arise from the outermost layer where both sorption and solute mobility slows down the diffusion. This limiting layer is very thin, only 10% of the total mass of the CM. In remaining underlying layers sorption and solute mobility is much higher; in order to improve the permeability of the membrane the diffusion through the limiting upper layer needs to be enhanced. [6]

The rate of cuticular penetration in the limiting upper layer (J) can be defined as the product of the solute mobility (k^*), the length of diffusion path (l_{ls}) and the driving force, as can be seen in equation 2.7. The driving force is defined as the product of the partition coefficient and the concentration gradient. The driving force at the surface, during sorption, is driven by the concentration of the formulation and the wax/formulation coefficient ($K_{w/f}C_f$). In order for sorption to occur, a high value of $K_{w/f}C_f$ is needed. For example; a low concentration of the dissolved molecule and a solute that prefers to be in the solution instead of the membrane, low $K_{w/f}$, will not diffuse into the membrane to any great extent. Altering pH, temperature or adding adjuvants can increase the permeability. [6]

$$J = k^* l_{ls}(K_{w/f}C_f - K_{c/w}C_w) \qquad (2.7)$$

Once inside the membrane the driving force is determined by the concentration inside the plant together with the cuticular membrane/water ($K_{c/w}C_w$) [6]. In figure 2.5 the lipophilic diffusion path is illustrated; first a diffusion process from an aqueous drop across the cuticle into an aqueous receiver compartment.

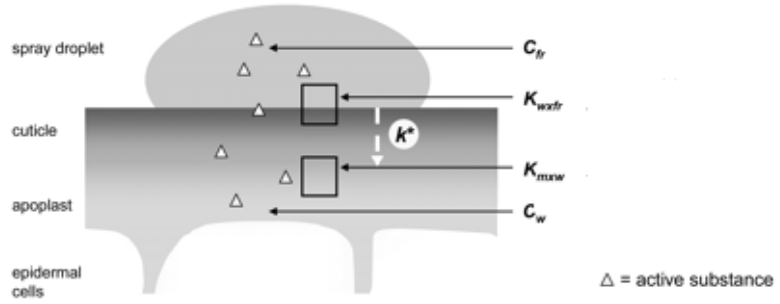

Figure 2.5. An illustration of the lipophilic diffusion path; from drop into the plant. The different parameters controlling the diffusion are marked. [6]

The molecular size of the penetrating substance affects the penetration since larger molecules have lower mobility than smaller ones. Buchholz et al [6] plotted solute mobility against molar volumes for different plant species and found no significant difference in size selectivity. This shows that there is an optimum size, regardless species, of the diffusing substance. This can be interpreted by the free volume theory; that the solute will diffuse through the free volume in the material, causing larger molecules to diffuse slower [11]. This volume can be permanent voids or temporary voids created from molecular movement in the barrier matrix.

In order to increase the penetration of lipophilic substances adjuvants can be used. Adjuvants can increase the mobility of the polymer chains and thereby increase the diffusivity. This type of adjuvants is called activator adjuvants (i.e. a plasticizer or fluidizer). However, for this to work the adjuvant and the solute must be penetrating the membrane simultaneously [6]. Plasticizers decrease the viscosity of the amorphous parts but they do not alter the physical properties of the wax and cutin polymers; no destruction of crystals or solubility of polymers.

Adjuvants might also have an additional effect on penetration by being retained on the surface of the membrane. This is achieved by increasing the concentration of the AI at the surface and increasing the partition coefficient between the solute and the cuticular surface, see equation 2.7. [6]

The hydrophilic pathway is more debated, but the main hypothesis is that the diffusion of hydrophilic compounds is believed to occur through aqueous pores in the CM. These aqueous pores arise from clusters of polar group’s imbedded in the lipophilic polymer matrix. The polar groups are permanent dipoles such as hydroxyl-, amino- and carboxylgroups. Upon hydration of the cuticular membrane the dipoles sorb water and the membrane swells. Both dipole-dipole bonds and ion-dipole bonds (for ionizable groups) arise between the polar groups and water. These bonds are very strong and make the polar groups insoluble in the polymer matrix and an aqueous phase/aqueous pore is formed in the cuticle. [12]

However, it has been showed that water can take two parallel paths of diffusion through the membrane; both the lipophilic way and the hydrophilic way. This can be seen from that extraction of wax from the membrane enhance the water diffusion, in the same way as for lipophilic substances. Whereas water may use different paths, for ions only aqueous pores are available for diffusion. [12]

Another possible diffusion path for hydrophilic substances is through the stomata. Schönherr et al [12] showed that accumulation of a hydrophilic dye proceeded more solely in areas that lack stomata. It was suggested that accumulation would occur where cuticular and stomatal transpiration takes place. However, this diffusion path is only available when the stomata is open.

2.4 Emulsions

Emulsifiable concentrate (EC) is one of the formulation types that occur in Agro applications and will be studied in this project. The constituents of an EC are most often AI, solvent and emulsifier, this formulation forms an emulsion upon dilution in water by the farmer. An emulsion is a dispersion of two immiscible liquids; often a hydrophobic liquid/oil in water. The medium inside the colloids/drops is called the dispersed phase and the surrounding medium the continuous phase. The colloids are in the size of 1-10µm [16]. In this application water is the continuous phase, and oil is inside the drops; which is the most common type of emulsion and the rest of the text will be regarding this type of system. The oil phase acts as a reservoir for the AI, by dissolving the AI, which has very low water solubility on its own.

However, just mixing oil and water will not create an emulsion on their own; in order to do that emulsifiers needs to be added. Emulsifiers are surfactants and are drawn to the interphase between oil and water. They lower the surface tension between the two liquids which initiates the formation of a larger surface through a decrease in droplet size. The emulsifiers also contribute with stabilizing properties, such as electrostatic and sterical. Smaller droplets are generally easier to stabilize. To produce an emulsion mechanical decomposition is often also needed in order to create the oil drops. [16]

The self-diffusion of the colloids in the solution can be described by equation 2.8, where the diffusion coefficient (D) is dependent on the particle size of the drop, the viscosity of the solution (η) and the energy needed in order for two colloids to merge together ($\boldsymbol{kT}$). [17]

$$D = \frac{\boldsymbol{kT}}{6\pi\eta R} \qquad (2.8)$$

Emulsions are not thermodynamically stabile systems and will eventually separate into two different phases. How long this will take depends on how well the emulsifiers are stabilizing the system. There are two ways to which emulsifiers can stabilize the emulsion; steric- and electrostatic stabilization. Electrostatic stabilization occurs for ionic surfactants. Ionic surfactants create a charge (positive or negative) at the drop surface and this charge is balanced by counterions of opposite charge from the surrounding solution. When two drops approach each other the concentration of counterions increases, which lowers the entropy. In order to restore the entropy of the system water will flow in between the drops and separate them. [16]

The counterions do not neutralize the surface charge of the drops completely. Binding counterions to the surface reduces the surface energy but a high concentration of ions at the surface reduces the entropy of the system. These two counteracting forces result in a nonuniform ion distribution around the drop and thereby a netcharge of the same nature as the surfactants. [16]

Steric stabilization occurs for nonionic surfactants, but the principal is the same as for electrostatic stabilization. Nonionic surfactants have long water-soluble polymeric chains

attached to the drop surface and instead of an increase in counterions an increase in chain concentration arise when two drops approach each other [16]. By adding both ionic and nonionic surfactants to the system electrosteric stabilization is established. Since a combined effect of both the electrostatic and the steric stabilization is given this type of systems provide the most stabile emulsions. A schematic picture of the different stabilization mechanism can be seen in figure 2.6.

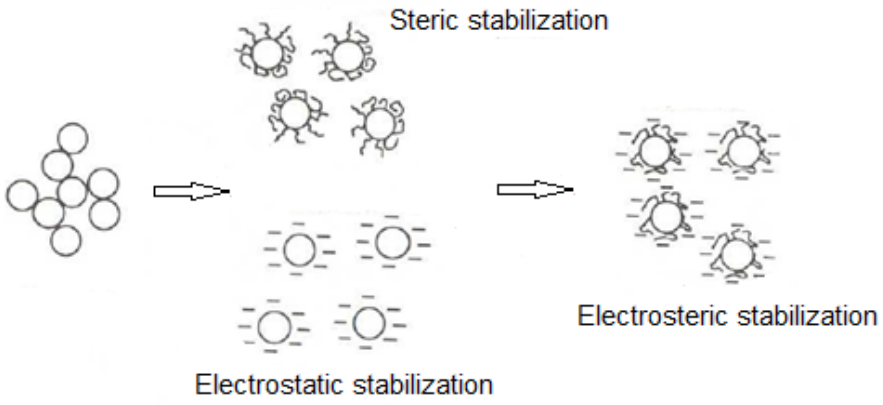

Figure 2.6. The different stabilization mechanism for creating a stabile emulsion (electrostatic-, steric- and electrosteric stabilization).

Besides using an ionic and nonionic surfactant to stabilize an emulsion, usually also a nonionic polymer is added in order to establish better blooming of the system. Blooming is the term for an EC to form an emulsion of small droplets spontaneously, making the emulsified sample look milky and white (i.e. the formulation blooms).

2.4.1 Colloidal stability

The colloidal stability is determined by the gravitation and the forces between the colloids. Since oil often has a lower density than water the oil-drops tend to end up on top. This phenomenon is called *creaming* and it is reversible by simply mixing the solution. With time gravity will affect the drops and creaming will happen again. The reverse, oil-drops with higher density than water, is also possible and then *sedimentation* occurs. Even if creaming and sedimentation is reversible, the possibility of drops coalesce increase when they pack close together. *Coalescence* is an irreversible process of the formation of fewer and large drops. In figure 2.7 a summation of the different colloidal mechanism is shown by a schematic picture. [16]

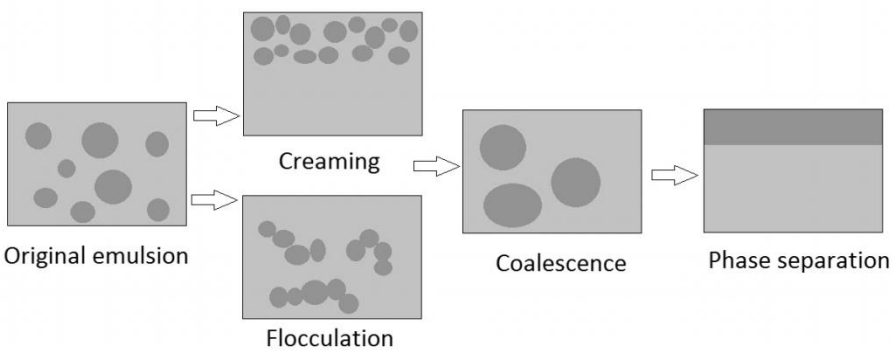

Figure 2.7. A schematic picture of different mechanism that eventually leads to phase separation. Creaming and flocculation are reversible processes that can be broken by stirring the solution. Coalescence is however irreversible and leads to phase separation.

Due to the random motion (Brownian motion) of colloids they will come in contact with each other and can then form flocks of drops. The difference between *flocculation* and *coalescence* is that when the drops flocculate they keep their structural integrity and do not merge into one big drop. Flocculation like creaming is a reversible process and the original emulsion can be restored by mixing the solution. With time bigger drops will grow on the cost of smaller and the system will go towards bigger and more even sized drops, this is called *Ostwald ripening*.

Colloidal stability also depends on attractive and repulsive forces between the colloids. These interactions can be described by the DLVO-theory that expresses the sum of the attractive van der Waals forces and repulsive electrostatic forces. The DLVO-theory is applicable on systems that are stabilized by electrostatic forces, not for steric stabilization. Van der Waals forces are always present between two bodies and if the bodies are identical they are attractive. [16]

In figure 8 the DLVO-theory is illustrated. At a large distance the attractive van der Waals forces dominates which makes the colloids approach each other. This is illustrated in figure 2.8 by a secondary minimum in potential energy. This minimum demonstrates a reversible process, for example flocculation or creaming. When the distance decreases the electrostatic forces increase and the colloids repel each other. In order to end up in the primary minimum, which illustrates an irreversible process, the colloids need enough energy to overcome the potential energy barrier (V_{max}). At very close distance the colloids once again repel each other, due to repulsive interactions between the atoms electron clouds.

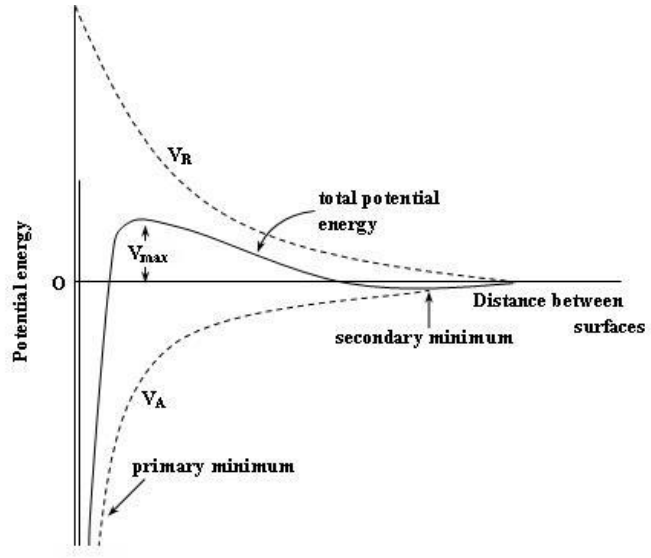

Figure 2.8. The DLVO-theory illustrated in a plot. [18]

Colloidal stability is also dependent on the size of the drop. Smaller and lighter drops are less affected by the gravity, that otherwise force the drops closer together. This can also be seen from equation 2.8, where a smaller drop size results in a higher self-diffusion. To get small drops sufficient mixing during formation of the emulsion is essential.

2.4.2 Agrochemical emulsions

Emulsions in Agro applications are constructed of an emulsifiable concentrate (EC), which consist of emulsifiers and AI dissolved in organic solvent (the oil phase), which the farmers

emulsify in water. The formed emulsion can then be sprayed onto the fields. In figure 2.9 the different steps of getting the formulation onto the leaf is illustrated.

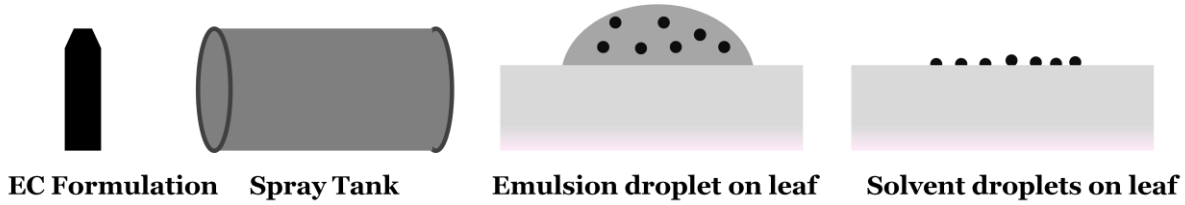

Figure 2.9. The EC formulation is emulsified in the spray tank and then spayed as droplets onto the plants. During dry down the water phase evaporates, leaving small oil droplets on the leaf surface. [19]

To supply sufficient mechanical mixing in order for an emulsion to form is hard in the farmers big spray tanks. To solve this problem “spontaneous emulsions” are used. Spontaneous emulsions requires very little or no mixing, instead it is the emulsifiers that through their migration from one phase to another drag water or oil into the other phase.

In order for the emulsions to last they must be very stable and therefore electrostatic stabilization together with steric stabilization is employed. Since small drops also helps stabilizing the system, blockcopolymers are chosen since they promote blooming and have affinity for both phases and will spread well in the solution creating small drops.

One way to study the uptake through cuticle from emulsifiable concentrates was done by Westbye et al [19].To be able to study the effect of such a system without the influence of emulsifiers simplifications had to be made, by setting up two extreme scenarios. Upon emulsification both the oil and water phase will be equilibrated in terms of, solvent, water and AI. The first scenario was formed after the assumption that all AI and solvent goes back into the oil phase and that all water evaporates. The second scenario assumes that no AI or solvent is forced back into the solvent phase and no water is evaporated from the solvent phase.

Experiments were carried out for different solvents; N,N-dimethyl decanamide and N-methyl,N-decyl formamide among others and the AI used was tebuconazole. It was observed that the amount of water dissolved into the solvent phase corresponded to the electron donating properties of the solvent. A high electron donating solvent (N,N-dimethyl decanamide) will interact stronger with water and thereby decrease the diffusion rate of tebuconazole dramatically from scenario 1 to 2.

The results from this measurement will be used in this study in comparison to the results from the emulsion system.

3. Materials and Methods

3.1 Materials

The two different solvent used: N,N-dimetyl decanamide and N-decyl-N-methyl formamide and emulsifiers were synthesized at AkzoNobel Surface Chemistry. Active ingredient: tebuconzole ((RS)-1-p-chlorophenyl-4,4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl)pentanol 97%, Nufarm UK Limited, West Yorkshire, UK). The solubility of the different solvents and tebuconazole can be seen in table 3.1 and 3.2. To illustrate the crops/weeds in the diffusion experiments *Clivia miniata* Regel was used, since it has thick cuticles that easily can be isolated. *Clivia miniata* (Lindl.) Regel var. *miniata* was obtained from "A fast flower". Standard water, prepared according to CIPAC MT 18.1 [20], was used in all the emulsion tests.

For the microscope images a non-water soluble dye: Fat Brown RR, Aldrich Chemical Company. Inc Milwakee wi 53233 USA, was used to color the oil phase.

Table 3.1. Characteristics of the tested solvents.

Solvent	Solubility: solvent in water (mg/L)	Solubility: water in solvent (%)	Solubility: Tebuconazole in solvent (g/L)
N,N-dimetyl decanamide	600	20,43	347
N-decyl-N-methyl formamide	200	17,79	337

Table 3.2. Solubility of Tebuconazole in water.

Agrochemical active	Solubility: Active in water (mg/L)
Tebuconazole	36

3.2 Methods

First the methods regarding the preparation of the emulsions, diffusion measurements and droplets dry down are stated, followed by different methods used to better understand the behavior of the formulated system (microscope images, solubility-, surface tension- and density measurements).

The results from the Franz cell measurements are presented as cumulative amounts of tebuconazole in the acceptor compartment. How tebuconazole concentration is converted into cumulative amounts is presented last in this chapter. When working with biological materials the variation of the measurements is quite high and how the data has been handled is also presented last in this chapter.

3.2.1 Emulsion preparation

The EC is prepared by mixing 9wt% emulsifiers and 25wt% tebuconazole in solvent. The EC is mixed by magnetic rod until a clear solution is obtained. The emulsion was prepared by adding EC to standard water. The mix is then slowly turned upside down ten times.

The emulsifier composition differs between the two solvents; for N,N-dimetyl decanamide the emulsifiers are a mix containing 24wt% dodecylbensensulfonat, 47wt% EO/PO-blockpolymer and 29wt% C16/C18- alkoxylate. For N-decyl-N-methyl formamide the emulsifiers consist of 15% phosphate ester 50% EO/PO-blockpolymer and 35% C16/C18- alkoxylate.

3.2.2 Diffusion experiment

The diffusion experiments were carried out in Franz cells (Inner diameter=9mm; $V_{Receptor}$= 6mL; V_{Donor} =1mL; SES GmbH, Analytical Systems Bechenheim, Germany). CM (approximately 50µm thick, containing both cuticle and epidermis) from Clivia miniata was used as membrane, surrounded by two rings of Parafilm (outer diameter=23mm; inner diameter=16mm) in order to avoid leakage. The CM was removed mid-leaf from the upper side using a 25mm dermatome (TCM 3000 BL,Nouvag AG, Goldach, Switzerland). The carrier formulations (1mL) were added to the donor compartment, facing the morphological outer side of the CM. The receptor compartment was filled with degassed water and 500µL were replaced at each sampling time. Parafilm was used to cover the cells during the experiment and the cells were kept under isotherm conditions at 25°C.

The procedures for diffusion experiment from a dried-down droplet were the same.

3.2.3 Analyze

The tebuconazole content was analyzed using HPLC together with an UV-detector (λ=222nm). A mix of water and acetronile (52+48 v/v), adjusted to pH 3,0 with phosphoric acid, was used as mobile phase. The flow rate was 2mL/min. The HPLC equipment consist of autoinjector (Varian Prostar Model 410), pump (Merck Hitachi L-6200), column (Agilant Hypersil 5µ ODS (C18); Software: EZCrome Elite) and detector (Waters 2487, Dual λ Absorbance Detector, Waters Inc, Milford, MA,USA).

3.2.4 Dry down

Larger parts of Clivia miniata were removed by dermatome and pinned to parafilm by the use of tape in order to avoid torsion of the membrane during dry down. The dry down took place under fixed conditions in a desiccator (T=24°C and RH=23%) during 1hour. The deposit area of the drops was marked to enable the making of membranes and 6µl of the formulation was added to the marked area in the form of one drop once inside the desiccator.

3.2.5 Solubility

The solubility of tebuconzole in 9% water solution of the emulsifiers used was determined by first adding tebuconazole in excess until a turbid solution was formed. The solution was agitated during two weeks, then the solution was filtrated two times (first trough a 0,45µm filter, then a 0,2µm filter) and a clear solution saturated with tebuconazole was obtained. The tebuconazole content was then analyzed using the same HPLC system as for the diffusion experiments.

3.2.6 Microscope images

To study the dry down of drops a light microscope with 0,8 times magnification was used. A non-water soluble dye was used to color the oil phase of the emulsion in order to tell where the oil phase ends up during dry down. Images were obtained every minute during the dry down using a camera.

3.2.7 Dynamic surface tension measurements

The dynamic surface tension for three different dilutions (1:10, 1:50 and 1:200) of the emulsion was measured using a Du Noûy ring: Tensiometer KSV Sigma 70. The principle of the method is that a platinum ring is submerged in the solution and then carefully pulled out. The force needed to detach the ring from the surface of the solution is measured and corresponds to the solutions surface tension.

3.2.8 Density measurements

The density of 25wt% tebuconzole solubulised in N,N-dimetyl decanamide was measured by simply weighing 50ml solution and dividing the mass by volume.

3.2.9 Data handling

The concentration of tebuconazole in the receptor compartment (see figure 2.3) at the different sampling times are converted into cumulative amounts by equation 4.1 and 4.2.

$$c_{cumulativ,1} = \frac{c_1 * V_d}{a} \qquad (4.1)$$

$$c_{cumilativ,n} = \frac{(c_{n-1} * V_s + V_d * c_n)}{a} \qquad (4.2)$$

$c_{cumulativ}$ is the cumulative concentration in µg/cm^3 , c is the concentration in the receptor compartment in mg/L, V_s is the sampling volume, V_d is the volume of the donor compartment and a is the area that the solution can penetrate through. For the first sampling the concentration is calculated according to equation 4.1. For the following samplings one has to take into account that previous samplings have been made and the cumulative concentration is calculated according to equation 4.2.

The standard deviation can be relatively high when working with leafs; the barrier properties of the CM are highly affected by temperature, humidity and maturation of the leaf. In order to still be able to get reliable measurements many replications are needed.

In order to exclude outliers a method where measurements outside the mean value ($\bar{m}$) +/- the standard deviation (s) is excluded, according to equation 4.3. Still the results should be considered in comparison to one another and not as absolute values.

$$\bar{m} \pm s \qquad (4.3)$$

4. Results and Discussion

For N,N-dimethyldecanamide an already optimized emulsifier system was used to study foliar penetration from an emulsified solvent system. For the N-decyl-N-methyl formamide the emulsifier system needed to be developed before being evaluated in the Franz cells.

For the droplet dry down experiments N,N-dimethyldecanamide was used to develop the method, before N-decyl-N-methyl formamide was tested for the comparative study.

4.1 Formulated systems

In order to be able to measure on formulated N,N-dimethyldecanamide the emulsifiable concentrate (EC) was prepared containing the solvent, emulsifiers and tebuconazole. In reality the ratio between EC and water is approximately 1:200. The emulsion-experiments have been carried out for three different concentrations, 1:50, 1:100 and 1:200; with the boundary conditions fixed (the solution was never allowed to dry on the CM-surface). The cumulative amounts of tebuconazole penetrating the CM are plotted in figure 4.1 and it can be seen that the penetrating amounts increase with an increase in EC-concentration. For all systems the tebuconazole concentration was 250 g/l.

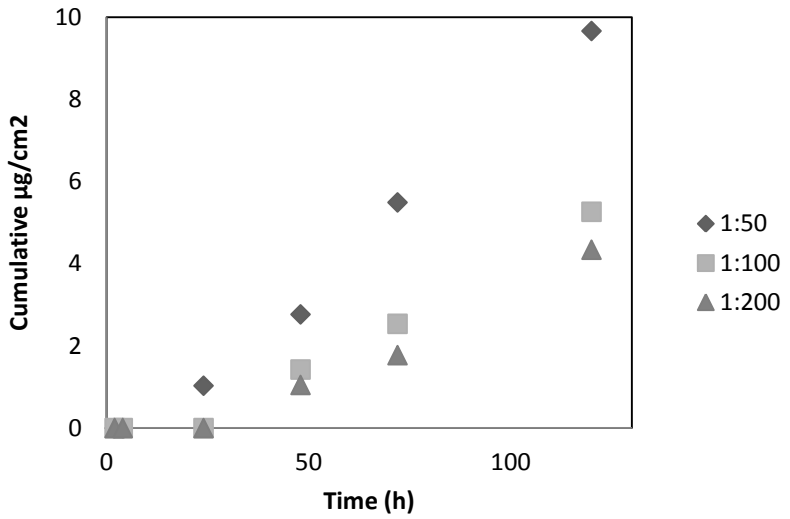

Figure 4.1. The cumulative amount of tebuconazole in the acceptor solution of the Franz cell is plotted against the time for each sampling. Each point is the mean of 6 measurements.

Even though a method as close to reality as possible is wanted, the lower amounts penetrating from the more diluted systems (1:100 and 1:200) makes it difficult to tell different systems apart. The higher concentration of 1:50 is therefore preferred when looking at formulated systems and will be used for the rest of the emulsion-measurements.

A comparison between the emulsion-system and other systems can be seen in figure 4.2 below. The lowest reading comes from water saturated with tebuconazole and the highest reading from tebuconazole solubilized in N,N-dimethyldecanamide. This shows that the solvent promotes the uptake, compared to water and acts as an adjuvant for this system.

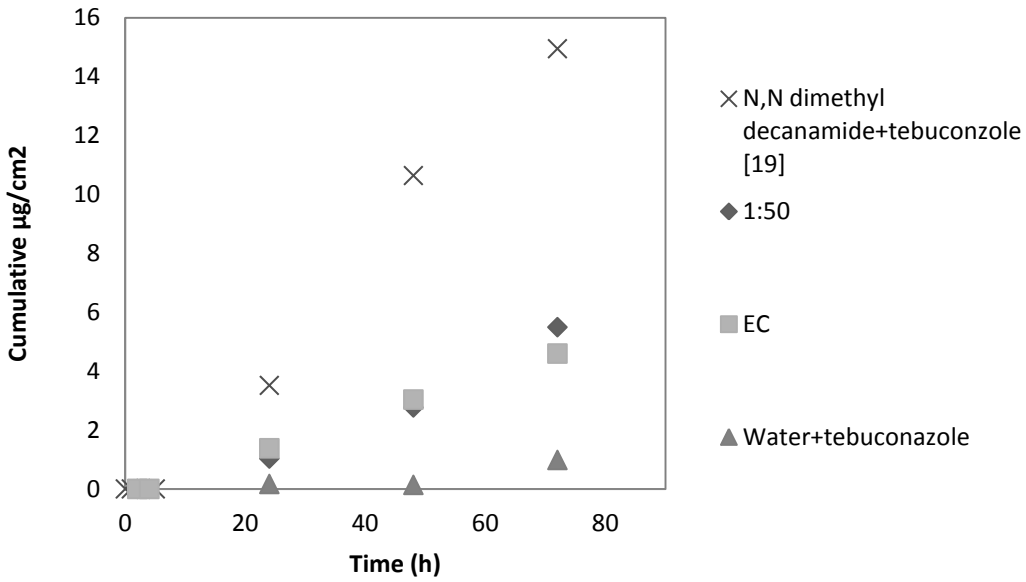

Figure 4.2. The cumulative amount of tebuconazole in the acceptor solution of the Franz cell for four different systems plotted against time. From the top; Tebuconazole solubilised in N,N-dimethyldecanamide, tebuconazole solubulised in N,N-dimethyldecanamide and water, emulsion formulation with the dilution 1:50 and finally the EC. Each point is the mean of 6 measurements.

However, looking at the results from running EC (i.e. solvent, tebuconazole and emulsifiers) in the Franz cells the penetrating amounts are quite low, even though the only difference between EC and tebuconazole solubilized in N,N-dimethyldecanamide is the addition of emulsifiers. This shows that the emulsifiers are not as good at promoting uptake as the solvents. However, the emulsifiers still enhance the uptake compared to tebuconazole solubilized in water.

Comparing the emulsion measurement with the EC they show similar penetration ability. A hypothesis that could explain this behavior would be that the oil phase in the emulsion creates a film close to the surface; this is likely since the Cuticle membrane (CM) is lipophilic and attracts the lipophilic substances. Figure 4.3 schematically illustrates this behavior and it is visualized that the film formation creates the same conditions over the membrane as for the EC and that would explain why the two systems behave alike.

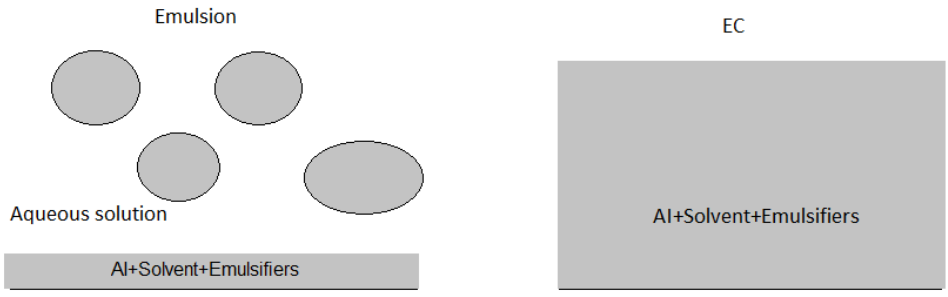

Figure 4.3. An illustration of a possible behaviour of the emulsion system compared to the EC.

For N-decyl-N-methyl formamide no emulsion-system had been developed which needed to be done before it could be tested in the Franz cell equipment. Since there is high similarity between N,N-dimethyldecanamide and N-decyl-N-methyl formamide the exact same

emulsifiers were first tested. However, the system proved to be instable and formed cream upon functionality tests. When altering the concentration of the surfactants the best results were obtained without any anion, this suggests that another anion is needed for a stable emulsion. In figure 4.3 below all the 16 different combinations tested with the new anion is visualized in a diagram.

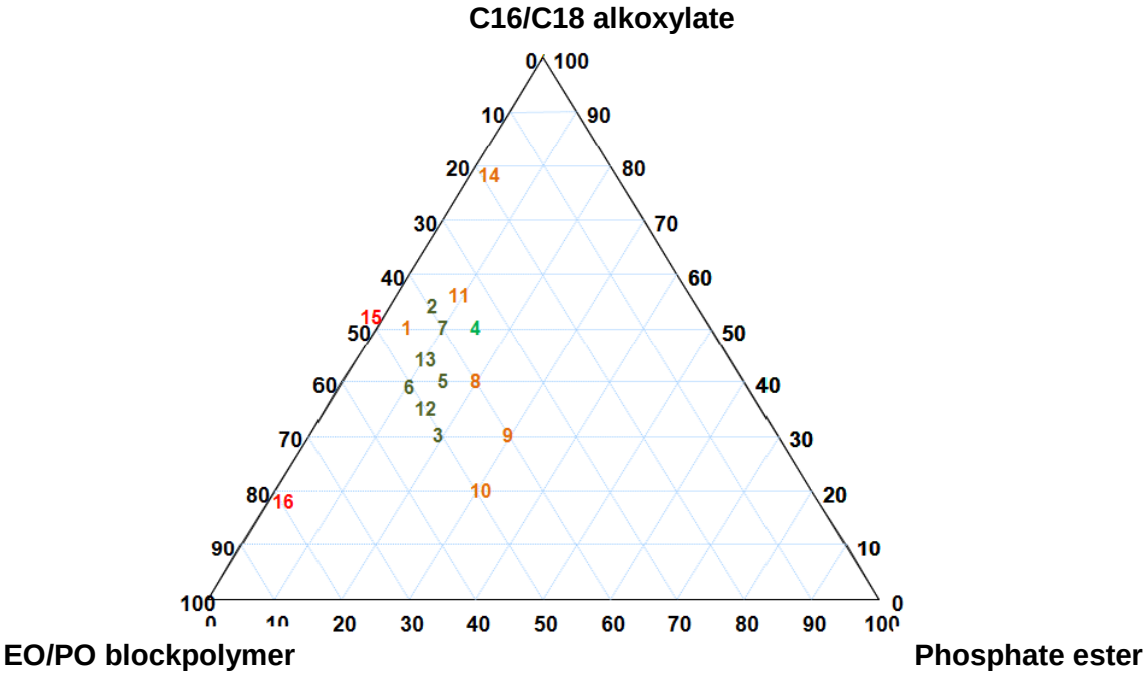

Figure 4.3. A diagram over the different combinations of emulsifiers that were tested for N-decyl, N-methyl formamide. Red colour indicates an instable system and the green colour indicates a more stable system.

Formulation number four (15% phosphate ester (anionic surfactant), 50% C16/C18 alkoxylate (nonionic surfactant) and 35% EO/PO blockpolymer) meets the stabilizations demands [21] and is used to create an emulsion formulation of N-decyl-N-methyl formamide. In figure 4.4 a comparison between N,N-dimethyldecanamide and N-decyl-N-methyl formamide as formulated systems at the ratio 1:50 is shown. The uptake for the two systems are very similar and indicates that N-decyl-N-methyl formamide is an as good solvent as N,N-dimethyldecanamide.

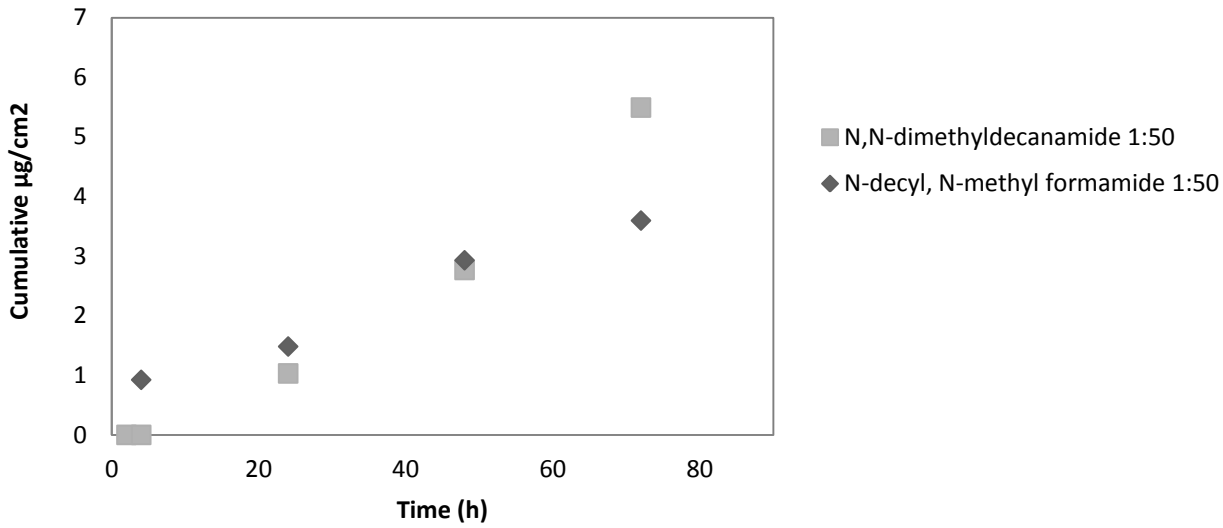

Figure 4.4. The cumulative amount of tebuconazole in the acceptor solution of the Franz cell plotted against time for the two different solvents. Each point is the mean of 5 measurements.

4.2 Dry down of droplets

To be able to dry down the droplets under fixed humidity and temperature a desiccators was used (RH=23% and T=24°C). Due to the low humidity, the dry down only took 1 hour. Formulated N,N-dimethyldecanamide was used to study the dry down of drops under microscope for 18 min (the dry down was speeded up by the heat from the microscope), see figure 4.5. As model-surface parafilm was used and to be able to see where the AI (mainly occurs in the oil phase) would end up the EC was dyed with a water insoluble dye (i.e.orange colour). As can be seen from figure 4.5 the flocculated oil drops is collected at the edge of the drop, creating a “coffering-pattern”.

Figure 4.5. Microscope-images of dyed EC formulated in water (1:10) after 1 min, 15 min and 18 min. The two bubbles in the centre of the drop are air-bubbles.

M.A. Faers et al [13] suggested that the density difference of the oil and water phase could cause the lighter oil to end up on top and consequently in the center of the drop during dry down. Density measurements of N,N-dimethyldecanamide compared to water can be seen in table 4.1. Since the addition of tebuconazole decreases the density difference between N,N-dimethyldecanamide and water this is not likely to be a factor in this system. Another factor that reduces the effect of the density difference is the size of the oil droplets in the emulsion. In our system the oil droplets are in the size range of 6µm in diameter which suggests that they are less affected by gravity than Brownian motion, this also points in the direction that the process of directing the oil droplets is more governed by the marangoni effect than gravity (i.e. oil drops end up at the edges of the droplet).

Table 4.1. Density measurements.

	N,N-dimethyldecanamide	N,N-dimethyldecanamide +tebuzonacole	Water
Density (g/cm3)	0,877 [22]	0,9435	1,0

MA. Faers et al [10] also states that for this density phenomenon to occur the surface tension of the drop needs to be at lowest 50mN/m. As can be seen from table 4.2 the surface tension for this system is not in that range. This all states that the oil phase is not likely to end up in the center of the drop but at the edge as the images in figure 4.5 suggests.

Table 4.2. Surface tension measurements for three different dilutions of EC in water, 1:10, 1:50 and 1:200.

	1:10	1:50	1:200
Surface tension (mN/m)	26,6	26,8	26,9

Since the surface tension of both the more diluted system (1:200) and the more concentrated systems (1:50 and 1:10) are nearly the same that indicates that an increase in concentration will not change the behavior of the system. The low surface tension means that the droplet will be pinned on the surface upon dry down, meaning that the area of the droplet will not change upon dry down (i.e. deposit formation). Since water evaporates at a higher rate from the edges of the droplet this means that a transport will start towards the edges of the droplet, which gives rise to so called coffee rings.

4.2.1 Franz cell measurements

Initial test for the ratio 1:50 and 1:200 as a dried down drop showed a very low penetration of active and long lag times. To overcome this concentration of the emulsions were increased to 1:10. The drops were dried down before starting the Franz cell measurements; hence the boundary conditions were no longer fixed.

In figure 4.6 the results from the dried down drop is compared to the measurements for the emulsions. The graph presents the penetration amounts per area and since the dried down drop takes up a smaller area this results in a higher penetrating amount/area. To approximate the area that the drops occupy the mean area of 10 drops where calculated by measuring their diameter. The drops areas turn out to be approximately 1/3 of the donor-compartment area, but the images in figure 4.5 indicate that the dried down drop (i.e. deposit) occupy an even smaller area, since the oil is not evenly distributed on the whole area covered by the initial drop (i.e. formation of coffee rings).

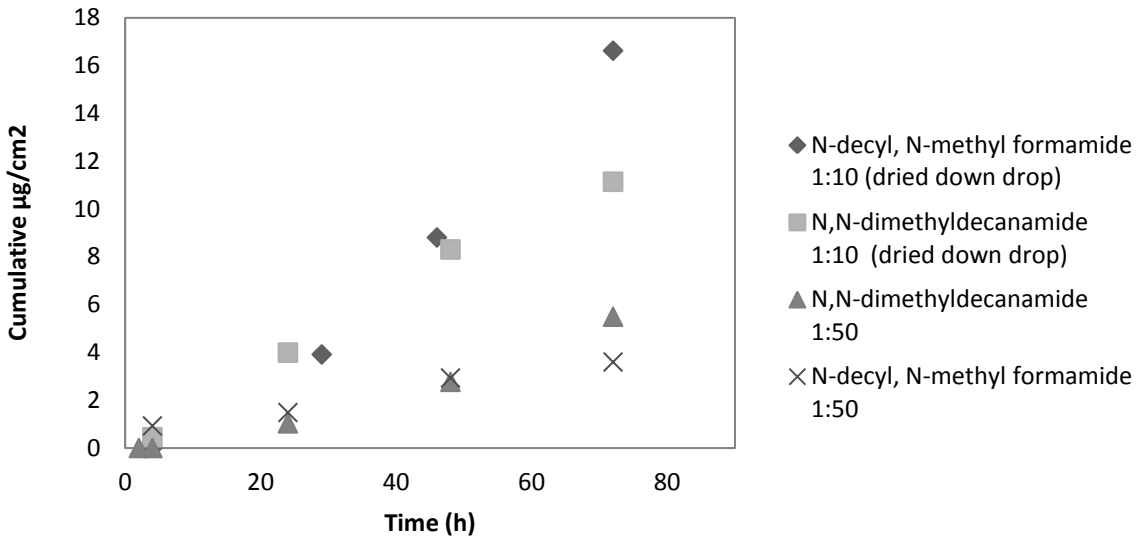

Figure 4.6. The cumulative amount of tebuconazole in the acceptor solution of the Franz cell plotted against the time for the two different solvents; both as solution and dried down drop. Each point is the mean of 6 measurements.

Figure 4.6 show that the two solvents show similar uptake ability in the form of a dried drop and as an emulsion solution which indicates that they behave alike. However, the dried down drops seems to penetrate more than the undried ECs. If this is due to the higher concentration or the process of drying down is impossible to say at the moment, but it would be an interesting future study.

5. Conclusions

In this thesis it has been showed that foliar uptake can be measured on formulated systems using Franz cells methodology. It has also been established that uptake measurements on dried down drops is possible and a method for doing this has been developed. A formulation for N-decyl-N-methyl formamide has also been developed and it was discovered that another anion than is used for stabilization of N,N-dimethyldecanamide was needed.

The results from the Franz cell measurements show that N-decyl-N-methyl formamide is equal to N,N-dimethyldecanamide regarding uptake and both solvents would be a good new candidate for a more environmentally friendly alternative. The studies on the dry down of drops indicate that the AI ends up at the edges of the drop, but in order to ensure this behavior more studies need to be done.

In this thesis it was also discovered that the emulsifiers and water suppresses the adjuvant effect from the solvent. However, tebuconazole penetrates more from the formulated solvent system than for a water system.

6. Future work

To further develop the method it would be ideal to store the plants and run the experiments under controlled environment (i.e. in a climate chamber). This would reduce the parameters affecting the barrier properties of the leaf and less replication might be needed in order to get reliable results. To be able to measure on more diluted systems, closer to reality, a more sensitive analyzing method could be implied, such as mass spectroscopy.

To reduce the lag time and speed up the measurements of the dried down drop one could try to manipulate the boundary conditions and force the formulation through the membrane. This could be done by having a solution saturated with the AI on top of the dried down drop and thereby keep the boundary conditions fixed and force the AI through the membrane.

It would also be interesting to investigate if other emulsifiers would be less negative for the uptake than the emulsifiers chosen for this study. Further, it would be of interest to study if there is a way to exclude incorporation from the water into the oil phase, and if this could help reduce the negative affect from the incorporation of water.

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Kajsa Lundberg

References

1. Börjesson J (2010) Enhancing deposition of agricultural sprays by the use of polymeric adjuvnt. Göteborg: Chalmers University of Technology (Master of Science Thesis, Department of Chemical and Biological Engineering).

2. Bergström L, Kirchmann H, Thorvaldsson G (2008) Widespread opinions about organic agriculture – are they supported by science evidence? Organic Crop Production – Ambitions and Limitations. Springer, Dordrecht. pp. 1-13.

3. Zabkiewicz J A (2007) Spray formulation efficacy – holistic and futuristic perspectives. Crop Protection 26, pp. 312-319.

4. Bergeron V (2003) Designing intelligent fluids for controlling spray applications. C.R. Physique 4, pp. 211-219.

5. Faers M A, Pontzen R (2008) Factors influencing the association between active ingrediente and adjuvante in the leaf deposito of adjuvante-containing suspoemulsion formulations. Pest Management Science 64, pp. 820-833.

6. Buchholz A (2006) Characterization of the diffusion of non-electrolytes across plant cuticles: Properties of the lipophilic pathway. Journal of Experimental Botany, Vol. 57 No.11, pp 2501-2513.

7. Fagerström A, Kocherbitov V, Westbye P, Bergström K, Mamontova V, Engblom J (2013) Characterization of plant leaf cuticle model wax, phase behavior of model wax-water systems, PII: S0040-6031(13)00457-7 DOI:http://dx.doi.org/doi:10.1016/j.tca.2013.08.025,TCA 76606, pp1-28.

8. Fagerström A, Kocherbitov V, Ruzgas T, Westbye P, Bergström K, Engblom J (2013) Effects of surfactants and thermodynamic activity of model active ingredient on transport over plant leaf cuticle. Colloids and Surfaces B: Biointerfaces 103 pp 572-579.

9. Åberg C (2009) Steady-State Diffusion on Complex Amphilic Films,Lund: Lund University (Doctoral Thesis, Center for Chemistry and Chemical Engineering).

10. Schreiber L, Schönherr J (2009) Water and Solute Permeability of Plant Cuticles. Springer-Verlag Berlin Heidelberg, Germany.

11. Popp C, Burghardt M, Friedmann A, Reiderer M (2005) Characterization of hydrphilic and lipophilic pathways of Hedera helix L. cuticular membranes: permeation of water and uncharged organic compounds. Jurnal of Experimental Botany, Vol. 56, No. 421, pp. 2797-2806.

12. Schönherr J (2006) Characterization of aqueous pores in plant cuticles and permeation of ionic solutes. Journal of Experimental Botany, Vol 57, No. 11, pp. 2471-2491.

13. Faers MA, Pontzen R (2008) Factors influencing the association between active ingrediente and adjuvante in the leaf deposito of adjuvante-containing suspoemulsion formulations. Pest Management Science 64, pp. 820-833.

14. Hunsche M, Noga N(2011) Effects of relative humidity and substrate on the spatial association between glyphosate and ethoxylated seed oil adjuvants in the dried deposits of sessile droplets. Pest Management Science 68, pp 231-239.

15. Truskett VN, Stebe KJ (2003) Influence of Surfactants on an Evaporating Fluorescence Image and Particle Deposition Pattern. Langmuir 19, pp 8271-8279.

16. Holmberg K, Jönsson B, Kronberg B, Lindman B (2002) Surfactants and Polymers in Aqueous Solution. John Wiley & Sons, Ltd.

17. Evans DF, Wennerström H (1999) The Colloidal Domain: Where Physics, Chemistry, Biology and Technology Meet. Wiley-VCH, New York, USA.

18. University of Washington **http://depts.washington.edu/solgel/pages/courses/MSE_502/Electrostatic_Stabilization.html**, cited 20 December 2013

19. Westbye P, Andersson M, Bergström K (2013) Solubility and adjuvant properties of novel solvents for agrochemical formulations. ISAA 2013 10th international symposium on Adjuvants of Agrochemicals, Edit: Castelani P, Stock D, Moran-Puente D, pp187-193.

20. CIPAC Handbook MT 18:1 “Preparation of Standard Waters” for 34,2 pp calcium equivalents.

21. CIPAC 39.2, 1970, “Emulsifiable Concentrates and Solutions/Low Temperature Stability of Liquid Formulatuions”.

22. Westbye P, Andersson M, Hazen JL, Bergström K (2013) Design of Novel Solvents for Agrochemical Formulations via Solvatochromic Methods using N-Alkyl substituted Amides as Example. Pestecide formulation and delivery systems, STP 1569, Carmine Sesa, Ed, doi:10.1520/STP156920120154, ASTM International, West Conshohocken, pp1-14.