Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

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ESCUELA DE INGENIERIAS INDUSTRIALES

Grado en Ingeniería Química

Investigación sobre las propiedades de producción de las microcápsulas de cobre y su combinación con extracto de lúpulo microencapsulado.

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TÍTULO: Investigating the product properties of copper microcapsules and their combination with microencapsulated hop extract

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Bachelor thesis

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Paula Calvo de Diego

Index

List of abbreviations and symbols

1	**A**	Area
2	**C**	Concentration
3	**°C**	Celsius degrees
4	**CE**	Capillary Electrophoresis
5	**$CH_3COONa.3H_2O$**	Sodium acetate trihydrate
6	**$C_7H_6O_2$**	Benzoic acid
7	**$C_{initial}$**	Initial concentration
8	**$C_{obtained}$**	Obtained concentration
9	**Cu^+**	Copper ion (Charge +1)
10	**Cu^{2+}**	Copper ion (Charge +2)
11	**$Cu(OH)_2$**	Copper hydroxide
12	**$CuSO_4$**	Copper sulfate
13	**CuCaps**	Copper Microcapsules
14	**DNA**	Deoxyribonucleic acid
15	**e.g.**	Examples
16	**FAU**	Friedrich-Alexander University Erlangen- Nuremberg
17	**Fe-S**	Iron-sulfur
18	**g**	Gram
19	**iPAT**	Institute of Processing Machines and Apparatus Engineering
20	**HCl**	Hydrochloric acid
21	**L**	Liter
22	**m**	Meter
23	**m1**	Total mass of the starting microcapsules
24	**m2**	Mass of particles contained in the 25 mL in the remaining of the cylinder
25	**mAu**	Milliunit of area
26	**$(mAu)^2$**	Squared milliunit of area
27	**min**	Minutes
28	**M**	Molar
29	**NaOH**	Sodium hydroxide
30	**P. viticola**	Plasmopara viticola
31	**ROS**	Reactive oxygen species
32	**rpm**	Revolutions per minute
33	**S**	Suspensability
34	**t**	Time
35	**V**	Volt
36	**x_{10}**	Indicate a value up to 10 % of the amount of the particles, the microcapsules have a lower diameter than value
37	**x_{50}**	Indicate a value up to 50 % of the amount of the particles, the microcapsules have a lower diameter than value

38	**x_{90}**	Indicate a value up to 90 % of the amount of the particles, the microcapsules have a lower diameter than value
39	**x axis**	Abscissae
40	**y axis**	Ordinate
41	**%**	Percentage

1 Introduction

Downy mildew, whose epidemics can cause tangible harm both to leaves and grapes clusters, is one of the most harmful diseases to be found in the European vineyards. This pathogen can infect all the vegetative organs of the vine, for example the leaf, the tip, flower, cluster, bunch, stalk or young fruit, and can cause numerous infections during the grape growing season. Several symptoms which correspond to the different stages in the cycle of the disease can be observed: “oil stains”, whitish mould and necrotic tissue. Currently, the most common product to combat Downy Mildew is copper in different chemical formulas like Bordeaux mixture, Copper-oxychloride, Cupper-hydroxide, tri basic Copper-sulphate, Copper-oxide and Copper octanuat. The main problem in that the copper ion is found to be immobile in the ground, and therefore accumulation of this element in the ground is caused by repeated application. For this reason, wine growers try to reduce usage of this element. Other possible compounds to combat Downy Mildew, are plant or compost extracts which have shown to give good results in vines. [1]

Antibacterial activity from α-acid (humulone) and β-acid (lupulone) from hops has been shown to be a possible remedy [2]. On the other hand, the use of copper combined with hop has been studied previously and found to be effective in the fight against Downy Milde [3]. Therefore the use of both in pesticides would reduce the concentration of copper, partly solving the problem generated by accumulation of copper in the soil.

Another solution to the problem of accumulation would be to produce microencapsulated pesticides which allow controlled release of the active ingredients of the pesticide and therefore of the copper. [4] Development of this technique is already being carried out.

The Institute of Processing Machinery and Systems Engineering (iPAT) can be found at Friedrich-Alexander University Erlangen- Nuremberg (FAU). This institute has a spray cooling production department which develops microcapsules and which has already developed various types of microcapsules with hop extract and several types of copper microcapsules intended for use in the development of microcapsulated pesticides to combat Downy Mildew. [3]

The aim of this thesis is to investigate the product properties of copper microcapsules and their combination with microencapsulated hop extract for their future use as pesticide.

These different types of microcapsules were produced using a spray cooling process and their properties were then examined. Distribution of size and stability of suspension, which is important for the applicability of microcapsules in the field, were achieved using different combinations of microcapsules and different combinations of suspensions. Furthermore, an evaluation of the release of copper from the microcapsules, both of those containing copper and combination of copper microcapsules was executed, therefore the development of a method was carried out to measure the concentration of copper in water. To do this, a method involving analysis of the copper concentration in the Capillary Electrophoresis (CE), which led to the development of an adequate buffer for measurement, was implemented.

2 Theoretical background

2.1 Use of copper against downy mildew

In this section, downy mildew and use of copper against downy mildew are explained.

2.1.1 Downy Mildew

Downy mildew, caused by the obligately biotrophic peronosporomycete *Plasmopara viticola*, is one of the most destructive criptogamic grapevine diseases that occurs worldwide. The classical cultivars of *Vitis vinifera*, are all susceptible to *P. viticola*, resulting in severe epidemics under warm and humid conditions. [5] The pathogen attacks all green parts of the vine. Only a few of the older leaves may develop symptoms if the growing season has been hot and dry. [6] Cluster and blossom infection with *P. viticola* before, during or after bloom may result in poor fruit set and quality, and considerable crop loss [7]. The pathogen was introduced to Europe in the last quarter of the 19th century and in 1878 the first symptoms were observed in the Bordeaux area. In the following decade all classical European grapevine cultivars showed to be highly susceptible, resulting in a severe pandemic throughout the continent. Combatting the disease requires a huge investment in fungicides every year. [5] [8]

The main symptoms of downy mildew in leaves are oily green or yellow patches on the upper side of the leaf. These patches become brown, necrotic or spotty. The underside of the leaf presents a grey-white fuzzy growth. The patches on the upper side of the leaf sometimes merge and become brown and brittle. These leaves wither and detach from the vine. This leaves the grapes on a mildewed plant are usually not good quality grapes. Shoots and tendrils which are affected by mildew appear to have waterlogged areas and it is here that the fuzzy white growth appears. Shoots and tendrils after becoming distorted, wither and dry up completely. [6]

Fruit can be affected by mildew at two different stages during growth. Firstly, the young grapes become fragile and occasionally mildew growth can be seen on the fruit. Secondly, the grapes can become infected in late summer or at beginning of autumn. These grapes change colour, start to resemble a raisin and detach from the branch. [6]

Mildew in vines is caused by a fungal type organism, called *P. Viticola*. This disease normally appears in excessively warm, wet years, but not in hot years. Rain is considered

to be the main factor for the spread of this disease. When damp nights are followed by rainy days, risk of infection from this pathogen is increased. Temperatures play an important role in the development of this disease, Mildew on a vine can develop in temperatures between 10 ºC and 29 ºC, the optimum temperature for development being between 18 ºC and 22 ºC. The most serious epidemics of this disease have been detected following damp winters and warm damp summers with rainfall every eight – fourteen days. Infected plants often become less resistant to other diseases. [6] [10]

P. viticola is a parasite which obtains nutrient from living tissue. It reproduces within the host tissue. The sexual spore produced in an oospore. Oospores can survive the winter either in the soil or in the host leaf. Germination generally takes place just after bud break. The oospore creates a germ tube with a sporangium on the tip. The sporangia houses zoospores. When released, the zoospores germinate and enter the leaf through healthy stomata. Both sporangia and zoospores have a short lifespan when exposed to sunlight and dry weather, in these conditions they can die after a couple of hours. In cooler, more humid climate, they can survive on their host for over 24 hours. After infection, the pathogen grows intercellulary, producing haustoria. Sporangia can also cause the spread of Mildew. They are produced in sporangiophores and are released though the stomata and are carried either by the wind or rain splash. [9] [10] [11] Some symptoms of downy mildew can be observed in Figure 1.

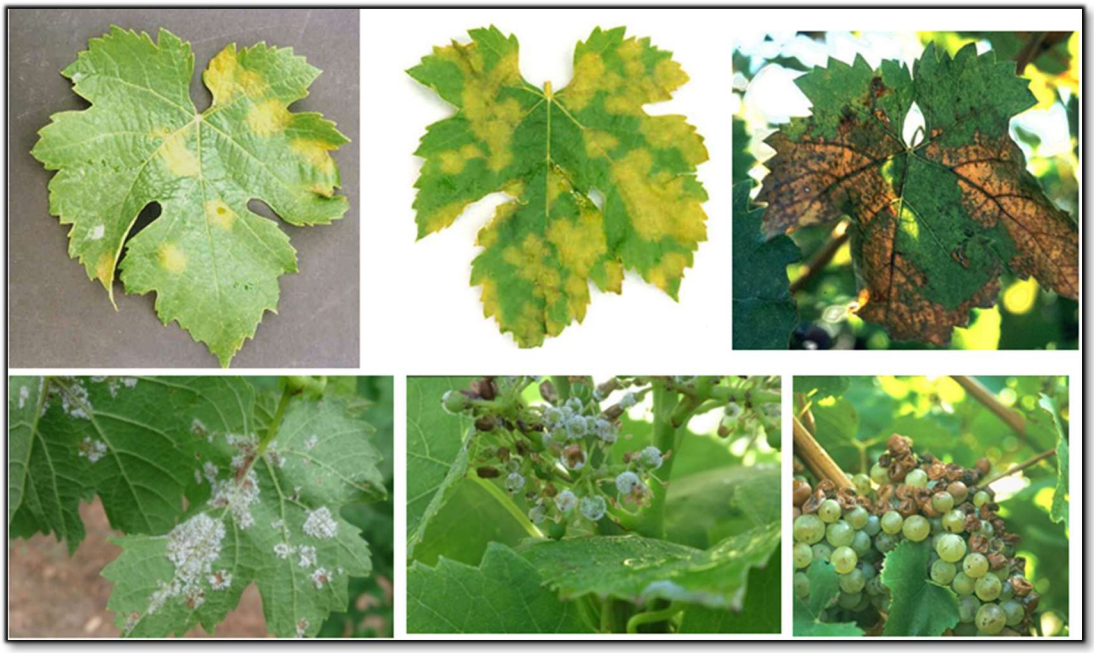

Figure 1: Images of the symptoms of downy mildew on vine leaves and fruit. [9]

2.1.1 Copper, potential ingredient in the fight against Downy Mildew

Copper has been used by man for over 10.000 years. Scientists now have a renewed interest in this metal, due to its antimicrobial properties. Recent studies have focused on the mechanism where microbes are "killed" on coming into contact with a copper surface and which can reduce contamination. Although human tissue has a high tolerance to copper, and is only affected negatively when exposed to very high concentrations, copper is highly toxic for microorganisms. The biocidal effect of copper is a mechanism whereby the cell death caused is multifactorial rather than one simple mechanism. To make its toxicity more effective, as decrease of oxidation of copper changes, between Cu^{+} and Cu^{2+}, it is able to accept and donate single electrons. The copper can then act as a catalyst to generate reactive oxygen species (ROS) like hydroxyl radicals and super anions. Vital cell constituents such as proteins, nucleic acids and lipids (cell membrane included) are vitally damage by these ROS. Fe-S (Iron-sulfur) clusters in cytoplasmic enzymes required to form branched-chain amino acids can be damaged when copper ions inactivate proteins. For these reason, the research involved in this project focused on copper as a potential pesticide to treat Downy Mildew. [12]

2.2 Microencapsulation: technologies

Microencapsulation is the process of enclosing a solid, liquid or gaseous substance within a shell on a tiny scale. Capsules produced can measure from less than one micron to several hundred microns in size. The capsule shell surrounds the active ingredient or core. [13] Microcapsule with core and shell can be observed in Figure 2.

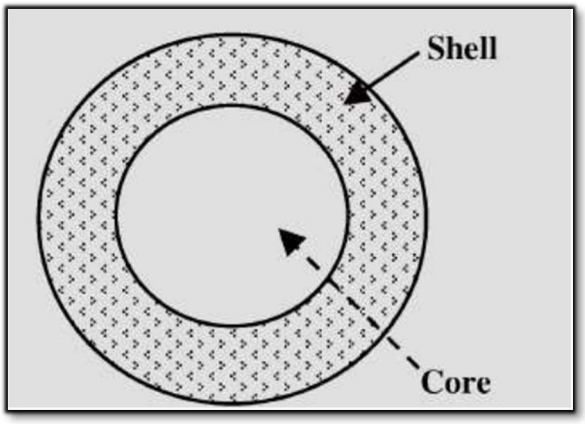

Figure 2: Microcapsule with core and shell. [13]

The use of microcapsules can be traced back to the 1950's. Green and Scheicher first microencapsulated dyes to produce carbonless copy paper. These same microcapsules are still used nowadays. By the 1960's, microcapsules were used to produce thermosensitive display systems. By the 1960's, microcapsules had revolutionised not only the pharmaceutical industry but also many other areas of production. Examples of microencapsulated products are pesticides and fertilizers. These are of special interest as the aim of the experiments carried out is to reduce leaching of certain products into the ground and microcapsules are able to locally limit release, that is, the use of these can carry out the release of the active components in a controlled manner. [4][14]

The reasons for microencapsulating a substance can range from:

- Confining core material within a capsule for a set length of time.
- To release a substance gradually through the shell (eg. in drugs and pesticides).
- So that external conditions melt, break or dissolve the outer shell.
- Separation of components due to incompatibility.
- Convert substances from liquid to free flowing solids.
- To avoid core material decreasing in effectiveness due to explosive substances.
- To hide tastes or odours.
- Reduce toxicity.

The permeability of the outer shell determines to a certain extent the usage of the microcapsule. The shape, size, shell composition, shell degradability and biocompatibility are all fundamental when selecting materials for microcapsules. [4]

There are many ways of microencapsulating substances. The morphology depends on both the core material and the break down process of the shell. There are three main types of microcapsules, as seen in Figure 3 [4][13]:

- Mononuclear, which consists of a simple shell surrounding the core, in other words one hollow chamber inside the capsule.
- Polynuclear, which consists of many cores or chambers surrounded by a shell.
- Matrix, which consists of evenly distributed core material incorporated into the actual material of the shell.

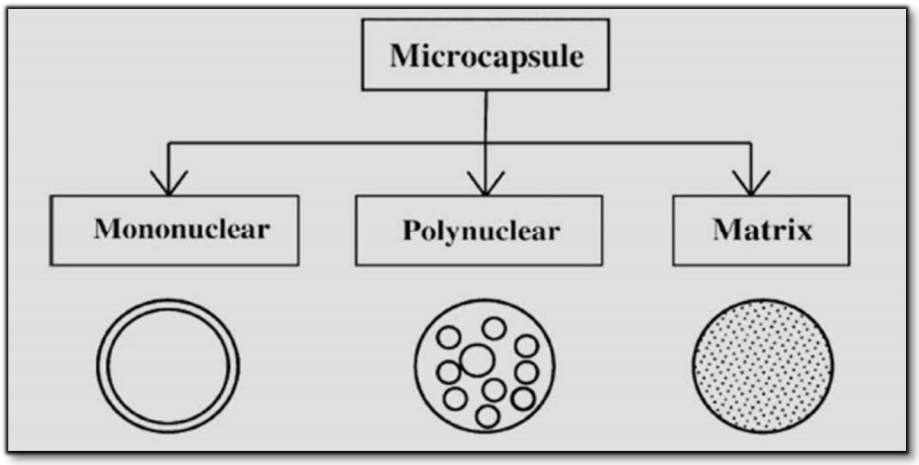

Figure 3: The three main types of microcapsules. [13]

These microcapsules can also be made up of various shells or clusters of microcapsules.

One of the most important characteristics of microcapsules is that their tiny size allows for a large surface area. 1 µm of hollow microcapsules having a diameter of 0.1 µm gives a total surface area of about 60 m. The total surface area is inversely proportional to the diameter. Therefore, such a large area allows a good contact with the exterior that allows a good release of the active components. [4]

2.3 Copper microcapsules (CuCaps) fundamentals

In the following section, the basics of copper microcapsules, named CuCaps are explained.

In the tables below, the compounds from which copper microcapsules are produced and the percentage of each one, can be observed. Table 1 represents the composition of the A104 CuCaps and Table 2 represents the composition of A123 CuCaps.

Table 1: Components of A104 microcapsules and their percentage.

CuCaps A104	**Percentage (%)**
Hardened canela oil	56.83
Benzoic acid	12.76
Tween 65	7.73
Copper (Ions) from Copper Sulfate	18.84
Copper (Ions) from Copper Hydroxide	3.84

Table 2: Components of A123 microcapsules and their percentage.

CuCaps A123	**Percentage (%)**
Hardened canela oil	71.95
Tween 21	12.7
Copper (Ions) from Copper Hydroxide	15.35

These two types of copper microcapsules were used in experimental processes which are explained in later sections. The main aim of the microencapsulation of these compounds, which are rich in copper, is the microcapsules to be used as pesticides to combat Downy Mildew. These microcapsules are of matrix type.

The principal difference between both types of microcapsules is that A123 has copper ions in the form of only copper hydroxide and A104 has a higher concentration of copper sulphate and a lower concentration of copper hydroxide, where copper sulphate is more soluble than copper hydroxide in water.

All the microcapsules contain emulsifiers for wettability, suspension stability and controlled release.

The principal aim of the microencapsulation of these substances is to control the release of the compounds in certain conditions and to achieve wettable powder. For this reason, many of the experiments carried out aim to find out the extent of the release of copper from the microcapsules as well as other parameters of interest.

2.4 Hop microcapsules

In the following section, the basics of hop microcapsules are explained.

Hop microcapsules are microcapsules formed using hop extract as a basic ingredient of its composition. In Table 3, the main components for manufacture of hop microcapsules, can be found.

Table 3: Components of H61 microcapsules and their percentage.

Hop microcapsules H61	**Percentage (%)**
Hardened canela oil	55
Hop-extract	15
Brij S20	15
C-1616	15

Due to hop extract being one of the potential ingredients of pesticide against Downy Mildew, the possible combination of hop microcapsules and CuCaps as a pesticide to treat Downy Mildew has been considered.

This type of microcapsules contains emulsifiers, which become wettable powder to be more stable in a suspension and also to control the release.

For this reason, the experiments carried out focus on analysing parameters of the hop microcapsules and CuCaps and a combination of both.

2.5 Capillary electrophoresis (CE): Fundaments and technology

In 1937, Tiselius (Nobel Prize Winner) introduced a separation technique named "Electrophoresis". This separation technique consisted of ions in an electrical field being attracted or repelled. Sample components of proteins combined in a buffer were found to migrate at different rates and in different directions according to charge and mobility when placed in a tube and subjected to an electrical field. [15]

Nowadays, capillary tubes of 25 to 150 μm interior diameter are usually used to carry out Capillary Electrophoresis (CE). These tubes commonly contain only a buffer. Using this capillary tubes is particularly advantageous where the harmful effects of Joule heating are concerned. Capillary tubes can stand very high electrical fields of 100 to 500 V/m and so little heat is generated when a high electrical field is applied. CE is considered to be highly useful as it can separate such a diverse range of compounds, despite originally being used to analyse macromolecules. Examples of current CE usage are separations of amino acids, pesticides, organic acids, and DNA (Deoxyribonucleic acid) restriction fragments to mention just a few. [15]

CE does not require complicated instrumentation. Electrodes are placed in reservoirs containing buffer so as to make electrical contact between a high voltage power supply (connected to the reservoirs and a narrow calibre fused silica capillary tube). The ends of the capillary, which is filled with the same buffer as the reservoirs, are plunged into the reservoirs. [15] Figure 4 represents a diagram of components of the CE.

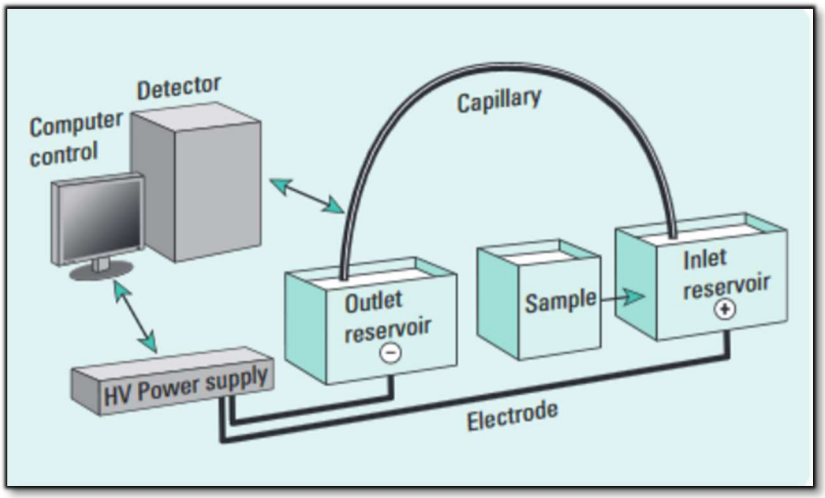

Figure 4: Diagram of components of the CE. [15]

By exchanging one buffer reservoir (anode resevoir) for a sampe reservoir and exerting external pressure by applying and electrical field, the sample can be injected into the capillary tube. The separation is carried out. Separation can be oberved at the opposite end through the wall of the capillary, where there is a detector to transmit the information to the computer. [15]

The buffer is a solution with a concentration of ions which has the capacity to form a soluble complex with the molecules to be analysed, and also, this solution solution is able to conduct the electricity. A buffer for use in the CE should possess the following properties: [15]

- Good buffering capacities in the pH range.
- Low absorbance at the wavelength of detection.
- Low mobility to minimize current generation.

The requiered time for a solute to migrate to the point of detection in called “the migration time”, which is sensitive to: [15]

- Voltage power of electrical field.
- Concentration of solute to be analysed.
- Characteristics of buffer like type of ion or pH.

3 Material and methods

The chemical products, equipment used and experimental procedures are explained in detail in this section.

3.1 Chemicals

In this section, the chemical products, their compounds, supplier and uses are explained in detail.

Table 4: List of chemical products used.

Name	Compound	Supplier	Use
Copper sulfate	$CuSO_4$	Carl Roth	Production of CuCaps Analysis in the CE Calibration for CuCaps analysis
Copper hydroxide	$Cu(OH)_2$	Carl Roth	Production of CuCaps Analysis in the CE Calibration for CuCaps analysis
Sodium acetate trihydrate	$CH_3COONa.3H_2O$	Carl *Roth*	Buffer production
Hydrochloric acid	HCl	Carl Roth	Buffer production
Sodium hydroxide	NaOH	Carl Roth	Buffer production
Hardened canela oil	-	-	CuCaps production Hop microcapsule production
Hop extract	-	-	Hop microcapsule production
Brij S20	-	Croda Iberica SA	Hop microcapsule production
C-1616	-	Mitsubishi Chemical Foods Corporation	Hop microcapsule production
Benzoic acid	$C_7H_6O_2$	PuroxB Food/Pharma	CuCaps production
Tween 65	-	Croda Iberica SA	CuCaps production
Tween 20	-	Croda Iberica SA	CuCaps production

3.2 Equipment and methods

The equipment used and experimental methods are explained in detail in this section.

3.2.1 Characterization of particle size

The Malvern Mastersizer 2000 with dispersion unit Hydro 2000s, which can be seen in Figure 5, is an instrument to analyse particle size distributions.

Figure 5: Photography of the Malvern Mastersizer 2000 with dispersion unit Hydro 2000s.

It is based on the diffraction of light by using a laser. This means that large particles disperse the light at low angles and small particles disperse the light at high angles. [16][17]

This instrument is able to measure the energy of the light dispersed over a range of angles and can transform said information into distributions of particles.

To analyse in the Mastersizer, 45 cycles of measurements were carried out.

- The first 30 cycles were carried out at a rotation speed of 2500 rpm.

- The following 10 cycles at a rotation speed of 2500 rpm and 10 % ultrasounds were activated.
- The remaining 5 cycles were carried out without ultrasounds at the same speed as the first 40 cycles, of 2500 rpm.

Using the data obtained, the values x_{10}, x_{50} and x_{90} will be represented in graphs. Where x_{10}, x_{50} and x_{90} indicate a value up to 10 %, 50 % and 90 % of the amount of the particles, the microcapsules have a lower diameter than value.

These results (x_{10}, x_{50} and x_{90}) were shown in graphs which represented the behaviour of the microcapsules during the 45 cycles.

3.2.2 Suspensibility test

The aim of the suspensibility test is to find out the capacity of the sample to being in suspension, that is, the particles are maintained, do not float or sediment in the suspension. These measurements were carried out with different concentrations of microcapsules or mixing different types of microcapsules, in this case, two types of copper microcapsules and one type of hop microcapsules (H61), as well as a combination of CuCaps and hop microcapsules.

In these tests, an amount of microcapsules was weighed on a laboratory scale and mixed with tap water at 20 °C in a beaker until it reached 50 g in total. To get the desired concentration different amounts of microcapsules were used. The mixture was stirred with a magnetic stirrer for one minute at 800 rpm. After, the mixture was introduced in a 250 mL measuring cylinder, which can be seen in the first picture of Figure 6, and filled with tap water. Immediately, the cylinder was closed and gyrated 30 times. Having waited for 30 minutes, the first 225 mL of the liquid from the upper area of the measuring cylinder was extracted by peristaltic pump and the remaining 25 mL was filtered to remove the excess of water using a centrifuge pump Sigma 3-16 SIGMA and the solid particles were retained in filter paper, which was left to dry for a day in a plastic container.

Before weighing the solid, the filter paper was weighed together with the plastic container. After one day, the dried solid was weighed on the filter paper and the container, which can be seen in the second picture of Figure 6.

Finally, the suspensibility was calculated applying the following equation:

$$S = 111 * \frac{m1 - m2}{m1} \quad [1]$$

Where S is the supensibility, m1 is the total mass of the starting microcapsules and m2 is the mass of particles contained in the 25 mL remaining in the cylinder.

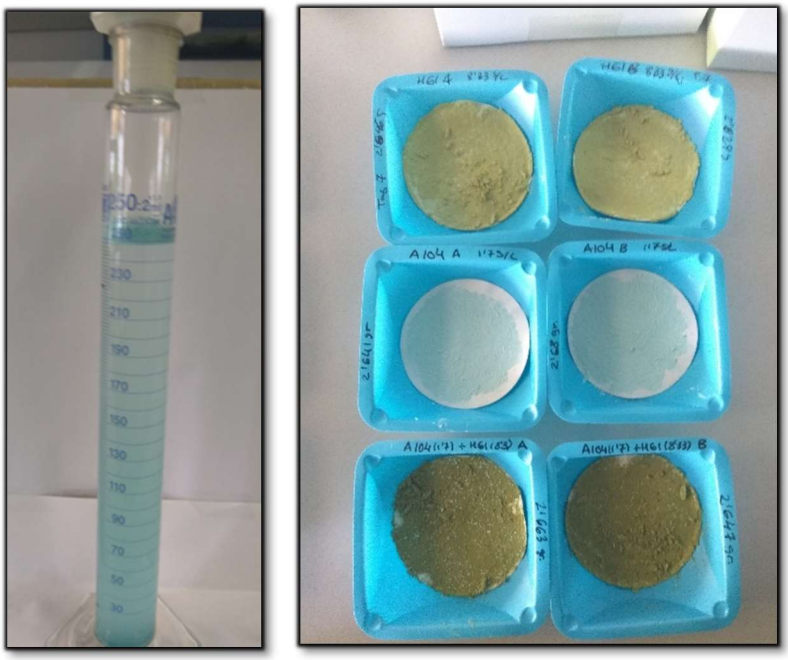

Figure 6: The first picture is the 250 mL cylinder and the second picture is the solid in the filter in the plastic container.

3.2.3 Capillarity electrophoresis (CE)

The results are obtained from a graph in which peaks appear. In the abscissae (x axis) the apparition time of the molecule is represented. In the ordinate (y axis), the units of area are shown. The area under the peak is proportional to the concentration of the molecule in the solution.

In these tests, an amount of microcapsules was weighed on a laboratory scale and mixed with destilled or tap water (depending on the measurement) in a beaker until it reached 75 g in total or in a volumetric flask (according to the required concentration). To get the desired concentration different amounts of microcapsules were used. The mixture was

stirred with a magnetic stirrer for 10 minutes at 800 rpm. After this time, the mixture was filtered using cellulose microfilters for the hop microcapsules, and nylon microfilters for de copper microcapsules. In combined CuCaps and hop microcapsules tests, the microfilters were used as the objective was the analysis of the concentration of copper.

From the previously filtered mixture, a quantity was taken and mixed at 50 % with the buffer. One mL of this mixture was introduced in the CE, which can be seen in Figure 7, to be analysed. The process in CE was the next:

- Cleaning program:
    - 2 min with HCl 1M
    - 2 min with destilled water
    - 2 min with NaOH 1M
    - 2 min with destilled water
    - 2 min with Buffer
- Pre-conditioning:
    - 2 min with Buffer
- Injection:
    - The separation voltage: -20 kV
    - The injection was performed by applying 50 mbar for 8s
- Post-conditioning:
    - 3 min with HCl 0.1M
    - 3 min with destilled water
    - 3 min with NaOH 0.1M
    - 3 min with destilled water
    - 3 min with Buffer

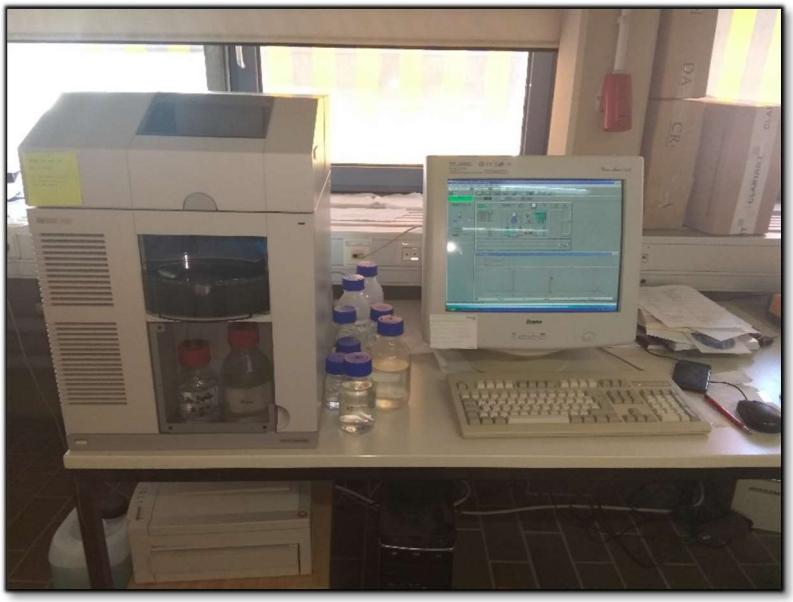

Figure 7: Photography of Capillary Electrophoresis.

3.2.4 Production process of microcapsules

For the production process of microcapsules, the spray cooling technique was carried out, both for CuCaps and hop microcapsules. For the production, a spray tower was used, which had a height of 5.3 m and 0.45 m diameter.

The mixture of the different components of microcapsules was executed in a 400 mL glass beaker by heating a bath of water. The temperature of this bath of water was measured by IKATRON ETS-D5 controller and the mixture of components was carried out by a Turrax IKA Ultra Turrax T 18 for two minutes at 20.000 rpm.

The peristaltic pump IKA Labortechnik PA-SF was used to transport the mixture of the components to the tower. One end of the tube was inside the glass beaker containing the mixture and the other end was connected to a nozzle of 500 µm through which two substances were sprayed to disperse the mixture in the tower. A nitrogen flow at 5 bar was introduced around the flow of the mixture to disperse the mixture in the tower. It is necessary to pre-heat the flow of nitrogen because the components of the mixture contain fat, which could solidify before arrive to the tower, if it comes in contact with nitrogen without pre-heating.

The microcapsules were immediately formed when the mixture passed through the nozzle. Once the microcapsules were retained in a filter in the bottom of the tower, they were separated from the air in a cyclone, which was connected to a vacuum cleaner. Once the microcapsules were separated, they were introduced into a bottle for future analysis. In Figure 8 a diagram of the process is represented.

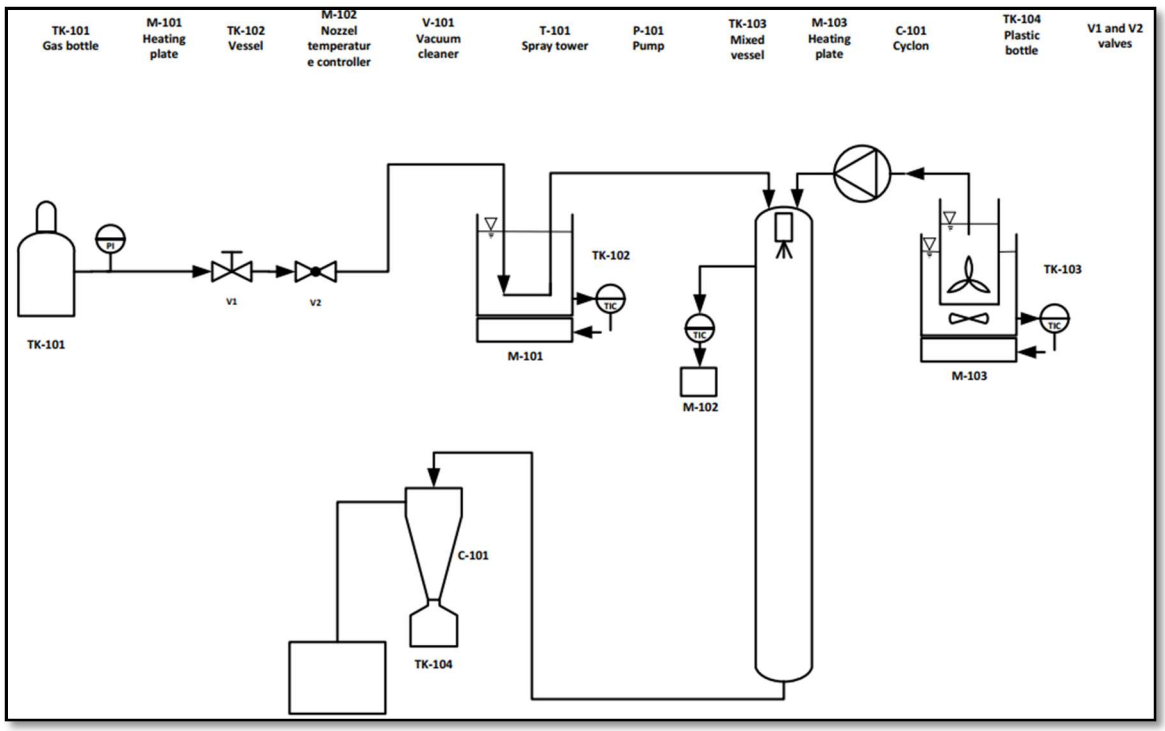

Figure 8: Piping and instrumentation diagram of the production process of microcapsules.

4 Results and discussions

4.1 Determination of particle size

In this section, the results obtained in the Mastersizer 2000 are analysed to find out the size-distribution of the microcapsules. Two types of CuCaps were measured, the A123 and A104, as well as a hop type of microcapsule, H61. Two types of CuCaps combined with H61 in a solution were then analysed.

Figure 9 represents the distribution of the particle sizes of the A123. It can be observed, that differences between initial and final values of the sizes exist. It means that initial agglomerations are destroyed after applying ultrasound at minute 25, because particle size is lower than before and remains constant afterwards.

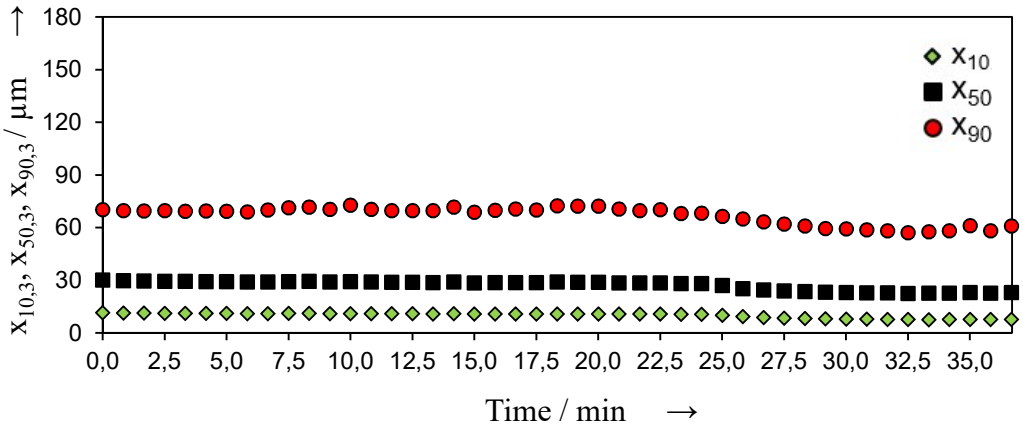

Figure 9: Graph showing the distribution of size of the A123 microcapsules.

Table 5 represents the values x_{10}, x_{50}, x_{90} initially and at the end, and the difference between both, the aim being to find out if agglomerations of particles exist. Significant differences can be observed therefore demonstrating that agglomerations of microcapsules do exist.

Table 5: Differences in particle size distribution of A123 in the initial cycle and end cycle.

	Beginning	End	Difference
X_{10} (µm)	11.47	7.68	3.79
X_{50} (µm)	30.04	22.97	7.08
X_{90} (µm)	70.17	60.91	9.26

Figure 10 represents the distribution of the sizes of the A104. In the same way as in A123 there is a significant variation between the values obtained in the first cycles and the last ones. Additionally, a rapid decline in particle sizes can be noted after the activation of ultrasound.

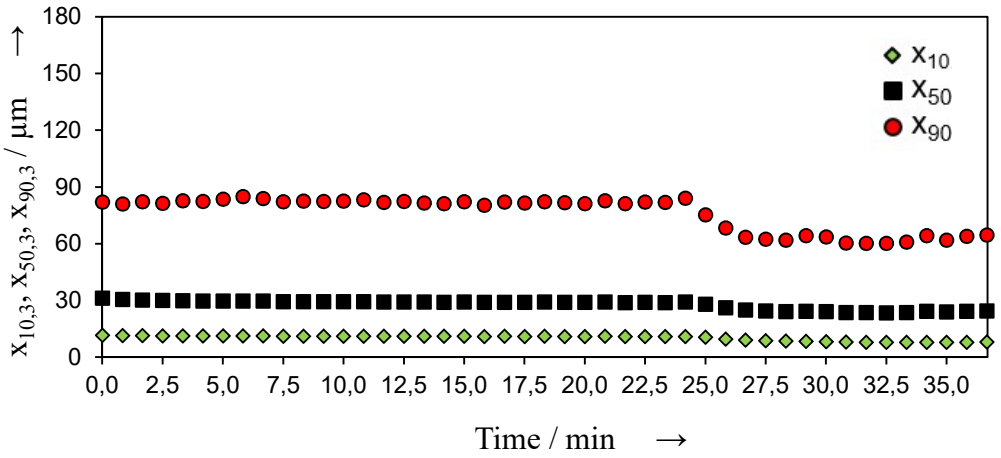

Figure 10: Graph showing the distribution of size of the A104 microcapsules.

If we compare the values of the CuCaps A123 and A104, it can be observed that the values x_{10} and x_{50} are very similar, but at the beginning of the analysis, x_{90} is somewhat larger in the case of the A104 and are more similar at the end of the analysis. This means that the A104 form larger agglomerations.

Table 6: Differences in particle size distribution of A104 in the initial cycle and end cycle.

	Beginning	End	Difference
X_{10} (µm)	11.67	8.03	3.63
X_{50} (µm)	31.35	24.51	6.84
X_{90} (µm)	82.06	64.66	17.40

Figure 11 represents the distribution of the sizes of the microcapsules H61. It can be observed how the initial size of these microcapsules is much larger than those of the CuCaps. This shows that H61 has bigger agglomerations initially, which can, however, be partially destroyed by the low-energy input of the stirrer. The end values on the other hand show, that the distribution of sizes present lower values than those of the CuCaps. This means that the size of the hop microcapsules are generally smaller after ultrasound. This might be due to possible erosion of the hop microcapsules when ultrasound is applied.

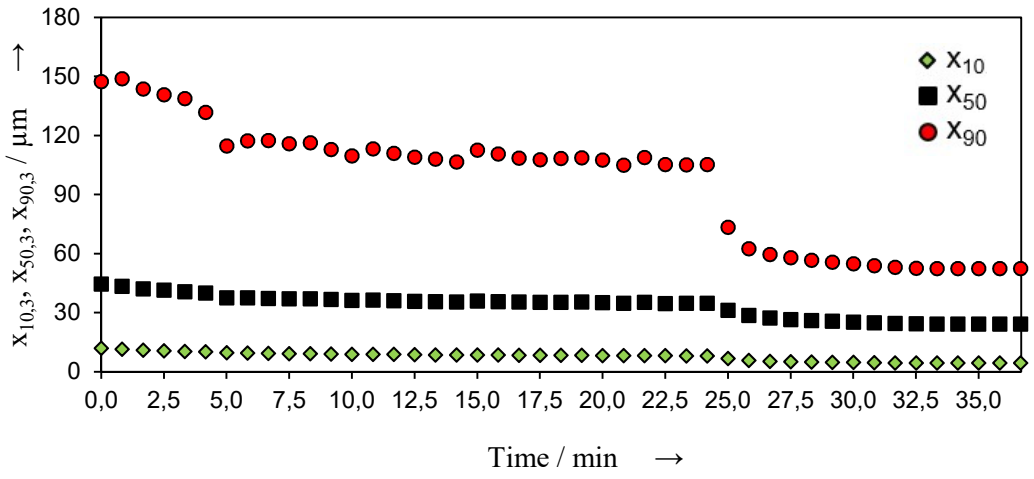

Figure 11: Graph showing the distribution of size of the H61 microcapsules.

Table 7: Differences in particle size distribution of H61 in the initial cycle and end cycle.

	Beginning	End	Difference
X_{10} (µm)	12.00	4.44	7.56
X_{50} (µm)	44.61	24.35	20.27
X_{90} (µm)	147.47	52.55	94.92

The next step was the analysis of the A123 with H61 combination. The results of this analysis are shown in Table 8 and Figure 12.

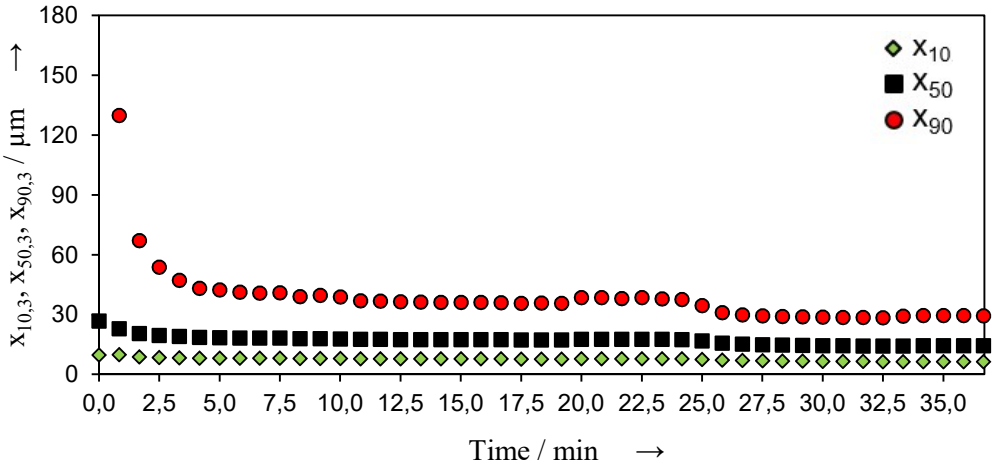

Figure 12: Graph showing the distribution of size when analysing the combination of A123 and H61 microcapsules.

In Figure 12, a very steep decline in x_{90} during the first minutes whilst only stirring can be observed. And also, a smaller decline can be appreciated after addiction of ultrasound. But in the case of x_{10} and x_{50}, the results are different. It can be noted that both lines do not suffer abrupt changes even after ultrasound. The first steep might be coincidental, if a very large agglomerate was added.

In Table, the differences in the initial cycle and end cycle of x_{10}, x_{50} and x_{90}, where the biggest difference is that of x_{90} can be observed, corroborating the results of the graph in Figure 11.

Table 8: Differences in particle size distribution of the combination of A123 and H61 in the initial cycle and end cycle.

	Beginning	End	Difference
X_{10} (µm)	9.90	6.57	3.33
X_{50} (µm)	26.84	14.44	12.40
X_{90} (µm)	328.18	29.47	298.71

The last analysis was of the combination of the A104 and H61 microcapsules. The results of this analysis are shown in Table 9 and Figure 13.

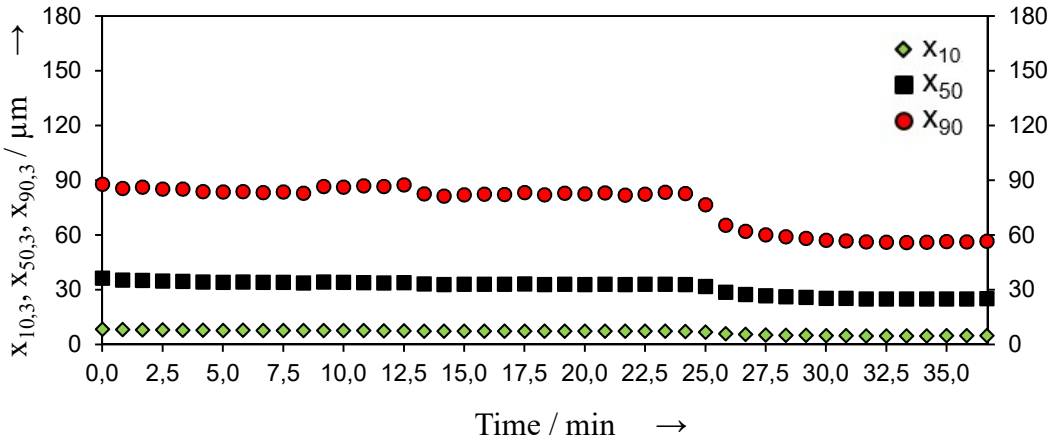

Figure 13: Graph showing the distribution of size when analysing the combination of A104 and H61 microcapsules.

In Figure 13, a small decline in x_{90} can be observed after addition of ultrasound and also a smaller decline in x_{50} can be appreciated in the same time. But in the case of x_{10}, the results are different. It can be noted that the line does not suffer abrupt changes even after ultrasound.

In Table 9, the differences in the initial cycle and end cycle of x_{10}, x_{50} and x_{90} can be observed.

Table 9: Differences in particle size distribution of the combination of A104 and H61 in the initial cycle and end cycle.

	Beginning	End	Difference
X_{10} (µm)	8.49	4.89	3.60
X_{50} (µm)	36.52	25.21	11.31
X_{90} (µm)	87.89	56.65	31.24

If the combinations of A123 with H61 and A104 with H61 are observed, both having been prepared in a suspension, it can be seen that initially the combination A123 and H61 presents a distribution of sizes with much higher values than these obtained in the combination of A104 and H61. However at the end of the experiment, the opposite occurred, the distribution of the combination A104 and H61 being higher.

In both combinations, it can be observed that there is a significant difference between the initial values and the final ones, which confirms the existence of agglomerations between the two types of particles.

4.2 Suspensibility measurements

In this section the results obtained in the suspensibility tests for the different microcapsules and their combinations were analysed.

In Figure 14 the percentages of suspensibility of different microcapsules (H61, A104 and A123) are represented with two different concentrations of each one. H61 with 8.33 g/L and 3.32 g/L and A104 and A123 with 3.32 g/L and 1.7 g/L concentrations.

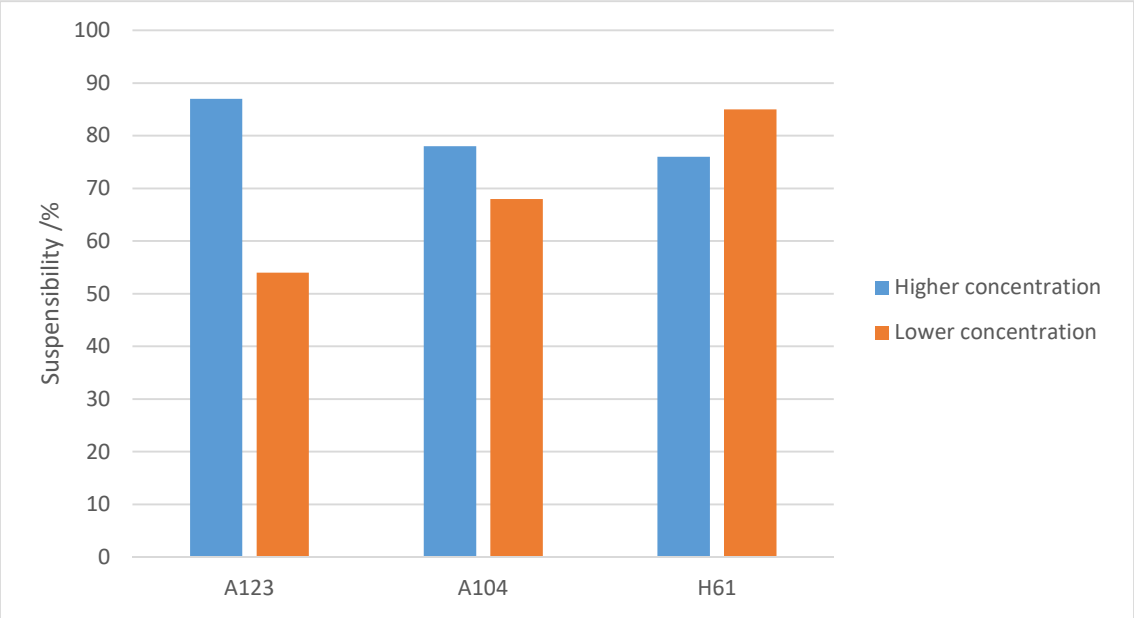

Figure 14: Percentage of suspensibility of different microcapsules (H61, A104 and A123) with different concentration.

Higher concentrations of the A104 (3.32 g/L concentration) have higher suspensibility than A104 (1.7 g/L concentration). The same occurs with A123 microcapsules. In this case, the opposite of the previous point occurs being the expected result.

If H61 with 8.33 g/L concentration is compared with H61 with 3.32 g/L, it can be observed that the suspensibility of the most concentrated sample is lower. This is strange as the opposite results were expected. At a higher concentration, the speed of sedimentation was expected to be lower therefore, the suspensibility would be higher. However, different phenomena can occur for which a lower suspensibility is obtained. Like for instance, interaction exists between the particles of the suspension, forming agglomerations which make the speed of sedimentation increase and therefore causing the suspensibility to decrease. Another possible reason for lower suspensibility is that on sedimentation, the particles form an ascending flow of liquid which causes the particles to take up lower positions and so sedimenting faster and in turn decreasing the suspensibility. [19, p. 237-239]

In Figure 15 the percentages of suspensibility of H61 with 8.33 g/L, A104 with 1.7 g/L and the both combination with a total concentration of 10.03 g/L are represented.

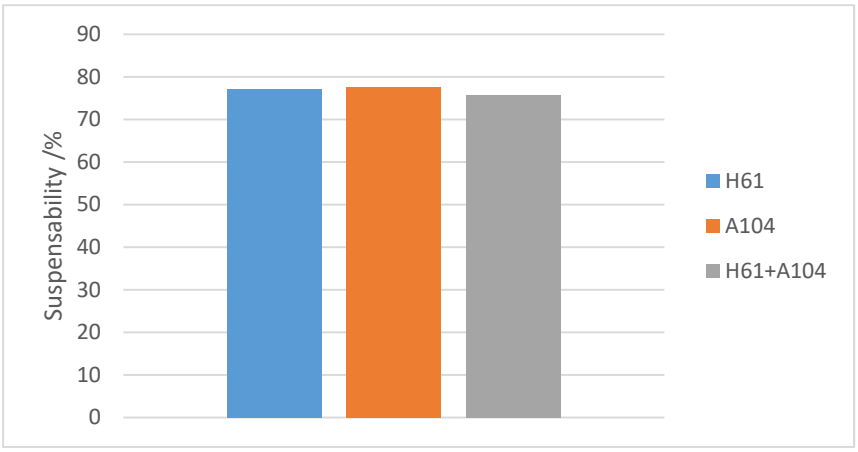

Figure 15: Percentage of suspensibility of H61 with 8.33 g/L, A104 with 1.7 g/L and the combination of both with a total concentration of 10.03 g/L.

H61 and A104 have similar suspensibility as does H61 combined with A104 although this combination has a slightly lower value.

In Figure 16 the percentages of suspensibility of H61 with 8.33 g/L, A123 with 1.7 g/L and the combination of both with a total concentration of 10.03 g/L are represented.

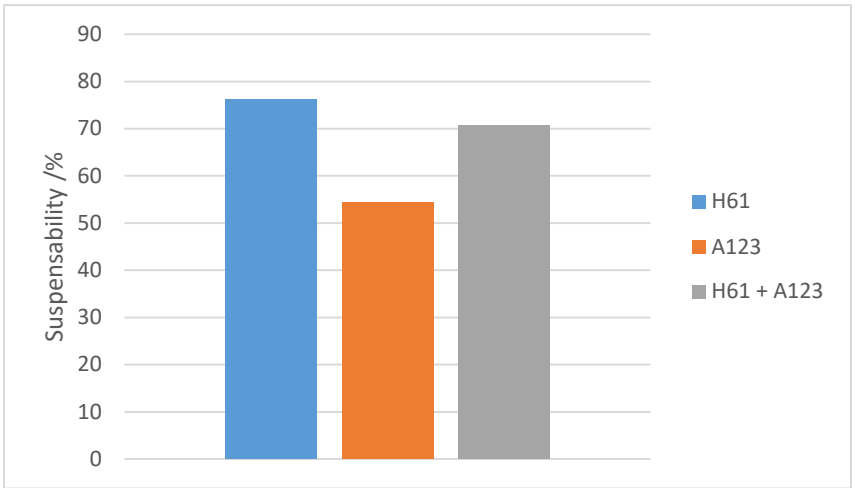

Figure 16: Percentage of suspensibility of H61 with 8.33 g/L, A123 with 1.7 g/L and the both combination with a total concentration of 10.03 g/L.

If H61 is combined with A123 the suspensibility maintains values close to 70 %, being slightly lower than H61 when not combined, but much higher than the A123 when not combined, that is, because the concentration of H61 in the combination is higher than A123 for this reason the suspensability of the combination is similar to the suspensibility of H61.

If H61 with A123 and If H61 with A104 are compared, it can be observed that the suspensibility in both are very similar.

4.3 Concentration analysis

The aim of this section is to analyse the results obtained in the CE to collect information about the percentage of release of the microcapsules by measuring the concentration of copper in water.

4.3.1 Development of buffer for the CuCaps analysis

As no analysis of CuCaps was carried out previously in the CE, it was necessary to develop a new appropriate Buffer to analyse the concentration of copper. Therefore, different methods from literature where selected and tested.

The first buffer, Buffer 1 which was tried, was made up of a solution of 2 mM sodium acetate with a pH of 8.7, the aim being to measure the copper in a complex of pyridine [18]. However, the results were not obtained neither on analysing the complex which should have appeared at a wavelength of 608 nm, nor on measuring the copper alone, which should have appeared at a wavelength of about 240 nm, but there is not an adequate base line and the peaks obtained are abrupt and small. The results are in Figure 17.

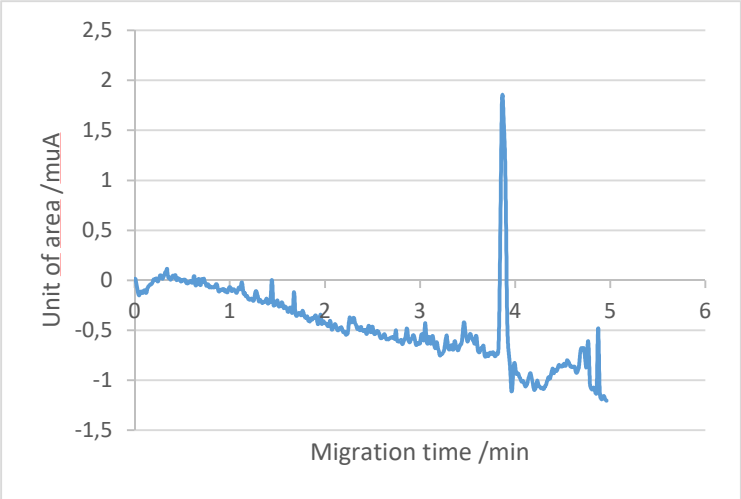

Figure 17: Results of the measurement with Buffer 1 at 240 nm of wavelength.

Therefore, Buffer 2, made up of a solution of 20 mM of phosphate with a pH of 8 was developed. Again, the results obtained were inadequate. Changes to a wavelength of 240 nm were detected, however, there are various peaks but no one defined peak of copper appears. These results could be because phosphate forms an insoluble complex with copper. The results are in Figure 18.

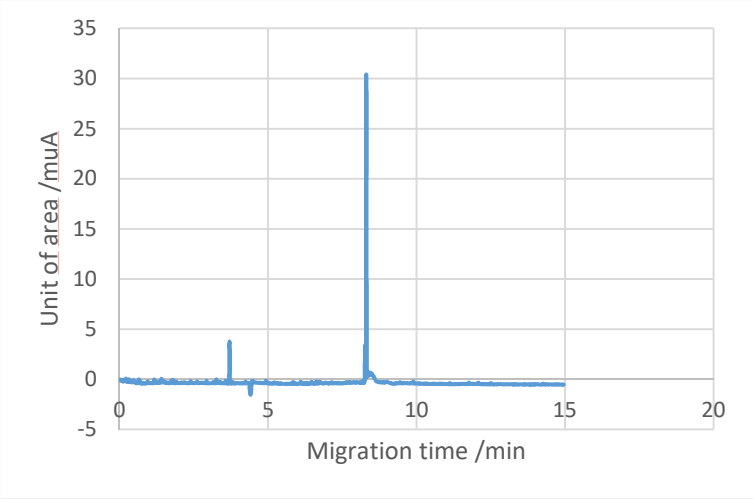

Figure 18: Results of the measurement with Buffer 2 at 240 nm of wavelength.

This lead to the development of Buffer 3, whose results are in Figure 19, made up of acetate with a pH of 8, but this time with a concentration of 20 mM. In this case, similar

results to those of Buffer 2 were obtained but were not useful for the same reasons like Buffer 1.

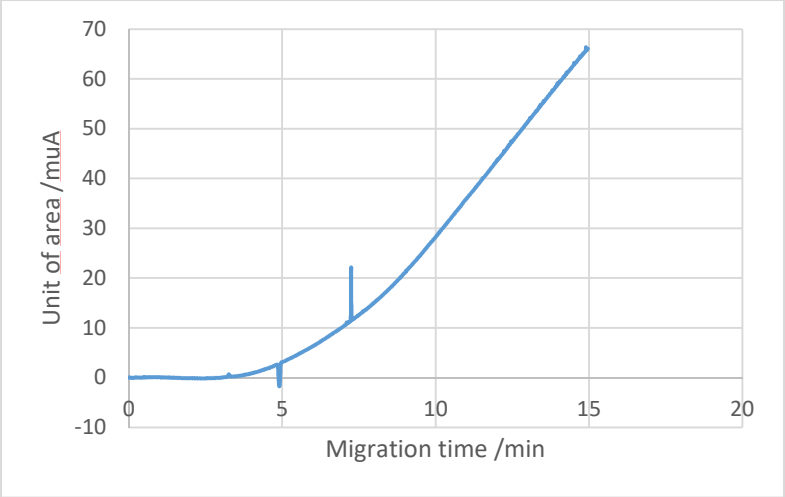

Figure 19: Results of the measurement with Buffer 3 at 240 nm of wavelength.

Due to this, a more acidic pH in the buffer was considered necessary and Buffer 4 had the same composition as Buffer 3 but with a pH of 5. This time, Buffer 4, whose results are in Figure 20, provided well defined copper peaks at a wavelength of approximately 240 nm. For this reason Buffer 4 was used for the measurements.

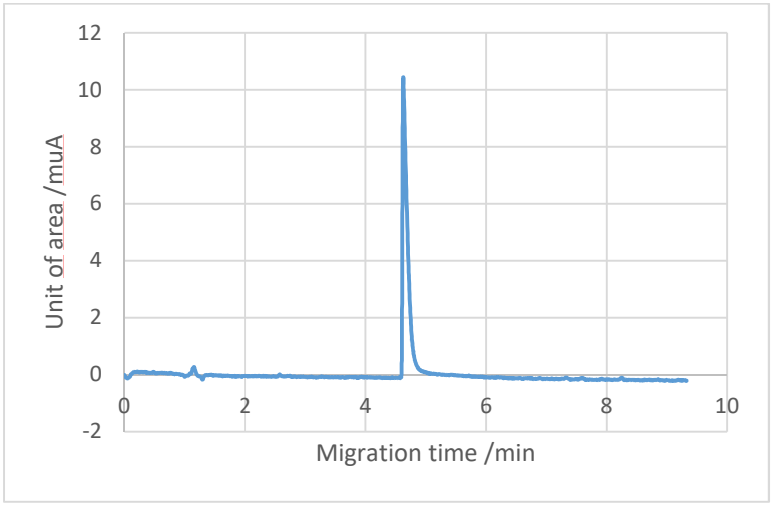

Figure 20: Results of the measurement with Buffer 4 at 240 nm of wavelength.

Lastly, Buffer 5 was developed to analyse the compounds of copper hydroxide which were found to have a low solubility in Buffer 4. Buffer 5 had the same composition as Buffer 4, only with a pH of 3.8 (more acidic) with which the expected results were obtained. For this reason, Buffer 5, whose results are in Figure 21, was used for copper formulas with low solubility in Buffer 4.

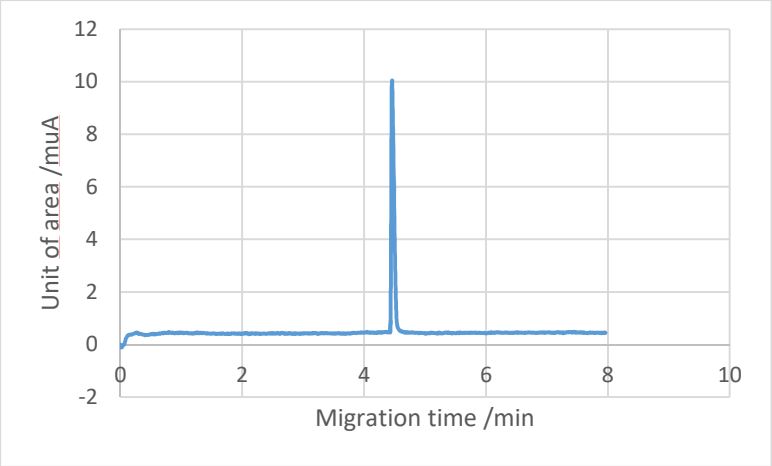

Figure 21: Results of the measurement with Buffer 5 at 240 nm of wavelength.

4.3.2 Calibration with $CuSO_4$ for analysis of CuCaps

Before analysing the microcapsules, two calibrations to measure the copper were carried out. To do so, $CuSO_4$ was used. Samples of five different known concentrations of copper with $CuSO_4$ (0.17 g/L, 0.085 g/L, 0.0425 g/L, 0.0213 g/L y 0.0106 g/L) were prepared.

It was analysed in the CE with Buffer 4 at a ratio of 1:1. For each value of concentration, an area below the copper peak was obtained, this being proportional to the value of the concentration in the samples. Therefore, the higher the concentration, the larger the area below the peak.

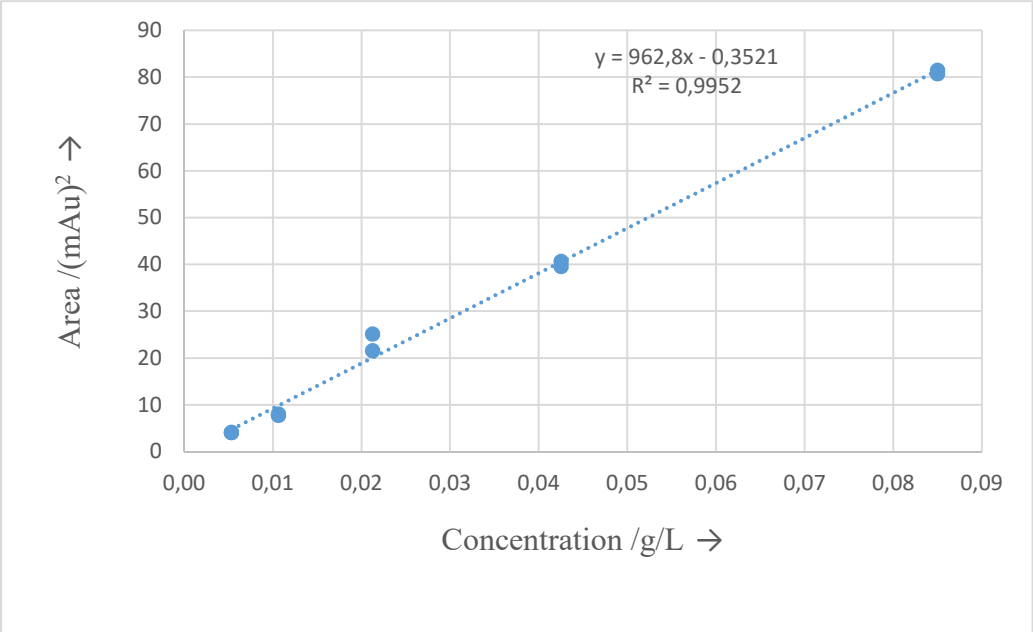

Figure 22: Line of calibration calculated using the values of concentration versus area obtained in the CE.

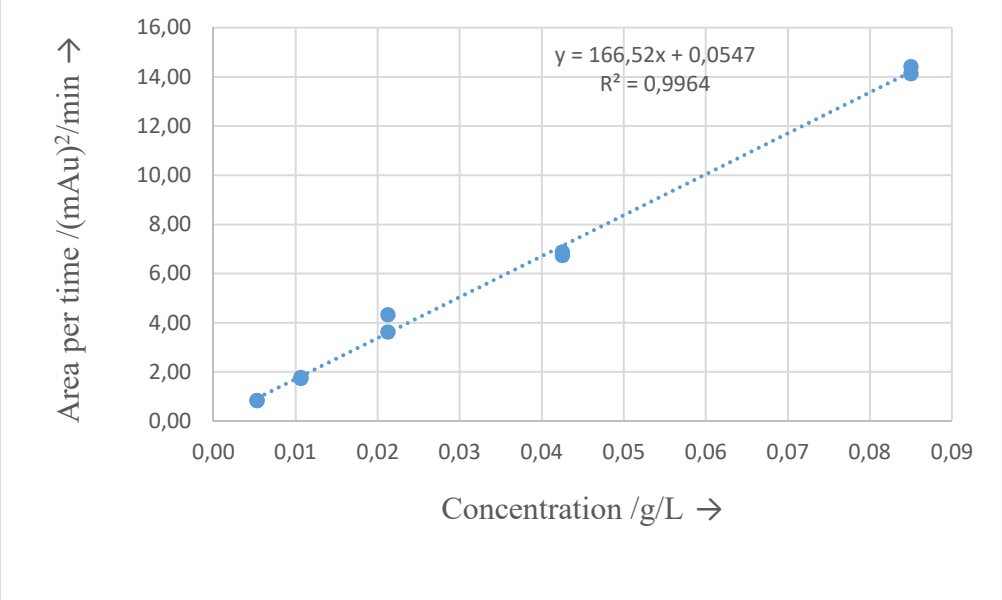

Figure 23: Line of calibration calculated using the values of concentration versus area divided by the time, both having been obtained in the CE.

Two calibrations were carried out to use the calibration whose results were closest to a straight line. Figure 22 represents the first calibration was carried out representing the

area obtained below the peak versus the corresponding concentration of each sample. Figure 23 represents the second calibration was carried out representing the area obtained below the peak divided by the time it took the peak to appear versus the corresponding concentration of each sample. The second calibration, which is in Figure 23, was more precise as the results were close to a straight line, because it considers the migration time, which changes in each measurement and also with the buffer. For higher concentrations the points obtained were closer to a straight line. But for lower concentrations, the points were further away from a straight line so there was an increased margin for error in the measurement of these lower concentration samples. Firstly, this could be due to mistakes when diluting the samples to obtain samples with lower concentration. Secondly, as the mass is lower in these lower concentration samples, when weighing them on the scales, it is easier to make mistakes in lower decimal numbers.

4.3.3 Possibility of calibration with $Cu(OH)_2$

In this section, the aim is to analyse the possibility of carrying out the calibration in the same way as in the previous section but with a different compound which contains copper, like $Cu(OH)_2$. To do this, samples with the same concentration of copper as in the previous section (0.17 g/L, 0.085 g/L, 0.0425 g/L, 0.0213 g/L y 0.0106 g/L,) but with $Cu(OH)_2$ and using Buffer 4 as dissolvent. The next step was to analyse the samples in the CE. The results obtained were the area which corresponded to each concentration. If the results are observed the area is much lesser for the samples of $Cu(OH)_2$ than for those of $CuSO_4$. This could be because the composition in $Cu(OH)_2$ did not completely dissolve in Buffer 4 and therefore part of the copper remained in the microfilter after filtration.

To solve this problem, the next step was to develop a new buffer with a lower pH (more acidic) to improve the solubility of the copper compound. Therefore, Buffer 4 (pH=5) was replaced by Buffer 5 (pH=3.8). To compare the analysis carried out with $Cu(OH)_2$ y $CuSO_4$, all the samples were analysed with Buffer 5 and the same concentration (0.085 g/L), the measurement was repeated three times for each composition.

The results of average and standard deviation of results of $CuSO_4$ samples and $Cu(OH)_2$ samples with Buffer 5 are represented in Figure 24.

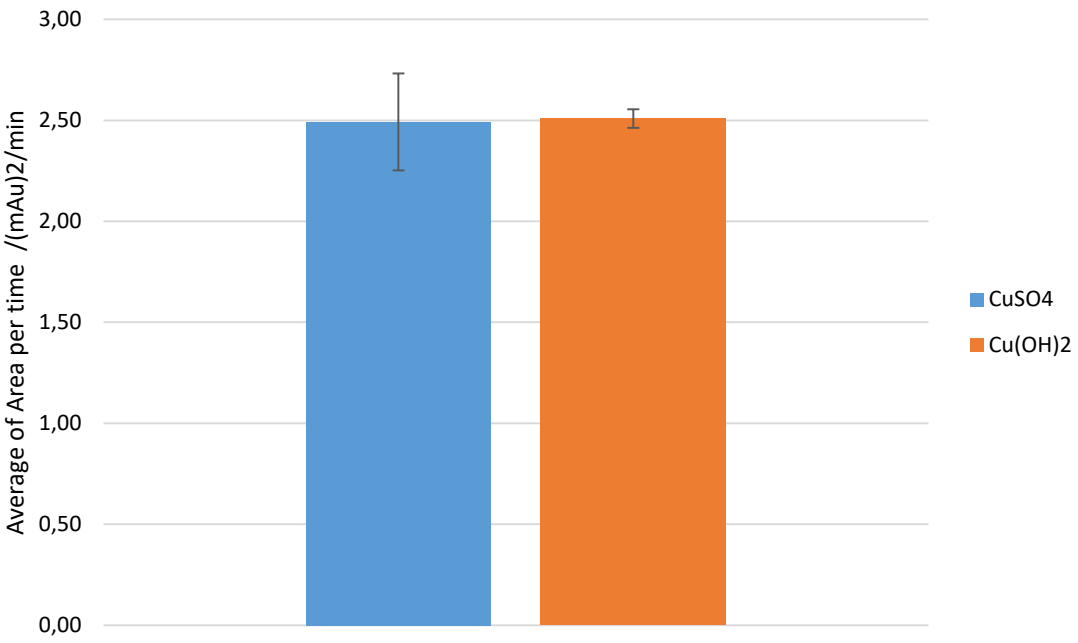

Figure 24: Average and standard deviation of results of $CuSO_4$ samples and $Cu(OH)_2$ samples with Buffer 5.

It can be observed that the results obtained are very similar to each other, showing only a slight deviation and the deviation being lesser for the results obtained from $Cu(OH)_2$ samples. The peaks obtained using Buffer 5 were less defined, the area below the peak being more complex to measure and being more open to errors when obtaining the value of the area.

Therefore, it has been demonstrated that calibration with other compounds of copper is possible, but in the case of $Cu(OH)_2$ as a calibration compound, the quality of the results will be worse due to the possible mistakes made when obtaining the area below the peak. Also, analysis will be done with copper already dissolved in water, so solubility in the buffer will not be a factor.

4.3.4 Release of copper from CuCaps

In this section the aim was to measure the released amount of copper from the CuCaps with CE.

To do so, dispersions of CuCaps A104 in water were prepared using two different concentrations (1.7 g/L and 0.85 g/L). The analysis of the samples was repeated several

times for each concentration. Having found the value of the area below the peak and thanks to the calibration line obtained in section 4.3.2, the concentration was calculated.

The results of the average of concentration obtained and shown in Figure 25:

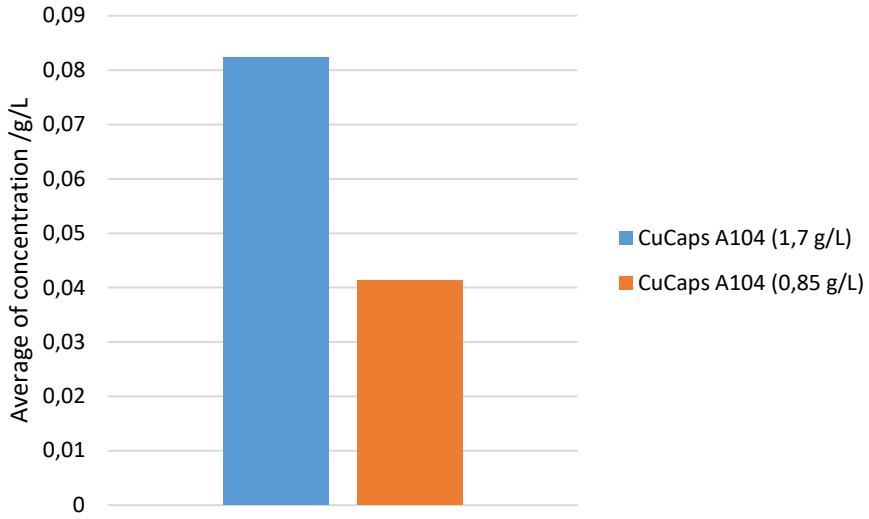

Figure 25: Released amount of copper in water from CuCaps with two different concentrations.

It can be observed that the area obtained for the 1.7 g/L samples of CuCaps is double that of the area obtained in the 0.85 g/L samples of CuCaps. This result was expected. By doubling the concentration, it is to be expected that double the concentration of CuCaps will be released.

The results of percentages of release calculated using the average for the CuCaps with two different concentrations are shown in Figure 26:

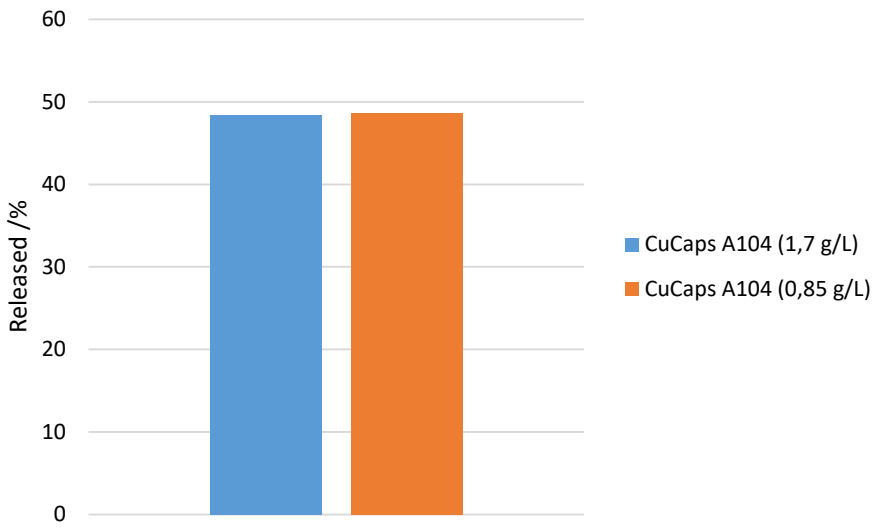

Figure 26: Percentages of release calculated using the average for the CuCaps with two different concentrations.

It can be observed that the percentage of release is similar in both concentration samples, being practically the same.

On trying to carry out the same experiment with a different type of CuCaps, the so-called A123, no results were obtained from these microcapsules. It means that no release could ber measure from the A123 CuCaps, because A123 has only copper ions in form of copper hydroxide, which has a low solubility in water.

4.3.5 Analysis of hop microcapsules and CuCaps combination. Comparison of release using different concentrations

In this section the percentage of release for the following samples will be analysed:

- A104 CuCaps with 1.7 g/L.
- CuCaps and hop microcapsules combination using different concentrations of hop microcapsules (H61).

The aim of this analysis is to see if the variation of copper released increases or decreases when various quantities of hop microcapsules are added.

The combination of CuCaps A104 and hop microcapsules H61 was carried out, maintaining a constant concentration of CuCaps and varying the concentration of H61 in three different concentrations (0.833 g/L, 1.7 g/L and 8.33 g/L). The results are in Figure 27.

The combination of CuCaps A104 with 1.7 g/L concentration and hop microcapsules with 8.33 g/L concentration was used in the field tests.

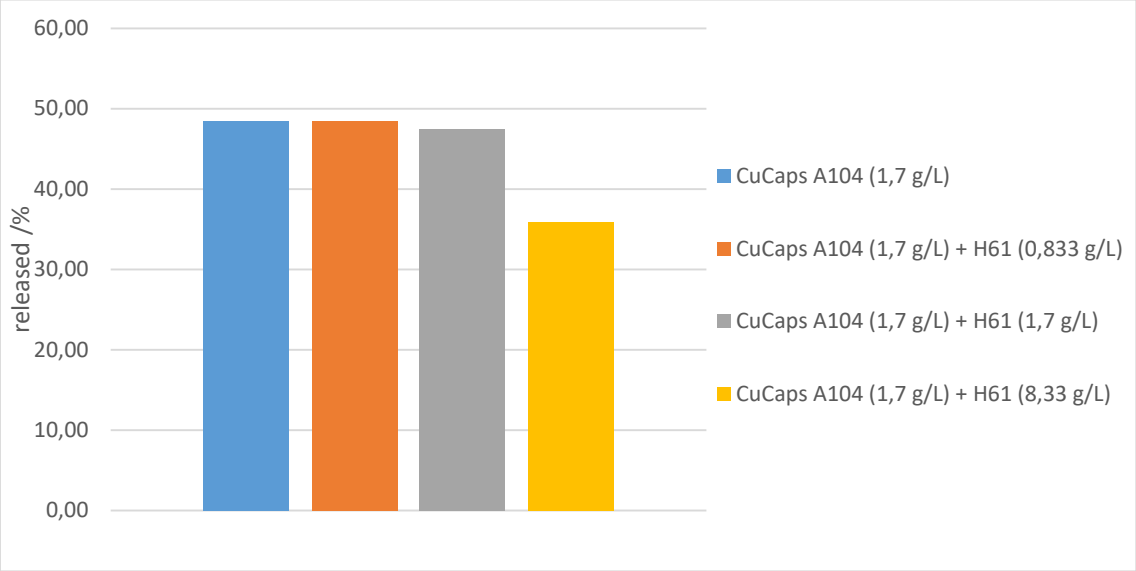

Figure 27: The percentage of release calculated using the average for the CuCaps and CuCaps combined with H61 hop microcapsules in different concentrations.

For A104 alone and A104 with hop microcapsules lower concentrations of H61, it can be seen that the release is similar. But A104 with hop microcapsules of higher concentration has a lower release. This may be due to for two reasons:

- Complex formation between hop actives and copper compounds occurred, as the hop ingredients can chelate with divalent cations.
- Competition between complex with buffer and complex with copper H61.

5 Conclusion

The aim of this thesis was to investigate the product properties of copper microcapsules and their combination with hop microcapsules. The combination is used against Downy Mildew. And by microencapsulation, the accumulation of copper in the soil should be reduced. To investigate the product properties of microcapsules, one type of hop microcapsules (H61) and two types of copper microcapsules (A104 and A123) were produced by spray congealing. Some tests were carried out with these microcapsules to find out their properties.

The analysis of the particle sizes were carried out in the Mastersizer 2000 using a constant rotative speed and ultrasounds. Two types of CuCaps were measured, the A123 and A104 as well as a hop type of microcapsules, H61. Two types of CuCaps combined with H61 in a solution were then analysed. It can be observed, that differences between initial and final values of the sizes exist in all of the microcapsules and combinations. This means that the initial agglomerations are destroyed after applying ultrasound, because particle size is lower than before and remains constant afterwards. The most representative distribution was x_{90} because it presents the most significant agglomerations. It can be observed that all types of microcapsules (A123, A104 and H61) have a similar real size in the last part of the graphs, H61 being initially bigger, because H61 has bigger agglomerations. Also, it can be concluded that A104 and A104 combined with H61 have a similar distribution of sizes during the entire measuring time. And H61 combined with A123 initially has a higher distribution of size in comparison with H61 combined with A104, but in the end the opposite occurred.

The suspensibility of the different microcapsules and their combination was analysed with different concentrations. The suspension using a higher concentration of copper microcapsules has a higher suspensibility than the suspension using a lower concentration. The opposite occurs with the H61, the suspension using H61 with a higher concentration has lower suspensibility due to the formation of agglomerations which increases the speed of sedimentation or the particles form an ascending flow of liquid which causes the particles to take up lower positions and so sediment faster. If H61 with A123 and If H61 with A104 are compared, it can be observed that the suspensibility in both are very similar, although, the suspensibility of A123 is lower.

A new method for copper analysis in the CE was developed, furthermore a buffer and a calibration were also developed. The buffer used is composed of a solution of 20 mM of acetate with a pH of 5.

In the analysis of copper microcapsules, it can be observed that the area obtained for the 1.7 g/L samples of CuCaps is double that of the area obtained in the 0.85 g/L samples of CuCaps, that is, by doubling the concentration, it is to be expected that double the concentration of CuCaps will be released. And the release in both concentrations is practically the same.

No results were obtained from A123 microcapsules, in other words, no release was measured from this type of copper microcapsules, because A123 has only copper ions in form of copper hydroxide, which has a low solubility in water.

The release of copper from microcapsules was measured from the A104 microcapsules and their combination with hop microcapsules H61. A104 alone and A104 combined with a low concentration of H61obtained similar release. But A104 combined with a higher concentration of H61 has a lower release, a decrease in release of copper was expected because of hop-actives form complex with divalent cations. The decrease may be for two reasons: complex formation between H61 and A104 compounds occurred or the complex with buffer and the complex with copper H61 competed with each other.7

Positive or negative effect of combination is not known yet, because it would be necessary to know the results of the field tests.

For future investigations, other types of copper microcapsules should be analysed to measure the release in combination with hop microcapsules. Moreover, other types of hop microcapsules should be combined with copper microcapsules to measure the release to find out the best combination of both types of microcapsules to use in the field.

6 Literature

[1] Hofmann, U. Optimization of downy mildew (Plasmopara viticola) control in organic viticulture with low copper doses, new copper formulations and plant strengtheners, results of 20 years of on farm research [Online]. *Ecoconsultant.* [Access at: June 2018]. Available under: http://www.uwe-hofmann.org/Downy%20mildew%20controlU%20Hofmann.pdf

[2] Sakamoto, K. Konings. W.N. Beer spoilage bacteria and hop resistance. *International Journal of Food Microbiology.* 2003, 105-124. ISSN 302-0106.

[3] Maudes, G. Development of an innovative plant protection product in the treatment against downy mildew aimed to reduce copper accumulation in soil, Master thesis, FriedrichAlexander-Universität Erlangen-Nürnberg, 2014.

[4] Dubey, R. Shami T.C. and Bhasker Rao, K.U. Microencapsulation Technology and Applications. *Defence Science Journal*. 2009, Vol. 59(1), 82-95.

[5] Boso Alonso, S. and Kassemeyer, H.H. Different susceptibility of European grapevine cultivars for downy mildew. 2008, Vol. 47(1), 39–49.

[6] Downy Mildew of grape. *Illinois Commercial Small Fruit and Grape Spray Guide*. 2004, No. 705, P.345.

[7] Reuveni, M. Activity of the new fungicide benthiavalicarb against Plasmopara viticola and its efficacy in controlling downy mildew in grapevines. *European Journal of Plant pathology*. 2003, Vol. 109(3), P. 243-251.

[8] Boso, J.L, Santiago, V. Alonso-Villaverde, P.Gago, M.C. Martinez, H-H. Kassemeyer. (2008) Susceptibilidad a Plasmopara vitiola (Berk. & Curt.) Berl. & de Toni, en diferentes variedades de vid. 34:387-397.

[9] Ash, G. Downy mildew of grape. *The American Phytopathological Society*. 2000 [Access at: June 2018]. Available under: https://www.apsnet.org/edcenter/intropp/lessons/fungi/Oomycetes/Pages/DownyMildewGrape.aspx

[10] Reyes Barrachina, R. Coscollá, V. Badía y F. Fabado. Lucha contra el Oídio y Mildiu de la vid con Estrobilurinas. *Comunitat Valenciana Agraria*. P. 48-58.

[11] Damon Smith. Mildiu de la Vid (Downy Mildew). *Extension*. 2012. [Access at: June 2018]. Available under: http://articles.extension.org

[12] O'Gorman, J. Humphreys, H. Application of copper to prevent and control infection. Where are we now? *Journal of Hospital infections*. 2012, Vol. 81(4), P. 217-223.

[13] Jyothi Sri, S., Seethadevi, A., Suria Prabha, K., Muthuprasanna, P., Pavitra, P. Microencapsulation; a review. *International Journal of Pharma and Bio sciences*. 2002 Vol. 3. P.509-31.

[14] Boh, B., Šumiga, B. Microencapsulation technology and its applications in building construction materials. *RMZ – Materials and Geoenvironment*. 2008, Vol. 55(3), P. 329-344.

[15] Lauer, H.H. and Rozing, G.P. *High Performance Capillary Electrophoresis.* Germany:Agilent Technologies, 2014. ISBN: 5990-3777EN

[16] Martínez García, A. D. Análisis y caracterización de microplásticos presentes en limpiadores faciales y su impacto sobre el medio ambiente, Trabajo Fin de Máster, Escuela Técnica Superior de Ingeniería Industrial (ETSII), 2014.

[17] Malvern Instruments Ltd. *User manual. Mastersizer 2000 essentials*. 2007.

[18] Basheer, C. Lee, H.K. Determination of copper(I) and copper(II) ions after complexation with bicinchoninic acid by CE. *Electrophoresis,* 2007, Vol. 28(19), p. 3520-3525.

[19] Sinnott, R. K., Coulson, J. M., & Richardson, J. F. *Coulson & Richardson's chemical engineering*: 2005, Vol. 6. Oxford: Elsevier Butterworth-Heinemann, P. 237-239. ISBN 0-08-041865-1.