Cannabis Booklet
Theory & Applications

Cannabis Application Notes

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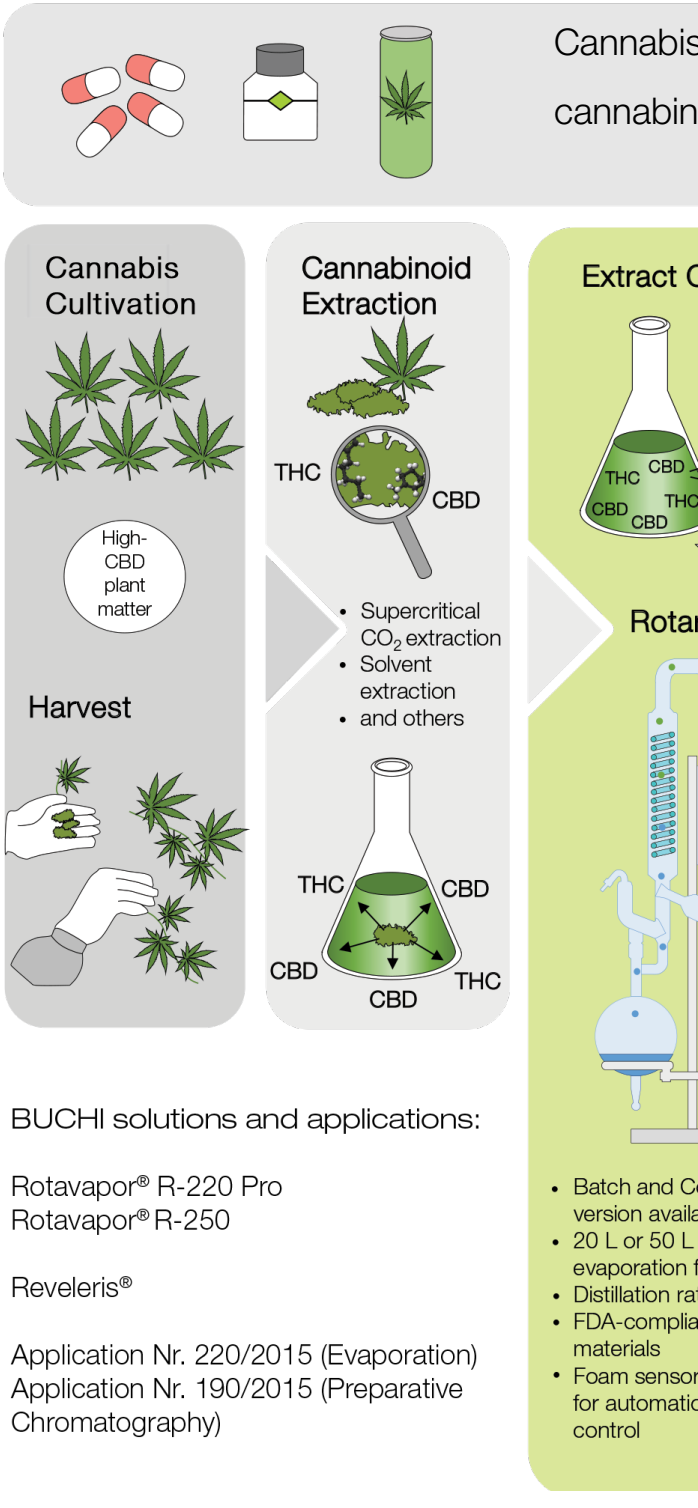
Cannabis

1. Introduction

Cannabis makes the headline of the news these past years. The laws are changing and its decriminalization for medical and recreational use in many parts of the world is opening a new market. Many people have heard of THC (tetrahydrocannabinol), the substance giving their high to the recreational users. Few people are however aware of the hundreds of other cannabinoid chemicals such as CBD (cannabidiol) or terpenes available in the plant, many of which are under pharmaceutical investigation. Cannabis is a bit like a treasure chest of compounds and those who use medical marijuana claim that the herb and its compounds can treat a wide range of conditions such as acute and chronic pain, kidney disorders, Alzheimer's disease, opioid and nicotine dependence or post-traumatic stress disorder.
The cannabis industry needs to determine how the hundreds of chemical components of cannabis can be developed into licensed pharmaceutical drugs in order to treat some of the above mentioned diseases. In order to do so, the active compounds need to be isolated and investigated. Through its diverse portfolio of instruments and its profound application knowledge, BUCHI can offer the cannabis industry instruments for a smooth cannabis production workflow together with starting parameters and typical applications for the different production steps used up to the end product.

This booklet aims to summarize the different process parameters achievable using BUCHI instruments. It contains starting points for typical applications used at different production steps in the cannabis industry. Those parameters are starting points and should be subject to optimization for more effective results.



BUCHI solutions and applications:

Rotavapor® R-220 Pro
Rotavapor® R-250

Reveleris®

Application Nr. 220/2015 (Evaporation)
Application Nr. 190/2015 (Preparative Chromatography)

s products with
oids (e.g. CDB)

Cannabis contains > 80 Cannabinoids
Therapeutic effects include
- Inflammatory diseases (e.g. MS)
- Degenerative illnesses (e.g. Parkisons)
- Diminish side effects (e.g. of chemotherapy)

Tetrahydrocannabinol (THC)
- Psychoactive

Cannabidiol (CBD)
- Non-psychoactive

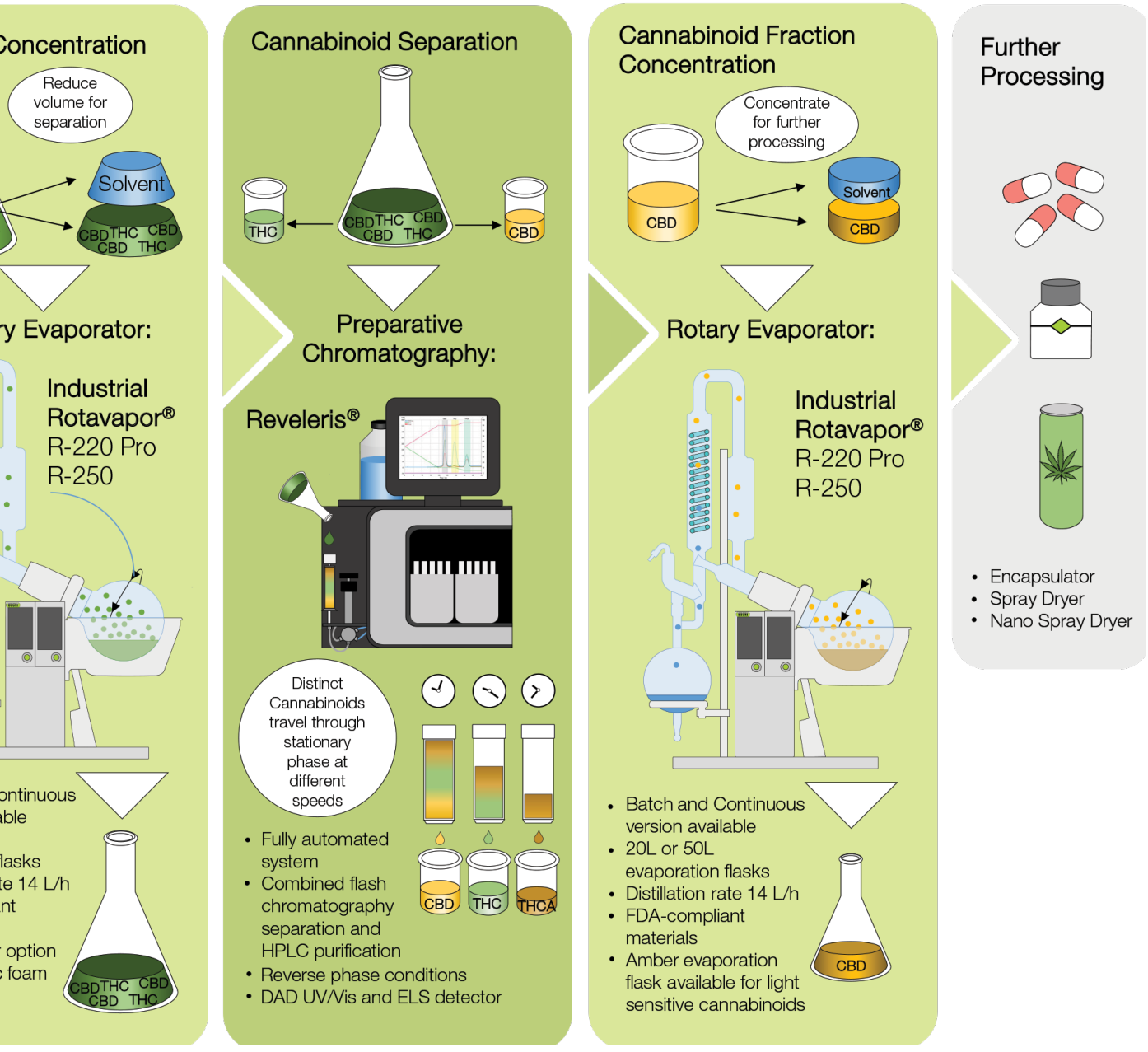


Fig.1.: Optimal solutions for convenient cannabis processing

Application notes

Industrial Rotavapor® solutions for cannabis products
Post chromatography solvent concentration

1. Introduction

Aim: Concentration of purified CBD by solvent evaporation
Use: Product concentration before formulation
Why: The large amount of ethanol present as solvent from the chromatographic fractions needs to be evaporated to obtain concentrated purified CBD
How: Industrial Rotavapor® R-220 Pro

2. Equipment and Chemicals

· Industrial Rotavapor® R-220 Pro
· F-325 Chiller
· Vacuum Pump V-600

· CBD fraction from purification process

3. Procedure

Fill the evaporating flask with 10 L of product and install the flask at the Rotavapor®, or mount the flask, establish a weak vacuum of about 800 mbar and then feed the evaporating flask with 10 L of product via the feeding-valve. Set a heating bath temperature of 60 °C and start heating. Set the working pressure at 150 mbar and the chiller at a temperature of 10°C (if tap water is used, ensure a sufficient water flow of 120 - 150 L/h). Lift up the heating bath completely and start the rotation at a set speed of 150 rpm.
If needed, the performance can be adjusted by increasing the bath temperature.

4. Results

The Rotavapor® R-220 Pro enables a reduction of supervison on the device to a minimum since it automatically fills and drains the system. An evaporation rate of 8.5 L/h can be expected for this process, allowing for drying of large quantities of refined cannabinoids into dry mass with relative ease and speed.

5. Conclusion

The BUCHI Industrial Rotavapor® line has already been proven to be the ideal solution for the concentration of CBD solution after separation by liquid chromatography. This is due to the low required investment and low running costs. The Rotavapor® R-220 is successfully applied in the process and production work-flow of cannabis product for both extract and fraction concentration.

Industrial Rotavapor® solutions for cannabis products
Post extraction solvent concentration

1. Introduction

Aim: Concentration of cannabinoids by solvent evaporation
Use: Extraction and winterization of cannabis oil with 96 wt. % ethanol is commonly performed on an industrial scale. Before performing chomatography, it is advantageous to reduce the amount of solvent thus concentrating the cannabis oil.
Why: A large amount of solvent needs to be evaporated to obtain concentrated cannabinoids prior to using chromatography for cannabinoid isolation. Concentration of the crude oil ensures consistent reproducible isolation of cannabinoids.
How: Industrial Rotavapor® R-220 Pro

2. Equipment

· Industrial Rotavapor® R-220 Pro:
· With foam sensor
· With descending glass assembly
· F-325 Chiller

3. Chemicals

Diluted solution of cannabis oil in ethanol from solvent extraction or winterization process

4. Procedure

Fill the evaporating flask with 10 L of product and install the flask at the Rotavapor®, or mount the flask, establish a weak vacuum of about 800 mbar and then feed the evaporating flask with 10 L of product via the feeding-valve. Set a heating bath temperature of 60 °C and start heating. Set the working pressure at 150 mbar and the chiller at a temperature of 10°C (if tap water is used, ensure a sufficient water flow of 120 - 150 L/h). Lift up the heating bath completely and start the rotation at a set speed of 150 rpm.
Very soon the distillation will start. If needed, the performance can be adjusted by increasing the bath temperature.
Foam formation under vacuum can either be manually interrupted by pressing the 'Aearate' button in short steps to let the foam collapse or by using the foam sensor which will automatically resolve foaming with short aeration impulses when needed.

5. Results

The Rotavapor® R-220 Pro is an ideal solution to concentrate cannabinoid extract. An evaporation rate of 8.5 L/h can be expected for this process, allowing the evaporation of a large amount of solvent with relative ease and speed. The solvent can be collected and reused for a subsequent extraction process.
Additionally, time consuming monitoring of the process and cleaning of the glass assembly can be reduced thanks to the possibility to use an automated foam sensor.

Cannabis products of interest are light-sensitive and can be protected by amberized evaporating flasks. Such flasks are optionally available.

6. Conclusion

The BUCHI Industrial Rotavapor® line has already been proven to be the ideal solution for solvent evaportation in the cannabis industry. This is due to the low required investment and low running costs. The Rotavapor® R-220 Pro is successfully applied in the process and production work-flow of cannabis product for both extract and fraction concentration.

Purification of cannabinoids
Preparative chromatography with Sepacore® X50

1. Introduction

Aim: Purification of cannabinoids
Use: To produce THC free products, pure CDB or pesticide free extracts
Why: To fit regulations from authorities, to produce high quality material
How: preparative chromatography with Sepacore® X50

2. Equipment

· Sepacore® Flash System X50 (11058260)
· Funnels and hoses to connect with tanks
· V-Stand (048891)
· Cartridge holder XL (11065862)
· Additional accessories
· C-601 pump (054101) with C-610 pump controller (054111)
· ELSD C-650 (11059106)

3. Chemicals

· Hemp extract after extraction, winterization, nanofiltration
· Ethanol 96%
· Water
· FlashPure EcoFlex C18 cartridge 1500 g (140000057) or 3000 g (140000058)

4. Procedure

The hemp extract from the extraction and prepurification is dissolved 1:1 with ethanol. For chromatographic separation use the parameters from the table below.

Cartridge	1500 g	3000 g
Flow rate	140 mL/min	210 mL/min
Eluent A	Water	Water
Eluent B	Ethanol	Ethanol
Separation time and Gradient	0 – 60 min 70 – 90% B	0 – 60 min 70 – 90% B
UV Wavelength	230 nm 256 nm	230 nm 256 nm

Depending on the requirements of your separation and the composition of your extract the gradient, separation time and the sample loading can be different.

The cartridge should first be equilibrated with the starting mobile phase for 2 column volumes. After equilibration the sample is loaded on the cartridge using a syringe or an external sample

pump. The separation using the gradient from table 1 is started. Desired fractions can be collected by a threshold of the UV or by time (program collection).
After the separation we need to remove the solvent. For this step use the application for post chromatography evaporation (page 7).

After every 5-8 runs the cartridge should be flushed with acetone to clean the cartridge and to extend its lifetime. Under certain conditions cartridges can be used for up to 200 runs.

5. Conclusions

The separation of cannabinoids can be done by preparative chromatography on Sepacore® X50. Cannabinoids can be separated in order to be used as pure substances, THC can be removed from hemp extract or the extract can be purified from substances like pesticides. This can all be done in only one separation step. The advantage of Sepacore® is the modularity of the system. We can increase the flow rate up to 500 mL/min for gradients and up to 1000 mL/min for isocratic separations.

Purification of cannabinoids
Preparative chromatography with Reveleris® X2

1. Introduction

Aim: Purification of cannabinoids
Use: To produce THC free products, pure CBD or pesticide free extracts
Why: To fit regulations from authorities, to produce high quality material
How: preparative chromatography with Reveleris® X2

2. Equipment

· Reveleris® X2 (145164931)
· Rack for squared bottles 480 mL (145148873)
· Squared bottles (148623412)
· Dry air supply (145168621) or connection to pressurized air
· V-Stand (048891)
· Cartridge holder XL (11065862)
· Bypass column kit (145164530)
· Additional accessories
· Reveleris® sampling pump (145176861)

3. Chemicals

· Hemp extract after extraction, winterization, nanofiltration
· Ethanol 96%
· Water
· FlashPure EcoFlex C18 cartridge 1500 g (140000057) or 3000 g (140000058)

4. Procedure

The hemp extract from the extraction and prepurification is dissolved 1:1 with ethanol. For chromatographic separation use the parameters from the table below.

Cartridge	1500 g	3000 g
Flowrate	140 mL/min	200 mL/min
Eluent A	Water	Water
Eluent B	Ethanol	Ethanol
Separation time and Gradient	0 – 60 min 70 – 90% B	0 – 60 min 70 – 90% B
UV Wavelength	230 nm 256 nm	230 nm 256 nm

Depending on the requirements of your separation and the composition of your extract the gradient, separation time and the sample loading can be different.

The cartridge should first be equilibrated with the starting mobile phase for 2 column volumes. After equilibration the sample is loaded on the cartridge using a syringe or an external sample pump. The separation using the gradient from table 1 is started. Desired fractions can be collected by a threshold of the UV and / or ELSD signal or by time (program collection).
After the separation we need to remove the solvent. For this step use the application for post chromatography evaporation (page 7).

After every 5-8 runs the cartridge should be flushed with acetone to clean the cartridge and to extend its lifetime. Under certain conditions cartridges can be used for up to 200 runs.

5. Conclusions

The separation of cannabinoids can be done by preparative chromatography on Reveleris® X2. Cannabinoids can be separated in order to be used as pure substances, THC can be removed from hemp extract or the extract can be purified from substances like pesticides. This can all be done in only one separation step. The Reveleris® software is easy to use and the integrated ELSD detector shows more intensive signals than UV detectors for cannabinoids.

Microencapsulation with Encapsulator B-390
Formulation of CBD into gelatin beads

1. Introduction

Aim: Formulation of CBD into gelatin beads
Use: Edibles and medication
Why: Taste masking, better compliance, easier handling, controlled release
How: Microencapsulation with Encapsulator B-390

2. Equipment

· Encapsulator B-390 (11058210)
· Single nozzle set (11058051)
· Rotavapor® R-300
· Interface I-300 Pro
· Vacuum Pump V-300
· Recirculating Chiller F-305
· 0.5 L BUCHI drying beaker (034767)

3. Chemicals

· CBD from purification process
· Gelatin (Sigma Aldrich G-1890)
· Titanium dioxide (TiO_2)
· Miglyol 812 neutral oil

4. Experimental

A 25% solution of gelatin is prepared by dissolving 50 g of gelatine in 150 g of distilled water heated at 50 °C. 2.5 g of CBD and 2.3 g of TiO_2 were then mixed under agitation in 80 g of the gelatin solution. Soft gelatin beads containing CBD were produced by prilling the solution into a cold Miglyol bath following the parameters below:

	Encapsulation parameters
Gelatin concentration	25%
Gelling medium	Cold (10°C) Miglyol
B-390 Heater temperature	70 °C
Nozzle size	450 µm
Pressure	500 mbar
Frequency	250 Hz
Electrode	1200 V
Estimated flow rate	10 mL/min

Table 1: Encapsulation overview

Pump the gelatin solution, which is kept at 60 – 65 °C (by placing the bottle containing the solution into a water bath), through the heated nozzle to form a liquid jet, which is broken up into droplets by the vibrational frequency. The produced droplets will land into the cooling oil bath below, which should be agitated at a high speed (vortex size > 2 cm) using a magnetic stirrer. The distance between the surface and bottom of the cooling oil should be at least 10 cm to allow the gelatin bead to harden sufficiently before hitting the bottom of the vessel used to hold the cooling oil.

Allow the gelatin beads to harden for 30 min in the cooling oil and make sure this liquid remains below 10 °C during the production process. Remove excess oil by filtering and/or with a paper towel.

Store soft gelatin beads in an airtight container.

If necessary, the gelatin beads can be either dried over a period of 24 - 48h by leaving to air-dry at ambient conditions or within 8 - 24 h using a BUCHI Rotavapor® to evaporate the water. During the drying, beads should be constantly rotated to prevent sticking and clumping. To dry gelatin beads using the BUCHI Rotavapor® R-300 system with Interface, Pump and Chiller, sieve the beads and remove as much of the oil as possible using a paper towel. Place the beads in a 0.5 L BUCHI drying beaker with a paper towel (it helps to absorb the remaining oil) and start the drying process. Beads are dried for 24h at room temperature, low rotation speed and high vacuum (20 mbar).

4. Results

Parameters described in Table 1, a stable chain of droplets can be observed (Figure 1).

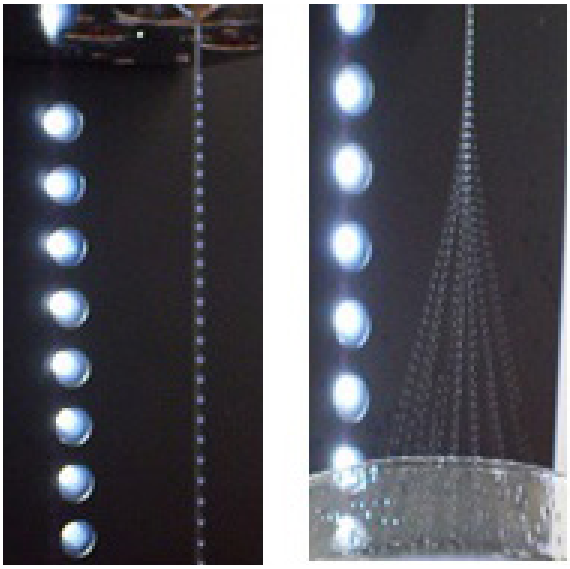


Figure 1: Stable chain of droplets consisting of an emulsion of oil and gelatin.

Capsule diameter: 800 um
CBD Oil loading: 10%
Encapsulation efficiency: > 95%

5. Conclusion

The formation of gelatin microbeads for the encapsulation of hydrophilic materials in a single step process is possible using the Encapsulator B-390. The heated nozzle helps maintain the temperature above the solidification point of gelatin. Different nozzle sizes available for the Encapsulator, the bead size can be chosen in the range of 200 - 2000 µm.

Microencapsulation with Encapsulator B-390/B-395 Pro
Formulation of CBD into soft oil core capsules

1. Introduction

Aim: Formulation of CBD into soft oil core capsules
Use: Edibles and medication
Why: Taste masking, better compliance, easier handling, controlled release medication
How: Microencapsulation with Encapsulator B-390/B-395 Pro

2. Equipment

· Encapsulator B-390 (11058210) with external syringe pump (11063610) or
· Encapsulator B395 Pro (11058220)
· Concentric nozzle set (11058051)

3. Chemicals

· CBD from purification process
· Calcium chloride dihydrate
· Sodium alginate, low viscosity grade, BUCHI (11058394)
· Miglyol 812

4. Procedure

Dissolve CBD in Miglyol at a concentration of 100 mg/mL and follow parameters in the table below:

	Encapsulation 1	Encapsulation 2
Alginate concentration	2%	2%
Calcium chloride concentration	0.1 M	0.1 M
Shell nozzle	400 µm	400 µm
Core nozzle	150 µm	300 µm
Pressure	500 mbar	500 mbar
Frequency	600 Hz	680-720 Hz
Electrode	2000 V	2500 V
Oil flow rate (syringe pump)[a)]	1.5 mL/min	4-6 mL/min
Estimated shell flow rate	10 mL/min	10 mL/min

a) for an accurate oil flow rate, the pump of the Encapsulator B-395 Pro needs to be calibrated before use. In case of an external syringe pump, please verify if calibration is required and react accordingly.

The core and the shell should be first pumped into the tubing until they reach the entry of the vibrating chamber. The shell polymer (alginate) should then be pumped through the shell nozzle and the parameters should be set up to obtain a stable droplet chain. The pumping of the oil through

the core nozzle can then begin. Slight adjustments to the set up values of both flow rates will finally be required to obtain a stable chain of mono-centric droplets which produce the liquid-core microcapsules.
The % of oil within the capsules can be calculated as followed:

%oil = Vm/Vc x 100 with the total volume of the capsule (Vc) and the volume of the oil core (Vm).

5. Results

Parameters described in Table 1, a stable chain of droplets consisting of an oil core envelopped within an alginate shell can be observed (Figure 1).

Encapsulation 1:
Capsule diameter: 800 um
Oil loading: 15%
Encapsulation efficiency: > 95%

Encapsulation 2:
Capsule diameter: 800 um
Oil loading: 50%
Encapsulation efficiency: > 95%

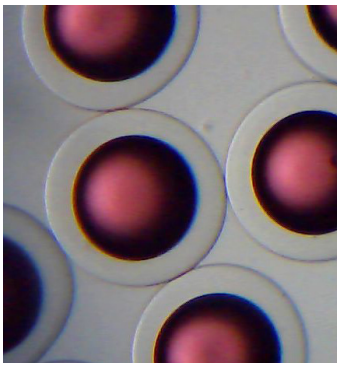


Figure 1: Microcapsules obtained with the parameters of encapsulation 2. Microcapsule diameter is approximately 800 µm and oil loading is around 50%. Red color was added to the oil for visual purposes.

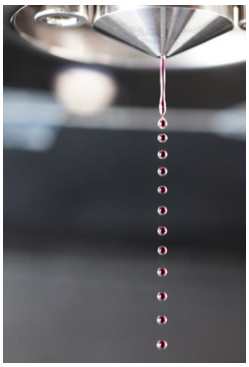


Figure 2: Stable chain of droplets consisting of an oil core and alginate shell.

6. Conclusion

The formation of core-shell capsules using the Encapsulator is straight forward and user friendly, it can be done with a high number of hygroscopic liquids and shell materials. Core loading of up to 50% can be reached with the different nozzle combinations and the capsule size can be chosen between 400 and 2200 µm. Capsules can remain in a wet state or be dried further using a drying technique. Whether capsules remain wet or are dried afterwards, encapsulation enables an easier handling of the oil containing the CBD, improving therefore patient compliance.

Spray Drying of a solution of CBD and polylactic polymer
Formulation of CBD into an inhalable dry powder

1. Introduction

Aim: Formulation of CBD into an inhalable dry powder
Use: Dry powder administration to lungs
Why: Direct delivery to the blood without injecting, even for protein-like actives
How: Spray Drying of a solution of CBD and polylactic polymer

2. Equipment

· Mini Spray Dryer B-290 Advanced (044699) with outlet filter (044754)
· Inert Loop B-295 (044701)
· Two fluid nozzle (0.7 mm / 1.4 mm)
· High performance cyclone (046368)

3. Chemicals

· CBD from purification process
· PLGA - Resomer R 202 H (Evonik Rohm GmbH, Essen, Germany)
· Dichloromethane (DCM)

4. Procedure

Prepare a 10% PLGA solution by disolving 4.2 g of PLGA into 38 g of DCM. Add 0.47 g of purified CBD into the PLGA solution and follow parameters in table below for the spray drying process:

Inlet temperature [°C]	45
Outlet temperature [°C]	26
Aspirator [%]	100
Pump speed [%]	30
Spraying air flow [mm]	50
Spraying gas	N_2
Cyclone	High performance[a)]
Inert Loop settings	-20°C

a) The high performance cyclone is used to improve collection of small particles and increase recovery yields.

In order to start the spraying process, select the parameters in the table above and preheat the spray dryer. Start spraying pure solvent. The spray cone should be symmetrical and located in the axis of the spray cylinder. Slight adjustments to the set up values maybe required. The spray quantity of pure solvent can be changed by changing the settings of the peristaltic pump. The spray flow strongly influences the outlet temperature, since the solvent draws energy from evaporation.

The outlet temperature can be regarded as the upper thermal load of the product, so make sure that the product is not damaged by an excessively high outlet temperature. As soon as the desired operating conditions have been achieved and are stable, change the feed tube from pure solvent to the prepared feed solution.

5. Results

With the parameters described in Table 1, a nice spray cone was observed and a dry powder was easily obtained in the collection vessel.

Particle size: 1-8 um (ideal for lung delivery)
Particle shape: spherical
CBD loading: 10% [w/w]
Yield: 36%

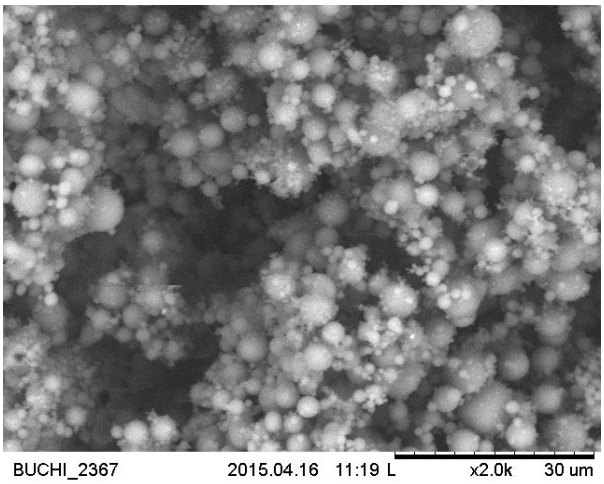


Figure 1: Spray dried powder containing 10% [w/w] CBD.

6. Conclusion

The preparation of an inhalable powder containing CBD can easily be done through spray drying in a one-step process. The use of PLGA as carrier enabled the production of uniform and stable encapsulated CBD for slow release formulations.

Spray Drying of an emulsion
Formulation of CBD into eadible powder

1. Introduction

Aim: Formulation of CBD into eadible powder
Use: Edibles
Why: Taste masking, easier handling, controlled release medication, increase shelf life
How: Spray Drying of an emulsion

2. Equipment

· Mini Spray Dryer B-290 Basic (044780) with outlet filter (044754)
· High performance cyclone (046368)
· Dehumidifier B-296 (optional) (040188)

3. Chemicals

· CBD from purification process
· Miglyol 812
· Maltodextrin DE 18-20
· Gum arabic

4. Procedure

Dissolve CBD in Miglyol at a concentration of 100 mg/mL. Prepare an emulsion of 5g oil, 10g of maltodextrin and 5g gum arabic (1:2:1) in 100 mL water using a high sheer mixer and follow parameters in table below for the spray drying process:

Inlet temperature [°C]	150
Outlet temperature [°C]	73
Aspirator [%]	100
Pump speed [%]	15
Spraying air flow [mm]	35
Spraying gas	Air
Cyclone	High performance[a)]
Accessories	Dehumidifier B-296[b)]

a) The high performance cyclone is not mandatory. It can be used to improve collection of small particles and increase recovery yields.
b) The dehumidifier B-296 is not mandatory in this process. It can be used to remove humidity from the drying air when ambiant humidity is too high, resulting in a not properly dried product.

In order to start the spraying process, select the parameters in the table above and preheat the spray dryer to the specified inlet temperature. Start spraying pure solvent. The spray cone should be symmetrical and located in the axis of the spray cylinder. Slight adjustments to the set up values can be required. The spray quantity of pure solvent can be changed by changing the settings of the peristaltic pump. The spray flow strongly influences the outlet temperature, since the water draws energy from the air by evaporation.
The outlet temperature can be regarded as the upper thermal load of the product, so make sure that the product is not damaged as a result of an excessively high outlet temperature. As soon as the desired operating conditions have been achieved and are stable, change the feed tube from pure solvent to the prepared feed solution.

5. Results

With the parameters described in Table 1, a nice spray cone was observed and a dry powder was easily collected in the collection vessel.

Particle size: 2-15 um
CBD loading: 2.5% [w/w]
Yield: 28%

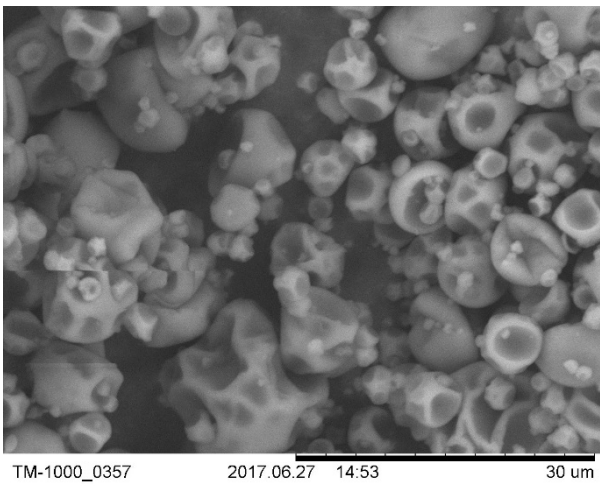


Figure 1: Spray dried powder containing 2.5% [w/w] CBD.

6. Conclusion

The production of an edible powder containing CBD can easily be produced through spray drying. The use of maltodextrin and gum arabic helps with taste masking of the oil and therefore makes it possible to disperse the powder in different types of food.

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