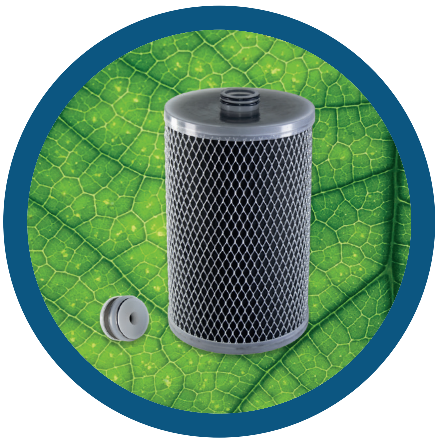
APPLICATION NOTE
#EP006-0

SiliaMetS® E-PAK®



Using E-PAK technology to remove chlorophyll in a biomass sample

The following study demonstrates the efficiency of E-PAK activated carbon cartridges to remove chlorophyll and other impurities from a biomass sample. Cannabis was herein chosen as the model sample.

The variables analyzed after a pre-purification step were:

- **Recovery of cannabinoids (*CBD*);**
- **The signal level of the chlorophyll by UV-visible spectroscopy (*qualitative test*);**
- **The saturation of the E-PAK cartridge.**

Using E-PAK technology to remove chlorophyll in a biomass sample

EXPERIMENTAL METHOD

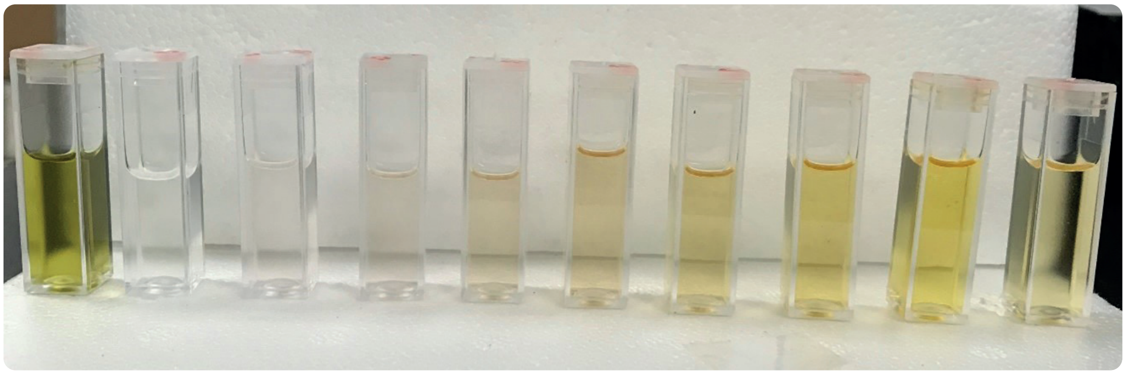
The extraction of the crude from the biomass was performed with ethanol at room temperature and at -20°C to obtain the samples for testing. Usually, extractions are done below freezing temperature to get cleaner extracts. In this experiment, an extraction at room temperature was done in order to test the efficiency of E-PAK for the removal of chlorophyll at a higher concentration.

Screening static tests (*bulk carbon suspended in ethanoic crude*) were first carried out with activated carbon at a 1:1 (*w/w*) ratio. A significant improvement in the appearance of the extract was obtained, changing from a dark cloudy solution to a clear orange color. Considering the objective was to approach large-scale conditions, subsequent tests were then carried out under dynamic conditions.

Out of the different types of carbon initially tested, Silia*Carb* HA Activated Carbon gave the best results. Therefore, E-PAK Silia*Carb* HA cartridges (*5 × 1 cm, PN: LS-CHA-51*) were chosen for the remainder of the dynamic tests. Prior to each purification process, the cartridge was conditioned with 3 column volumes, i.e. 150 mL of ethanol. Then, 350 mL of extract was passed through the cartridge. The flow rate per run was set to 7.5 mL/min to ensure optimum contact time and with a residence time of 2.5 min. Once a run was completed, the extract was collected and analyzed for its total solids, as well as the cannabinoid and chlorophyll contents (*qualitative analysis*). Using the same cartridge, this process was then repeated with new extract samples up to 8 times. The experiments were performed with solutes that were extracted at either -20°C or at room temperature to test the influence of the contaminant concentration. For extractions done on samples at -20°C, CBD was found to be less soluble getting a less concentrated solution of CBD (*1.32 mg/mL*) than at room temperature (*2.03 mg/mL*) but with lower amounts of impurities present with 0.3074 and 1.580 UV absorption, respectively.

A qualitative analysis with a UV spectrophotometer was carried out to ensure a follow-up of the chlorophyll during the passages on the E-PAK Silia*Carb* HA. The maximum absorbance wavelength of chlorophyll is 665 nm in ethanol.[1] This was validated with the UV analysis of the crude extract, showing maximum absorbance at 666 nm. A picture of the different extracts was taken showing the color evolution through the different runs (*Figure 1*).

Figure 1: Successive passages of cannabis extracts (*done at room temperature*) through E-PAK cartridge.



Each cuvette corresponds to 350 ml of extract (*15 g/L solutes*) passed through a Silia*Carb* HA Activated Carbon cartridge. From left to right: 1) extracted crude, 2) ethanol solvent, and from 3) to 10) runs 1 through 8 respectively.

RESULT

The results show that the E-PAK purification of the cannabis samples allowed to retain chlorophyll significantly. A substantial drop in the UV signal was observed compared to the crude extract.

Since the cartridge was not saturated after a single treatment (*even with highly contaminated extracts such as those obtained after extraction at room temperature*), it had been possible to easily purify several extract samples using the same E-PAK cartridge. The limiting factor for reusing cartridges is the pressure required to maintain a constant flow. It was found at the industrial scale testing that 500 L of extract could be passed through the 16.5 × 100 cm cartridge without having to increase the inlet pressure. It would also be possible to double this volume (*therefore 1000 L*), by increasing the pressure.

In respect to CBD, a very high recovery rate after each successive run was achieved, demonstrating that there is neither retention nor degradation on the cartridge. For the first extract, a recovery of only 50 % was obtained. This is explained by the fact that the activated carbon / extract ratio is very high for the first filtration, which leads to the retention of certain cannabinoids. However, the fact that the yields are higher than 100 % for the following runs demonstrates that the cannabinoids initially retained on the cartridge are released in subsequent runs. (*See Table 1*).

An average CBD yield of up to 98 % was obtained for the purification of extracts generated at room temperature although containing a higher concentration of impurities (*Table 1*). It is also consistent with the increase number of recirculations required (*greater than 6 runs*).

The end of life of the E-PAK cartridge is generally characterized by a gradual decrease and / or fluctuation in the flow. In addition, the appearance of a whitish / yellowish deposit on the carbon was observed once removed from its support (*Figure 2*).

Figure 2: E-PAK cartridge before (*left*) and after treatment (*right*).

Table 1: Purification results with UV (*665 nm*) of ethanoic crude on Silia*Carb* HA (*1/1*) obtained at -20°C and room temperature (*r. t.*) with average yield after CBD purification.

Purification results with UV (*665 nm*) of ethanoic crude on Silia*Carb* HA (*1/1*)				
Sample	CBD (*mg/mL*)*		UV Chlorophyll indice (*665 nm*)	
	-20°C	r. t.	-20°C	r. t.
Ethanoic crude	1.32	2.03	0.3074	1.580
Run 1	0.57	1.32	0.0022	0.000
Run 2	1.60	2.15	0.0017	0.009
Run 3	1.34	1.90	0.0054	0.0213
Run 4	1.38	2.08	0.0092	0.0285
Run 5	1.41	2.11	0.0145	0.0341
Run 6	1.31	2.01	0.0183	0.0172
Run 7		1.97		0.0196
Run 8		2.11		0.0220
Average yield (%)	96	98		

* Titration of CBD in solution. Each passage can therefore be compared to the reference value.

CONCLUSIONS

In summary, a 5 g cartridge of Silia*Carb* HA Activated Carbon had the capacity of processing between 6 and 8 runs (*350 mL/run*) of ethanoic crude samples of CBD extracted at room temperature. When scaling up to the 16.5 × 100 cm cartridge, the treatment capacity is estimated to be 1000 L of ethanoic crude sample, which corresponds to about 200 to 250 kg of biomass and yielding up to 20 kg of purified CBD. However, this result may vary depending on the type of biomass used and the extraction conditions.

References

1. Ritchie R. J., *Photosynth. Res.* **2006**; *89*; 27.

High Value Silica-based and Specialty Products for Chromatography, Analytical and Organic Chemistry, and Purification

DISCOVER OUR BROCHURES

METAL & ORGANIC SCAVENGING

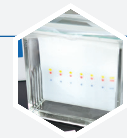
Silia*MetS*® – Metal Scavengers
Silia*Bond*® – Organic Scavengers
***E-PAK*®** – Fixed Bed Flow-Through Purification Cartridges




CHROMATOGRAPHY & PURIFICATION

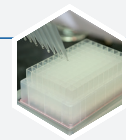
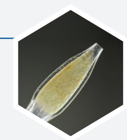
Silia*Flash*® – Irregular Silica Gels
Silia*Sphere*™ PC – Spherical Silica Gels
Silia*Bond*® – Chromatographic Phases
Silia*Sep*™ – Flash Cartridges
Silia*Plate*™ – TLC Plates



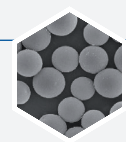
SAMPLE PREPARATION

Silia*Prep*™ – Silica-based SPE Cartridges & Well Plates
Silia*PrepX*™ – Polymeric SPE Cartridges & Well Plates
Silia*Prep*™ – Micro-SPE Tips


ANALYTICAL & PREPARATIVE CHROMATOGRAPHY

Silia*Sphere*™ – Spherical Silica Gels
Silia*Chrom*® – HPLC Columns

ORGANIC SYNTHESIS

Silia*Bond*® – Reagents & Oxidants




R&D SERVICES

Metal & Organic Scavenging Screening
Organic Synthesis
Extraction & Purification
Custom Column Packing
Material Science




SiliCycle Inc.
2500, Parc-Technologique Blvd,
Quebec City (Quebec) G1P 4S6
CANADA

Phone: **+1 418.874.0054**
Toll Free **+1 877.745.4292** (*North America only*)
Fax: **+1 418.874.0355**

Email: **info@silicycle.com**
Website: **www.silicycle.com**

Overseas Offices
SiliCycle Europe europe@silicycle.com
SiliCycle India india@silicycle.com
SiliCycle Shanghai contact@silicyclechina.com

Follow us: