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PRODUCTION OF MATERIALS WITH ANTIMICROBIAL PROPERTIES BY A COMBINED SUPERCRITICAL EXTRACTION AND IMPREGNATION PROCESS

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ABSTRACT

In this work, a combined process of supercritical fluid extraction and adsorption was presented as an efficient tool for incorporation of natural substances with antimicrobial activities into selected carrier materials. The process proposed offers numerous possibilities for combined process design and product development, and should be optimized in each particular case. Loadings of active substances up to 9% were obtained in the presented examples.

INTRODUCTION

The increasing resistance of microbes against common synthetic antibiotics, antimycotics and antivirals causes serious problems in medical treatment especially in open wounds, skin burns or surgery. In general a trend towards searching for natural antibacterial compounds is predominant in recent years. Anyway, these compounds are usually present in herbs only at small concentrations. For their recovery, organic solvents are increasingly replaced by harmless methods like supercritical extraction. Supercritical carbon dioxide ($scCO_2$) is not only known as a possible solvent for valuable compounds but also for its high diffusion ability in organic matter. The latter property may be used for impregnating solid matrices with an adsorbate. The mentioned properties of $scCO_2$ may be used in a combined process, introduced by Fanovic et al. [1], for extracting natural compounds with posterior application on a solid matrix. The envisaged compounds may either be absorbed within the solid or adsorbed on its surface as an adhering coating. In any case, kinetics and optimum processing conditions may differ between the extraction and the application step. In this presentation a process design comprising a closed solvent (supercritical CO_2) cycle is discussed which includes an extraction and an adsorption step that can be operated at independent conditions or with different contact times. Previously published [1-5], as well as new results are presented demonstrating a variety of available options in the process design provided by the experimental set-up. As a source of natural bioactive compounds lichens of *Usnea* genus (*Usnea barbata*, *Usnea lethariiformis*) [6], hops (*Humulus lupulus*) [7], and curry plant (*Helichrysum italicum*) [8] were selected for their antibacterial activity; thyme (*Thymus vulgaris*) [9] for antimitotic and antibacterial activities and lemon balm (*Melissa officinalis*) for antiviral activity of its isolates [10]. As base materials, polymeric materials (polycaprolactone (PCL), cellulose acetate, starch, chitosan, LDPE), medicinal textile (cotton gauze, polypropylene non-woven fabric (PP-NWF)) and organic-inorganic composites (polycaprolactone-hydroxy apatite (PCL+HA)) were used.

MATERIALS AND METHODS

Lemon balm (*Melissa officinalis*) cultivated in northern Serbia, curry plant (*Helichrysum italicum*) cultivated in Dalmatia and *U. barbata* collected in Former Yugoslav Republic of Macedonia were used for this study. PP non-woven fabric (40 g/m^2), cotton gauze (weaving density of 17 threads/cm^2) and LDPE (without additives, density 926 kg/m^3) were used as substrates. Commercial carbon dioxide (purity 99%) was supplied by Messer-Tehnogas.

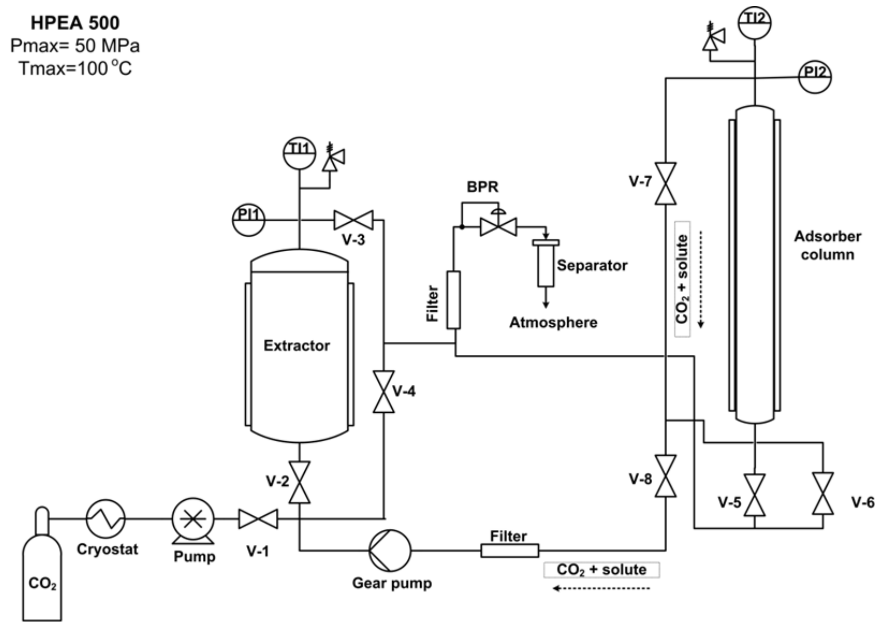

Fig.1 Schematic presentation of HPEA 500 unit for the combined process

The experimental set-up (High Pressure Extraction Adsorption 500 unit, Eurotechnica GmbH, Germany) is presented in Fig. 1. This laboratory unit includes an extractor vessel (500 mL) and adsorption column (100 mL) with a possibility of supercritical fluid circulation through the vessels. The extractor is filled with the raw material from which a target substance is to be extracted. The adsorption column is filled with a solid to be impregnated by the extract from the prior extraction step. The combined extraction-adsorption plant allows for the back pressure regulator (BPR) to be placed between the extractor and adsorber thus providing a possibility of conducting extraction and adsorption processes at different pressures. A high pressure view cell may be placed between the extractor and adsorber as well with the aim of optical detection of solute's concentration. Such a set-up was applied in adsorption of extracts isolated from lichen *Usnea lethariiformis* onto PCL and PCL+HA [1, 2]. Processing mode applied was a continuous flow of $scCO_2$ through both vessels operating under different conditions or a combination of the continuous flow with posterior circulation of the supercritical solution through both vessels operating under the adsorption conditions [1, 2]. In the experimental set-up presented in Fig.1 , the BPR is used to maintain the pressure in the entire process at one level. A gear pump is provided for circulation of supercritical solution through both vessels or optionally through the adsorption column only. In this set-up the extractor and adsorber operate at the same pressure, while contact time may differ due to the flow of the supercritical solution through both vessels or through the adsorber only. Since extraction is usually accomplished much faster than adsorption, such an option might be of interest when scaling up the prcess. This set-up was applied in adsorption of extracts from thyme [3], hops [4], curry plant [5], lemon balm and *Usnea barbata* onto selected carriers. The operation modes applied were: (a) Batch – $scCO_2$ circulates through both vessels for a certain time, and (b) Semi-continuous - a number of batch operations with continuous $scCO_2$ flow through the system between the batch cycles with the aim of fresh solvent supply. Optimum operating mode (e.g. number of cycles, combination of batch operation and continuous $scCO_2$ flow) were determined by gravimetrical measurements or optical methods under pressure.

RESULTS AND DISCUSSION

In order to select a proper experimental set up for a combined process, it is necessary to study the extraction and adsorption processes separately first. Extraction conditions that provide the highest recovery of target bioactive compounds should be defined. Behaviour of the selected solid carrier under supercritical conditions as well as influence of the rate of decompression on its physical properties should be thoroughly investigated. The presence of active compounds may also have an influence on the carrier properties (e.g. plasticizing effect).

Selected results on application of the combined process, including previously published ones [1-5], are presented in Table 1. As can be seen, loadings of active components of up to 9% were obtained. In the case of impregnation with *U. lethariiformis* onto PCL as a possible material for a scaffold with medicinal application [1], there was a need to perform the extraction at pressure of 30 MPa and temperature of 313 K in order to isolate target active components. However, under these conditions PCL undergoes melting. Therefore, conditions of lower pressure and temperature (15 MPa, 308 K), that are suitable for impregnation of PCL and its posterior foaming were kept in the adsorption vessel. This example illustrates a need to apply different conditions for the extraction and adsorption/impregnation when designing the combined process.

In the case of thyme, target compounds were monoterpene phenols which could be isolated under the pressure in the range 10-15 MPa. Therefore, both processes were conducted under the same conditions (15 MPa, 308 K), while the processing modes were varied (batch, semi-continuous) [3]. Selected results for incorporation of thyme extract into different carriers are presented in Table 1. Batch processing mode during 5h was shown to be advantageous providing considerable loadings to all substrates. The same mode (batch, 5h) was shown to be efficient in the case of hops [4], curry plant [5] and *U. barbata* extracts adsorption (Table 1) providing high loadings of active compounds in the process with the extractor and the adsorber operating under the same conditions. In future, it is of interest to perform the same experiments with the adsorption taking place at a lower pressure (10-15 MPa) for comparing the loading of active compounds and the bioactivity of final product. By varying conditions of the adsorption step, the chemical profile of adsorbed compounds can be tailored due to the change in $scCO_2$ density and its solvent power, and this may be a topic for future investigations. In the case of lemon balm, the target compounds are from its essential oil. Both processes (extraction and adsorption) were conducted at the same conditions of 10 MPa and 313 K and a considerably high loading was achieved in a semi-continuous mode (Table 1).

Presented results illustrate numerous possibilities for a combined process performance. The choice of working conditions and processing mode depends on chemical profiles of active compounds and carrier materials, as well as on interactions among all components present in the system and kinetic behaviour, especially the diffusivity. It should be also kept in mind that the raw material - to - carrier material mass ratio is another parameter that should be optimized.

Table 1. Results of the combined process application to development of materials with antimicrobial properties

<table>
<tr><th>Natural material [Reference]</th><th>Carrier</th><th>P[MPa], T[K] Extractor/Adsorber</th><th>Mode</th><th>Loading (%)</th></tr>
<tr><td rowspan="8">Thyme [3]</td><td rowspan="3">Cotton gauze</td><td rowspan="8">15, 308 /15, 308</td><td>Semi-continuous
2 cycles of 5h</td><td>8.99</td></tr>
<tr><td>Semi-continuous
6 cycles of 2h
(1h adsorber only)</td><td>1.08</td></tr>
<tr><td>Batch
5h</td><td>7.18</td></tr>
<tr><td>PCL</td><td rowspan="5">Batch
5h</td><td>9.04</td></tr>
<tr><td>PP-NWF</td><td>4.78</td></tr>
<tr><td>Cellulose acetate</td><td>1.44</td></tr>
<tr><td>Chitosan xerogel</td><td>0.96</td></tr>
<tr><td>Corn starch xerogel</td><td>0.70</td></tr>
<tr><td rowspan="3">Hops [4]</td><td>PCL</td><td>15, 308 /15, 308</td><td rowspan="3">Batch
5h</td><td>6.04</td></tr>
<tr><td>PP-NWF</td><td rowspan="2">29, 323 /29, 323</td><td>4.36</td></tr>
<tr><td>Corn starch xerogel</td><td>2.58</td></tr>
<tr><td>Usnea lethariiformis [1]</td><td>PCL</td><td>30, 313 /15, 308</td><td>Continuous 2h +
circulation 1h</td><td>2.8</td></tr>
<tr><td rowspan="4">Usnea lethariiformis [2]</td><td rowspan="2">PCL+ 10%HA</td><td>30, 313/ 17, 308</td><td>Continuous 2h +
circulation 1h</td><td>4.1</td></tr>
<tr><td>30, 313/30, 313</td><td>Continuous 2h</td><td>5.7</td></tr>
<tr><td rowspan="2">PCL+ 20%HA</td><td>30, 313/17, 308</td><td>Continuous 2h +
circulation 1h</td><td>5.9</td></tr>
<tr><td>30, 313/30, 313</td><td>Continuous 2h</td><td>1.7</td></tr>
<tr><td>Usnea barbata</td><td>LDPE</td><td>30, 313/30, 313</td><td>Batch
5h</td><td>3.05</td></tr>
<tr><td>Curry plant [5]</td><td>Corn starch xerogel</td><td>35, 313/35, 313</td><td>Batch
5h</td><td>1.26</td></tr>
<tr><td>Curry plant</td><td>PP-NWF</td><td>35, 313/35, 313</td><td>Batch
5h</td><td>3.99</td></tr>
<tr><td>Lemon balm</td><td>Cotton gauze</td><td>10, 313/10, 313</td><td>Semi-continuous
5 cycles of 1h</td><td>2.24</td></tr>
</table>

CONCLUSION

In this presentation a process design which combines processes of supercritical fluid extraction with posterior impregnation of the extract onto a solid carrier was presented for application of natural extracts with antimicrobial activity. The experimental set-up provides a variety of options in the process design related to the operating conditions and mode. The combined process should be optimized in each particular case. Presented experimental data indicated that considerably high loadings of the bioactive extracts are attainable by the proposed process.

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