Analysis of supercritical fluid extraction of lycopodine using response surface methodology and process mathematical modeling

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ABSTRACT

Supercritical CO_2 was used in this work to obtain the lycopodine alkaloid from the *Lycopodium clavatum* L. The objective was to investigate the best process conditions through the usage of the response surface methodology, evaluating the effects of extraction parameter such as pressure (200–300 bar), temperature (40–60 °C) and pre-treatment time of plant in an alkaline solution (0–2 h). The relationship between these variables and the yield of lycopodine extract was obtained by a second-order polynomial using the Box–Behnken design. The optimal extraction condition was found to be within the ranges investigated: 300 bar, 40 °C, no pre-treatment. These process conditions resulted in a yield of 20.29 lycopodine % of the final extract. From this result, the extraction curve was plotted as a function of time and simulation of the extraction process was performed using two models available in the literature, which are based on differential mass balances for the solid and fluid phases.

1. Introduction

Lycopodium clavatum L. is a widely distributed pteridophyte throughout the world. Its extract has been used in complementary medicine and is being investigated for the treatment of liver diseases and Alzheimer's disease. It is also used in homeopathy and in recent studies its dilutions showed hepatoprotective and anti-carcinogenic effects for liver cancer in rats (Mandal et al., 2010; Samadder et al., 2013; Pathak et al., 2006, 2009). Furthermore, studies have shown that *L. clavatum* L. extracts obtained via conventional extraction methods using organic solvents, such as hexane, petroleum ether and methanol have anticholinesterase, anti-inflammatory, antibacterial, antiviral and antifungal activity (Orhan et al., 2006, 2007; Konrath et al., 2012).

These studies show that alkaloids obtained from *L. clavatum* have great potential in pharmacological applications (Ayer, 1991; Ma and Gang, 2004). Lycopodine is the main alkaloid present in *L. clavatum* which can be identified by gas chromatography mass spectrometry GC/MS (Orhan et al., 2006; Mandal et al., 2010.) This compound showed substantial growth inhibition of Hela cells and had effect on human prostate cancer cells, reducing their proliferation and inducing eliminating cancerous cells (Mandal et al., 2010; Bishayee et al., 2013; Samadder et al., 2013).

Important drugs isolated using supercritical fluid extraction (Kohler et al., 1997; Ashraf-Khorassani and Taylor, 1997; Kim et al., 2001) has demonstrating to be more selective in comparison with conventional techniques in which organic solvents are used. Furthermore, when carbon dioxide is used

Nomenclature

A	immersion time of the plant in a sodium carbonate solution (h)
a_0	superficial area (m^{-1})
A_p	total area of particles (m^2)
c	extract concentration on fluid phase $(kg\,m^{-3})$
C_0	number of replicates of experiments at the center point
d	extractor diameter (m)
D_i	internal diffusion coefficient $(m^2\,s^{-1})$
e	extract mass in regards to N
h	bed height (m)
h	axial coordinate (m)
J	mass transfer rate $(kg\,s^{-1}\,m^{-3})$
K	inaccessible solute mass contained within the solid particles (kg)
K	equilibrium coefficient $(m^3\,kg^{-1})$
k	number of variables
k_f	mass transfer coefficient for fluid phase $(m\,s^{-1})$
k_s	mass transfer coefficient for solid phase $(m\,s^{-1})$
k_{TM}	internal mass transfer coefficient $(mm\,s^{-1})$
l	characteristic dimension (m)
M_t	accumulated mass (g)
M_∞	mass extracted in an infinite time (g)
N	solid phase mass free from solute (kg)
N	total number of experiments from design
O	initial solute mass in solid phase (kg)
P	easily accessible solute mass (kg)
P	pressure (bar)
p	descriptive p-value level
Q	solvent flow rate $(g\,s^{-1})$
q	specific quantity of solvent
q	extract concentrate in solid phase $(kg\,kg^{-1})$
q_m	specific amount of solvent at the beginning of extraction in the interior of particles
q_n	specific amount of solvent at the end of the extraction of easily accessible solute
q_0	initial concentration of extract in solid phase $(kg\,kg^{-1})$
q^*	concentration in the solid–fluid interface $(kg\,kg^{-1})$
$\dot{q}$	solvent mass flow rate in regards to N (s^{-1})
T	temperature (°C)
t	extraction time (s)
t_i	time for internal diffusion (s)
u	solvent superficial velocity $(m\,s^{-1})$
V	extractor volume (m^3)
x	fraction of solute in the solid phase (solute free basis)
x_0	initial concentration of free solute in the solid phase (mass fraction)
x_k	Initial extract concentration inside the solid particles (mass fraction)
x_p	easily accessible solute concentration (mass fraction)
y	solute fraction in fluid phase (free basis)
y_r	extract solubility on solvent
W	Sovová model parameter for the slow extraction period
Z	Sovová model parameter for the rapid extraction period

z_w	boundary coordinate between fast and slow extraction
α	significance level
ε	bed voidage
ρ	solvent density $(kg\,m^{-3})$
ρ_s	solid phase density $(kg\,m^{-3})$

as the solvent, supercritical carbon dioxide extraction has the advantage of being a clean technology (Hawthorne, 1990; Chester et al., 1998).

Among the factors that influence the efficiency of supercritical fluid extraction are temperature, pressure and fluid flow (Chester et al., 1998; Mukhopadhyay, 2009). Design of experiments and response surface methodology (Montgomery and Runger, 2003) are used as an effective alternative for the identification and optimization of the variables involved in the process. By using these tools, it is possible to evaluate the influence of several variables on one or more responses with a reduced numbers of experiments, therefore reducing time and cost (Anderson-Cook et al., 2009). This methodology has been widely used in the chemical engineering literature as a way of optimizing processes (Liao and Chung, 2011, 2013; Arami-Niya et al., 2012). The main design of experiments methods used in the optimization of supercritical fluid extraction are Taguchi, central composite design and Box–Behnken (Sharif et al., 2014). The Box–Behnken design has been used in several studies, for example, in the investigation of pressure, temperature and time factors in the extraction of xanthones from *Garcinia mangostana* L. (Zarena et al., 2012) and from rapesed oil (Cvjetko et al., 2012). It has also been used in the optimization of parameters such as pressure, temperature and amount of co-solvent in the extraction of flavonoids from *Pueraria lobata* (Wang et al., 2008) and of antioxidants from *Crataegus monogyna* (Shortle et al., 2013).

In order to determine process parameters that should be considered in the prediction, optimization and scale-up a supercritical extraction process, another important factor is the mathematical modeling of the extraction process curves (Vargas et al., 2013; Meireles et al., 2009).

As a result, many models have been developed for supercritical fluid extraction using different approaches: empirical models (Esquível et al., 1999), models based on mass transfer in a single phase (fluid phase or solid particle) (Gaspar et al., 2003; Bartle et al., 1990), models based on portioning thermodynamics and kinetic desorption (Tan and Liou, 1989; Desai et al., 2014), models that consider the mass transfer in both phases (Bulley et al., 1984; Sovová, 1994; Reverchon, 1996) models established by analogies to heat transfer (Reverchon, 1997) and combinations of these (Crank, 1975). A thorough review on the different aspects which are considered in mathematical models available in the supercritical fluid extraction literature can be found in Oliveira et al. (2011).

This work used the response surface methodology for the optimization of supercritical extraction of lycopodine from *L. clavatum* in order to evaluate the effects of CO_2 pressure and temperature and the effects of immersion time of plant in an alkaline solution through a Box–Behnken design. For the optimized conditions within the ones investigated here, the mass transfer parameters of two mathematical models developed from the differential mass balance for the fluid and solid