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Separation of ergot alkaloids by adsorption on silicates

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Abstract

A mixture of ergot alkaloids (agroclavine, elymoclavine, chanoclavine, and chanoclavine aldehyde) was separated from the *Claviceps purpurea* fermentation broth by adsorption on inorganic adsorbents containing silica. The uptake of alkaloids depended on the concentration of adsorbent and pH. The adsorption capacity for of inorganic materials increased with increasing content of inorganic oxides such as MgO and CaO in the adsorbent. Using statistical thermodynamics, a simple mathematical model describing the multicomponent adsorption equilibrium is proposed and a numerical method suitable for fast computer simulation of multicomponent adsorption was developed.

Symbols

C	concentration of alkaloid in the medium, g l^{-1}
F	Helmholtz free energy in Equations (2) and (3) or Faraday charge
g	amount of adsorbed alkaloid, g alkaloid g^{-1} adsorbent
G	concentration of adsorbent in the medium, g l^{-1}
k	Boltzman constant
k_i	adjustable parameters in Equation (4) or (7)
q	q_i is the partition function of a single component i in Equation (1)
Q	partition function of canonical distribution
M	number of active sites
N	number of active sites occupied by a component i
n	number of adsorbed components
p	exponent in Equation (7)
T	temperature, K
μ	chemical potential in Equation (3)

Indices

i	component i
max	maximum
pH	attributed to Equation (7)
0	initial

Introduction

Ergot alkaloids (Flieger *et al.* 1997) can be produced in submerged fermentation by *Claviceps* sp. or *Penicillium* sp., which are used for their industrial production. The final fermentation broth contains a complex mixture of alkaloids, salts, polysaccharides, fats, solids, etc., and organic solvent extraction is commonly used for the separation of ergot alkaloids from the broth. To avoid the formation of emulsions during the extraction, a novel separation process taking advantage of solid-liquid adsorption of alkaloids on

activated carbon, bentonite and other silicate sorbents was developed (Řeháček *et al.* 1986, Stuchlík *et al.* 1988). Similarly, Payne & Shuler (1988) used selective adsorption on polycarboxylic ester resin XAD-7 to isolate alkaloids from plant cell cultures. Adsorption increased the yield of alkaloids up to two-fold as compared with a corresponding solvent extraction process.

Later, the Kawaken Fine Chemicals Company submitted two patents (1990a,b) for isolation and purification of ergot alkaloids from *Claviceps* sp. fermentation broth by adsorption on porous, synthetic polymer, ion exchanging adsorbents such as styrene divinylbenzene copolymer or phenolic resin. The fermentation broth was passed through a packed bed column and subsequently washed by water and eluted by methanol. Methanol was removed under reduced pressure. The residue was extracted with ethyl acetate and dried with a yield of 68% and purity 94%.

The design and scale-up of reliable adsorption unit operation require a thorough understanding of the multicomponent sorption equilibrium of the complex mixture. However, quantitative characterization of adsorption equilibrium in terms of fundamental thermodynamic properties of solute, solvent and adsorbent is rather difficult (Stumm 1987, Ching 1997). In this study, the simultaneous uptake of agroclavine, elymoclavine, chanoclavine and chanoclavine aldehyde by inorganic sorbents was investigated. These ergot alkaloids can be characterized by a similar chemical structure (Budvari 1996, Flieger *et al.* 1997) as shown in Figure 1. They are sparingly soluble in water, though partially soluble in acid.

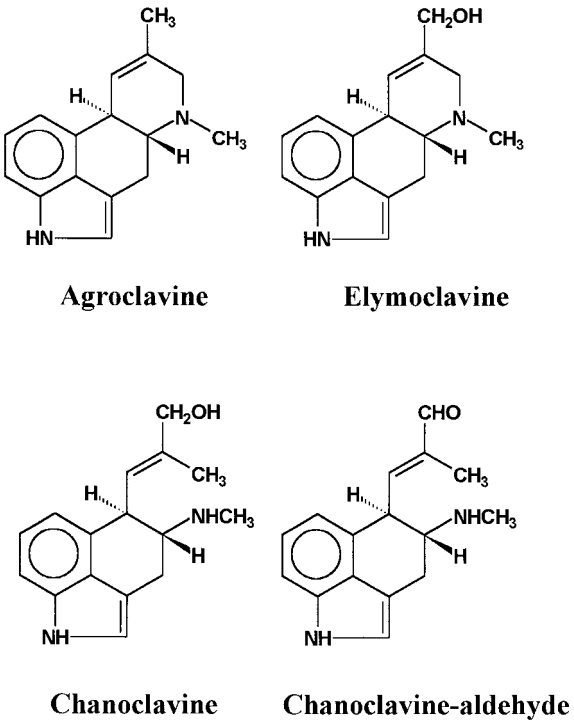

Fig. 1. Chemical structure of ergot alkaloids under study.

Material and methods

Sorbed species

Filtered fermentation liquid, obtained in a batch cultivation (Pažoutová *et al.* 1981) of *Claviceps purpurea 59* (Pažoutová *et al.* 1987) contained (g l^{-1}): agroclavine 0.326, elymoclavine 0.224, chanoclavine 1.075 and chanoclavine-aldehyde 0.403. When necessary, the sample pH was adjusted to desired value by NH_4OH.

Sorbents

The following sorbents were examined throughout: laboratory grade alumina (Lachema, Brno), natural bentonite (Lachema, Brno), kieselguhr for beer filtration (BCI, Hamburg), silica gel based sorbents Separon SGX (50 μm) and Separon SGX C-18 (50 μm) octadecyl modified produced by Tessek, Prague, Spheron 40, Spheron 100, molecular sieve Florisil distributed by Lachema, Brno, and analytical grade bentonite Veegum K offered by Erbslöh, Krefeld. To evaluate the separation efficiency of the sorbents, simple contact experiments were performed using Erlenmeyer flasks (100 ml) with 30 ml of fermentation liquid and different known amounts of the solid. The flasks containing these dilute suspensions were placed on a shaker, allowing enough time (2 h) for developing the sorption equilibrium. The content of each flask was filtered and the filtrate analysed for residual ergot alkaloid concentration.

Analytical assay of ergot alkaloids

The content of ergot alkaloids in samples of the filtrate after sorption was determined by reversed phase HPLC (Křen *et al.* 1985). Conditions: column reverse phase; Separon SGX C-18 (Tessek, Prague), particle size 10 μm, 25 $\times$ 0.4 cm; mobile phase; water/methanol/NH_4OH (sp.gr. 0.880) (35:65:0.1, by vol.); flow rate 1 ml min^{-1}; detection UV at 225 nm.

Other details of the analytical procedure were described previously (Pažoutová *et al.* 1987).

Thermodynamic analysis of multicomponent adsorption

The simultaneous multicomponent adsorption of soluble compounds from the aqueous phase on solid sorbents has not been extensively treated in the chemical engineering literature. Assuming that the sorption takes part between a solid surface with M active centers and $N_1, N_2, \ldots, N_n$ molecules of n components are dissolved in the fermentation liquid, the partition function can be written in the form:

$$Q_m = M! \prod_n q_i^{N_i} / [(M - \sum_n N_i)! \prod_n N_i!], \quad (1)$$

where q_i is the partition function of a single component i which describes the energy of the bond between an active center and the adsorbed molecule i. Using partition function Q_m to define Helmholtz free energy F we can relate the molecular phenomena with the macroscopic scale:

$$F = -kT \ \ln \ Q_m. \quad (2)$$

The symbol k used here is the Boltzmann constant. In the next step we evaluate the chemical potentials of a single molecule of species i according to the relation:

$$\mu_i = (\partial F / \partial N_i)_{T,V,[Ni]} = \mu_i^0 + kT \ \ln \ C_i. \quad (3)$$

Since N_i and M are very large numbers, we can employ Stirling's formula for approximation of ln $N!$. After a few commonly known algebraic manipulations (e.g., Hill 1968) we receive the final formula for a simultaneous multicomponent adsorption isotherm.

$$g_i = g_i^{\max} k_i C_i / (1 + \sum_n k_j C_j); \quad i, j = 1, 2 \ldots n, \quad (4)$$

where g_i is the amount of adsorbed alkaloid in the solid phase and C_i is the equilibrium concentration of ith component in the liquid phase. The symbols $g_I^{\max}$, $k_1, k_2 \ldots k_n$ are parameters that have to be estimated experimentally. Equilibrium concentrations of adsorbed compounds C_i can be calculated with respect to initial concentrations C_i^0 and mass concentration of the solid material G using mass conservation law:

$$C_i^0 - C_i = G \ g_i; \quad i = 1, 2 \ldots n. \quad (5)$$

The set of Equations (4) and (5) fully describes the multicomponent adsorption equilibrium. However, for the purpose of practical calculation, Equation (5) can be differentiated and rewritten in the form:

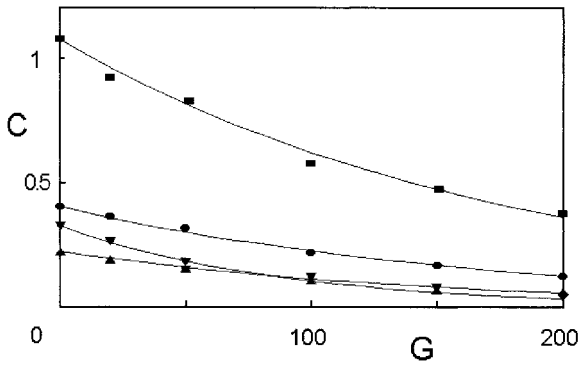

Fig. 2. Dependence of ergot alkaloid equilibrium concentration C (g l^{-1}) on the concentration of adsorbent Spheron 40 G(g l^{-1}). pH was adjusted to 4.25. ■ – chanoclavine, ● – chanoclavine aldehyde, ▲ – elymoclavine, ▼ – agroclavine, points – experimental data, lines – calculated by numerical integration of Equations (6) and (4).

$$\begin{aligned} &\mathrm{d}\, C_i / \mathrm{d}G = -g_i; \quad (G = 0, C_i = C_i^0); \\ &\quad i = 1, 2 \ldots n. \end{aligned} \quad (6)$$

The numerical integration of differential Equations (6) and (4) allows to calculate the relationship between equilibrium concentrations C_i and adsorbent concentration G in one simulation run on a PC (e.g., Bosch *et al.* 1993).

Results and discussion

Comparison of different silica based adsorbents

Ergot alkaloids uptake by inorganic adsorbents occurs rapidly with time constant from 5 to 10 min. Under the experimental conditions used here we took about half an hour to reach the equilibrium. In the first series of experiments, the pH value of the filtered fermentation medium was adjusted to 4.25. Figure 2 shows the representative course of alkaloid equilibrium concentrations depending on the amount of adsorbent. The parameters of multicomponent adsorption were estimated by numerical solution of Equations (4) and (6) using a customary nonlinear regression procedure described earlier (Volesky & Votruba 1993).

Table 1 summarizes the results of parameter estimation. The results are sized according to the increasing sorption capacity of the solids. The standard deviation between calculated concentration and data ranged from 0.01 to 0.03 g of alkaloid l^{-1}. The values of the square correlation coefficient varied within 0.98 and 0.99. The goodness of fit test based on chi-square calculation has shown that the model (Equations (4)

Table 1. Values of parameters g_i^{max} [g alkaloid g^{-1} sorbent] and k_i [l g^{-1} alkaloid] in Equations (4) characterizing the adsorption of ergot alkaloids on different solids at pH 4.25.

Sorbent	Elymoclavine		Chanoclavine aldehyde		Agroclavine		Chanoclavine	
	g_1^{max}	k_1	g_2^{max}	k_2	g_3^{max}	k_3	g_4^{max}	k_4
Alumina	0	–	0	–	0	–	0	–
Kieselguhr	0	–	0	–	0	–	0	–
Separon SGX C18	0.0007	5.9	0.0018	2.2	0.023	2.20	0.0012	1.4
Separon SGX	0.0022	7.1	0.0045	2.8	0.578	0.091	0.0095	1.3
Spheron 100	0.0050	7.2	0.0036	7.2	0.035	2.70	0.010	2.6
Spheron 40	0.0041	7.1	0.0099	2.6	0.023	2.58	0.0096	2.6
Florisil	0.075	0.37	0.053	0.36	0.112	0.40	0.052	0.33
Bentonite	1.81	0.098	0.50	0.16	1.47	0.12	0.81	0.12
Veegum K	4.33	0.046	2.26	0.061	6.4	0.028	1.3	0.12

and (6)) may be accepted for the measured data with the probability higher than 0.92.

The adsorbents based on pure $Al_2O_3$and SiO_2 such as alumina or kieselguhr did not exhibit sorption activity. The sorption capacity of silica gel based sorbents such as Separon SGX and SGX C-18, Spheron 40, Spheron 100 was comparable and much lower than that of the synthetic zeolite – Florisil or bentonites. Natural or synthetic zeolite (Parker 1997) is composed from 58–60% of SiO_2,, 15–17% Al_2O_3, 6–7% CaO, 0.1–0.3% MgO and 15% of water, approximately. By contrast, bentonite consists of 50% SiO_2,, 22% Al_2O_3, 2% CaO, 4–5% MgO and 20% of water, roughly. It seems that a lower ratio of SiO_2 to $Al_2O_3$and a higher content of MgO in the sorbent can improve the sorption capacity of the solids. Calles (1998) observed a similar influence of different Si/Al ratio on multicomponent adsorption equilibrium of carbon dioxide, ethylene and propane on zeolite ZSM-5. Stumm (1987) attributed the improvement of adsorption capacity of complex silicates to suppression of SiO_2 hydrolysis induced by $Al_2O_3$and MgO. The maximum sorption capacity g_i^{max} was observed on bentonite based sorbents. The reciprocal value of parameter k_i is proportional to the partition function of a single component q_i in Equation (1) and describes the energy of the bond between the active center and adsorbed molecule of species i. The values in Table 1. indicate that the energy of the bond increases with a higher content of MgO and decreases with increasing Al_2O_3 content in complex silicates.

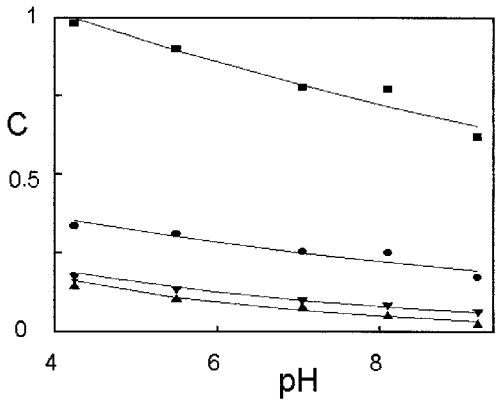

Fig. 3. Dependence of ergot alkaloid equlibrium concentration C (g l^{-1}) on pH. Concentration of adsorbent Separon SGX was 40 g l^{-1}. For symbols see Figure 2.

Effect of pH

Figure 3 shows the typical dependence of ergot alkaloid equilibrium concentrations on pH. The moderate decrease of alkaloid equilibrium concentrations with increasing pH is usually attributed to weak hydrogen bonding by electrostatic forces and formation of the Stern electrical double layer in water environment which is typical for silica and other insoluble inorganic oxides (Tamaru 1980). Following the Stern's theory of electrical double layer we can approximate the pH effect on g_i^{max} by a simple power function:

$$g_i^{max}(pH)/g_i^{max}(4.25) = k_{pH\,1}(1 - k_{pH\,2}[H^+]^p). \quad (7)$$

Using nonlinear regression the values of parameters $p, k_{pH\,1}$ and $k_{pH\,2}$ were estimated as 0.0283, 17.8 and 1.245, respectively. As shown in Figure 3, the

proposed model satisfactorily describes the pH effect on multicomponent adsorption in a wide range of pH values. When a dissociation reaction coupled to intermolecular interaction of adsorbed compounds with the solvent takes place, the value of p has to reflect the molecularity of the reaction. However, in our case the value of parameter p was much lower than expected and it is close to the numerical value of the product RT/F which, multiplied by ln(10), is the slope of linear relationship between electrical potential on solid-liquid interface and pH value. Thus, the observed weak effect of pH on the multicomponent adsorption of alkaloids can be attributed to the polarization of sorbent particles rather than to the hydrolysis of active sites.

Acknowledgement

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