

## Adsorptive Separation of Alkaloids

アルカロイドの吸着分離

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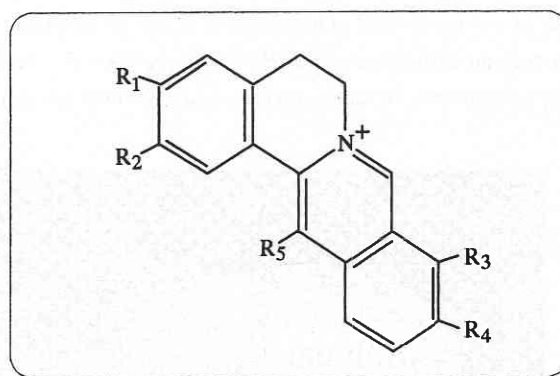
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## 1. INTRODUCTION

Recent years, the cultivation of plant cells in submerged culture has been proposed as an alternative for the factory production of alkaloids. However, the high cost of plant cell culture will limit this technology to high value products. To improve process economics through improvements in the primary recovery stages, adsorptive separation has been suggested as a better method for recovering alkaloids from their very dilute cultivation solutions economically<sup>1)</sup>.

In order to design a new adsorptive separation process, it is necessary to get a theoretical understanding of the characteristics of alkaloids adsorption onto an adsorbent, such as the preferential adsorption for a special alkaloid, the competitive adsorption among the alkaloids, and the chromatographic elution by different solvents etc.. The present study has been carried out to elucidate the fundamental adsorption and desorption mechanisms by using computer simulation method, and to study the selective adsorption and desorption of alkaloids by adapting the solvophobic theory to organize the computer simulation results. The main aim is to develop a methodology for predicting the preferential adsorption and selecting the appropriate eluent solvent.

The model alkaloids considered here were berberine alkaloids, a kind of antibacterial alkaloids found in *Coptis japonica* Makino. Figure 1 shows their structural formula. The solvents used were water, methanol, ethanol, acetone, cyclohexane and N,N-dimethylformamide (DMF). The basal graphite plane was



	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
<b>Berberine</b>	O-CH <sub>2</sub> -O		OCH <sub>3</sub>	OCH <sub>3</sub>	H
<b>Coptisine</b>	O-CH <sub>2</sub> -O		O-CH <sub>2</sub> -O		H
<b>Worenine</b>	O-CH <sub>2</sub> -O		O-CH <sub>2</sub> -O		CH <sub>3</sub>
<b>Jateorrhizine</b>	OH	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H
<b>Palmatine</b>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	OCH <sub>3</sub>	H

Fig. 1 Structural formula of berberine alkaloids

used as a model surface.

## 2. SIMULATION

## 2.1 Potential energy

The simulations were carried out by using the molecular mechanics (MM) and molecular dynamics (MD) methods. The DREIDING force field was used for the calculation of the total potential energy:

$$E = E_b + E_\theta + E_\phi + E_{inv} + E_{el} + E_{hb} \quad (1)$$

where, the first four terms represent the bond interactions, and the last three terms are non-bond interactions<sup>2)</sup>. The values of the

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potential parameters were from Mayo *et al*<sup>3)</sup>.

2.2 Molecular model of adsorption system

The liquid phase model consisted of a single solute molecule, a solvation shell constructed around the solute molecule with solvent molecules, and a graphite surface. To prevent the solvent molecules in the solvation shell from escaping, a thin reflecting wall of the solvent molecules was created outer the solvation shell. The schematic diagram is shown in Figure 2. In MM and MD calculations, all of the atoms in alkaloid molecule and solvent molecules of the solvation shell were movable, while the atoms of the solvent molecules composing the reflecting wall and those in the graphite surface were fixed<sup>2)</sup>.

2.3 Theory

The adsorption process of an adsorbate B onto an adsorbent C to produce an adsorbed complex BC in the presence of a liquid can be compared to the same process occurring in the gas phase

in the absence of solvent as schematically illustrated in Figure 3<sup>4)</sup>. The free energy change related to the effect of the solvent on the adsorption is obtained by subtracting the standard free energy change for the adsorption in the gas phase from that in the solvent phase under unitary standard state. On the other hand, these two processes can be related to each other by the free energy change of wetting the adsorbent,  $\Delta G_C^{se}$ , that of wetting the BC complex,  $\Delta G_{BC}^{se}$ , and that of dissolving the adsorbate into the liquid phase,  $\Delta G_B^{se}$ . A net solvent effect energy,  $\Delta G_{net}^{se}$ , defined to express the effect of the solvent on the adsorption, can be quantitatively given by:

$$\Delta G_{net}^{se} = \Delta G_{sol}^{assoc} - \Delta G_{gas}^{assoc} = \Delta G_{BC}^{se} - \Delta G_B^{se} - \Delta G_C^{se} \quad (2)$$

Although the individual terms involved in the right hand side of Equation (2) can in principle be calculated with the physicochemical properties of the system, it is a very complicated procedure<sup>5)</sup>. The attribution of this study is that we developed a molecular simulation approach to obtain the items required for calculating  $\Delta G_{net}^{se}$  purely from the molecular structure of alkaloid<sup>6)</sup>. This distinctive feature is particularly meaningful for the adsorbate whose physicochemical properties are limited available.

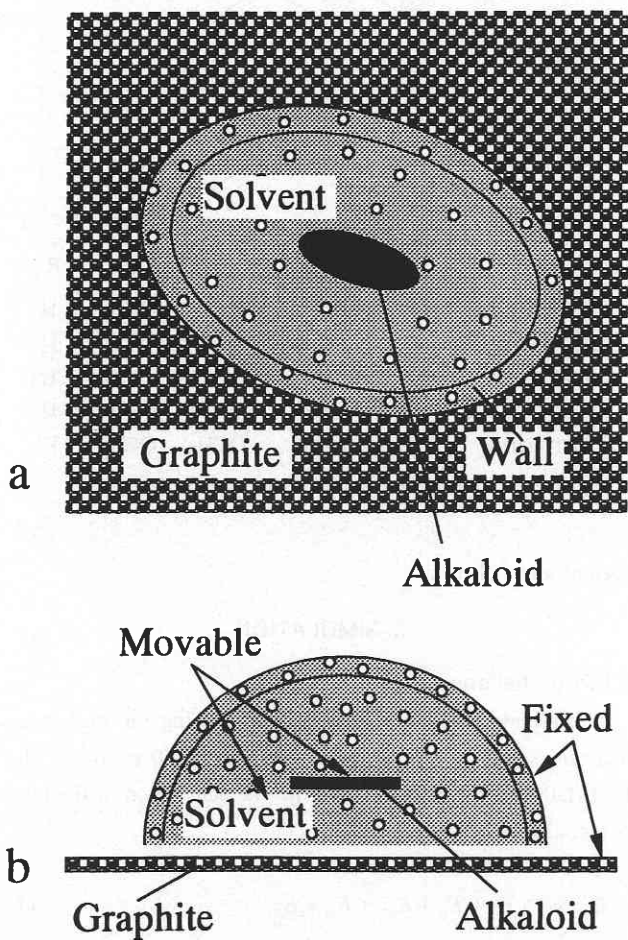


Fig. 2 Schematic diagram of molecular model of adsorption system. (a) top view, (b) side view

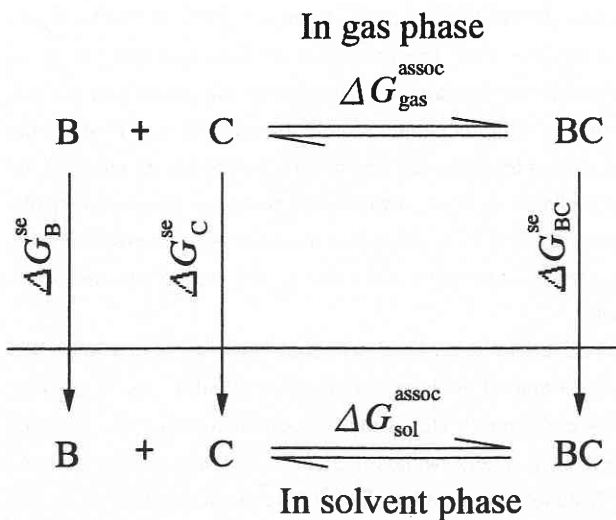


Fig. 3 The thermodynamic cycle showing the relationship between the binding of an adsorbate molecule onto an adsorbent surface to form an adsorbed complex in the gas phase and that when all species are in the solvent phase

### 3. RESULTS AND DISCUSSION

#### 3.1 Conformational energy change of alkaloid

It has been known that the physiological activities of biochemical are affected strongly by their molecular conformations. Therefore, the conformation examination is needed for investigating the possibility of the application of adsorption method to the separation of physiological active components such as alkaloids. As the first-step approximate consideration on the molecular conformation change, the conformational energies of the alkaloid molecule in various states were calculated by using Equation (1), and compared with that of a free alkaloid molecule under vacuum. Table 1 lists the calculated conformational energies  $E_B$  of berberine molecule in the solvents and on the surface as an example. It is shown that the conformation of berberine molecule changed little due to adsorption or dissolution.

Table 1. Conformational energies [kJ/mol]

Solvent	In solvent		On graphite	
	$E_B$ [kJ/mol]	$(E_B - E_B^0)/E_B^0$ [-]	$E_B$ [kJ/mol]	$(E_B - E_B^0)/E_B^0$ [-]
Vacuum	324.8*	-	335.1	3.2
Water	326.6	0.6	341.1	5.0
Methanol	325.7	0.3	340.9	5.0
DMF	325.7	0.3	328.6	1.2

\* $E_B^0$ , conformational energy of a free berberine molecule in vacuum

#### 3.2 Alkaloid-solvent interaction

Unlike adsorption in gas phase, adsorption from solutions is greatly affected by the interaction between adsorbate and solvent. Therefore, the alkaloids-solvent interaction becomes an integral part and necessary to be addressed in advance. Here, the alkaloid-solvent interaction energy,  $\Delta E_{BS}$ , was defined as:  $\Delta E_{BS} = E_{BS} - E_B - E_S$ , where  $E_{BS}$ ,  $E_B$  and  $E_S$  represent the total potential energies of the alkaloid/solvent system, the alkaloid molecule and the solvent, respectively.

The calculated results of alkaloid-solvent interactions are shown in Figure 4(a) for the part attributed to the vdw attractive interaction, and in Figure 4(b) for the total interaction energy, including all of the non-bonded terms listed in Equation (1). It is indicated by Figure 4(a) that the vdw attractive interaction between alkaloid and solvent becomes stronger with the increase in molecular surface area of alkaloid, and that the order of the vdw affinity strength of alkaloid for the solvents is DMF > acetone > cyclohexane > ethanol > methanol > water, which follows the same sequence as the molecular size of solvent

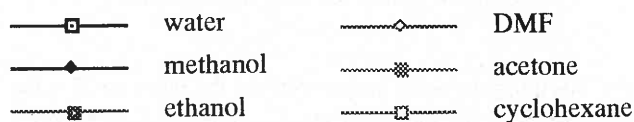
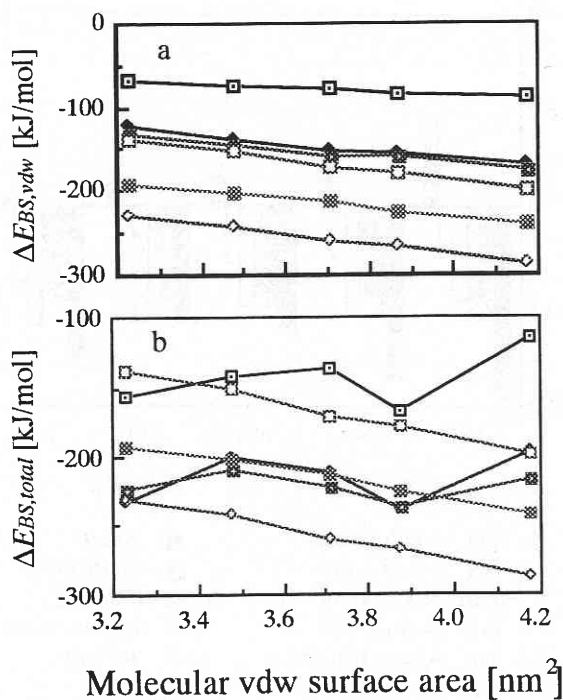


Fig. 4 Interaction energy between alkaloid and solvent (a) the van der Waals interaction (b) the total interaction

except cyclohexane.

Comparing Figure 4(a) with Figure 4(b), the following observations were found: 1) for the cases of acetone, cyclohexane and DMF, the total interaction energies were close to the vdw interaction energies, 2) for the cases of water, methanol and ethanol, the total interaction energies were smaller than the vdw interaction energies, and 3) the dependence of the total interaction energies on the molecular surface area of alkaloid was not very clear as compared to that of the vdw interaction energies. These facts suggest that the vdw dispersion force dominates the interactions of the berberine alkaloids and lower polar solvents, and that the total interactions are strongly affected by the hydrogen-bond interactions between the molecules of alkaloids and higher polar solvents<sup>7)</sup>.

#### 3.3 Selective adsorption and desorption

Based on the definition of net solvent effect energy given by Equation (2), we can know that, for the same adsorbate/adsorbent system, the more negative the value of  $\Delta G_{net}^{se}$  is, the easier

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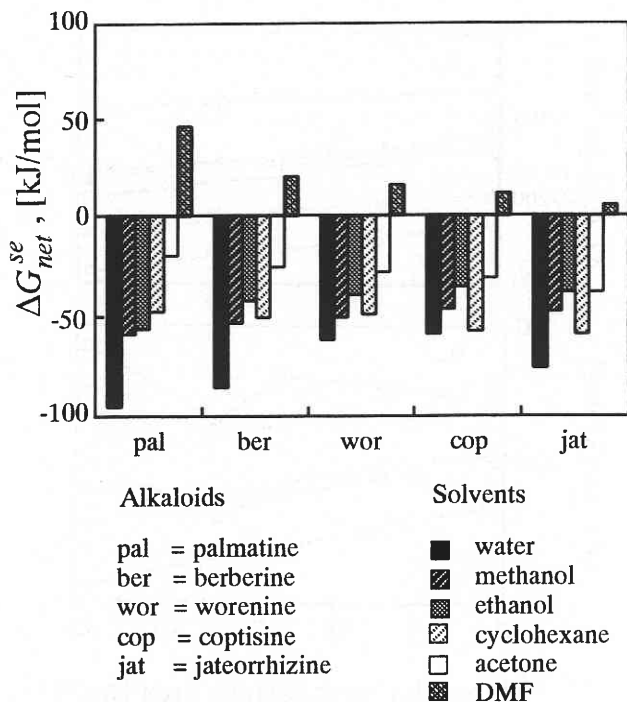


Fig. 5 Free energy change related to the solvent effect on the adsorption of alkaloid onto graphite in solvent

the adsorption occurs. On the contrary, the more positive the value of  $\Delta G_{net}^{se}$  is, the easier the desorption occurs. Figure 5 shows the net solvent effect energies obtained by the molecular simulations using Equation (2) for the adsorption of five berberine alkaloids onto the graphite surface from solvents. The results indicated that DMF was the most powerful eluent for desorbing the berberine alkaloids from graphite surface, since it held the most positive values of  $\Delta G_{net}^{se}$  among the six solvents. While the adsorption capacities of berberine alkaloids from their aqueous solutions were largest since the most negative values were held.

It is also noted that the solvent effect energies of the same solvent on adsorptions of different alkaloids were different. This phenomenon is mainly caused by the different strength of the interaction between the alkaloid and the solvent, and may be

attributed to the difference of the berberine alkaloids molecular structural parameters such as molecular size and molecular polarity. It is known that the value of  $\Delta G_{net}^{se}$  increased with the alkaloid molecular polarity increase for the case of high polar solvents such as water, methanol and ethanol. While in the case of the non-polar solvent, cyclohexane, and the lower polar solvents such as acetone and DMF, the opposite trend was observed. This result is useful for the selection of an appropriate eluent solvent.

#### 4. CONCLUSIONS

A calculation approach for discussing the solvent effect on the adsorption in liquid phase was developed. The calculation of the solvent effect energy was purely from the molecular simulations, without any properties of adsorbate needed. By comparing the values of the calculated solvent effect energy of berberine alkaloids in each solvent, the knowledges about the preferential adsorption and the eluent strength of solvents were obtained. These results are useful for studying the selective adsorption and desorption of alkaloids, and for desiring a new adsorptive separation process.

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