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Modelling selective separation of trypsin and lysozyme using mesoporous silica

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ABSTRACT

The selective separation of biomolecules is a critical process in food, biomedical and pharmaceutical industries. Due to its size and properties, mesoporous silica offers many advantages as a separation media for biomolecules such as proteins and enzymes. In this paper, we investigate mathematically the separation of proteins trypsin and lysozyme using mesoporous silica materials. These proteins are modelled as densely packed spheres, while the silica pore is assumed to have a cylindrical structure. The Lennard–Jones potential together with a continuum approximation is employed to determine the interaction among the proteins and the interaction between a protein and a silica pore. For these systems, the total interaction energies are obtained analytically as functions of the protein size and the pore dimensions. We find that the pore radii which give rise to the maximum adsorption energies for trypsin and lysozyme are 21.74 Å and 17.74 Å, respectively. Since the binding energy between any two protein molecules is found to be three orders of magnitude lower than the adsorption energy of the protein into the silica pore, proteins prefer to be separated and stay inside the pore. Further, we find that using silica pores with radii in the range between 17.23 Å and 21.24 Å allows the entrance of only lysozyme, as such separating lysozyme from trypsin. These results agree with previous experimental study, confirming that mesoporous silica pores may be used to separate trypsin–lysozyme mixture.

1. Introduction

Mesoporous silica materials have been identified as attractive supports to adsorb and immobilize proteins and enzymes, such as cytochrome c, lysozyme and trypsin [1–3]. This is due to their narrow pore size distributions, their high surface area and pore volume and well-ordered hexagonal pore structure [4]. Silica is a chemically inert, nontoxic, polar and dimensionally-stable amorphous form of SiO_2. This material can have different surface areas, pore volumes and particle sizes, depending on the synthesis process [5]. As a result, it is possible during the manufacturing process to adjust the pore size to best fit application needs. To be able to effectively use mesoporous silica as a support for protein and enzyme immobilization, this paper investigates the relationship between its pore size and the sorption energy for two proteins, namely trypsin and lysozyme.

Trypsin and lysozyme are often used in biochemical and pharmaceutical engineering and food industries as an antibacterial agent. Trypsin is a globular protein found in the digestive system of many vertebrates with a chemical composition $C_{35}H_{47}N_7O_{10}$. Lysozyme is an enzyme that can damage bacterial cell walls; its chemical composition is $C_{36}H_{61}N_7O_{19}$. Here, both trypsin and lysozyme are modelled as densely pack spheres [6–11]. The hydrodynamic diameters for trypsin and lysozyme, 38 Å and 30 Å respectively, are taken from the work of Kisler et al. [12].

Takahashi et al. [1] study the adsorption of a horseradish peroxidase enzyme on FSM-16/51 and MCM-41/50 silica mesoporous. They find that the relative pore size and the diameter of the enzyme are the key factors to determining whether the enzyme molecule can be adsorbed on the silica pore. The adsorption of cytochrome c onto mesoporous silica is studied by Deere et al. [2]. Similarly, these authors find that the size of protein and the dimensions of the pore play important roles in the adsorption process. Further, Yang et al. [3] investigate the adsorption behaviour of trypsin and lysozyme by mesoporous silicate. They conclude that the pore volumes, surface chemistry and the binding energy of the proteins affect the adsorption mechanism.

In this paper, we confirm through applied mathematical modelling the effect of silica pore size on the adsorption of proteins. In particular, given a globular protein of certain radius, we predict the dimensions of the silica pore that will readily adsorb and immobilize the protein molecules. Furthermore, we determine the critical pore size that will maximise the protein sorption capacity. In addition, we consider a mixture of trypsin–lysozyme proteins and show that the interaction between two proteins is lower than the interaction between protein and the pore, indicating potential separation of proteins using the silica pore. Finally,

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in order to use silica pores as a molecular sieve, we prescribe a range of pore size that will allow only the entrance of lysozyme, as such separating lysozyme from trypsin. Here, we assume the physisorption of proteins and silica pores where we model the van der Waals interaction between protein molecules and a pore of silica using the Lennard-Jones potential and a continuum approach. In the continuum approach, we assume that atoms comprising the molecule are uniformly distributed over its surface or its volume. Therefore, the total interaction energy between two molecules can be evaluated utilizing an integral technique. For more details of this approach we refer the readers to Girifalco et al. [13] and Cox et al. [14,15].

In the following section, the Lennard-Jones potential together with the continuum approximation is detailed. In Section 3, we derive the interaction energy between two spherical molecules and determine the adsorption energy for a spherical protein and a silica pore. Section 4 presents our numerical results and finally, conclusions are given in Section 5.

2. The Lennard-Jones potential and a continuum approximation

We employ the Lennard-Jones potential and a continuum approximation to determine the molecular interatomic energy between two molecules. The 6–12 Lennard-Jones potential is given by

$$\Phi = -\frac{A}{\rho^6} + \frac{B}{\rho^{12}},$$

where ρ denotes the distance between two atoms, $A = 4\epsilon\sigma^6$ and $B = 4\epsilon\sigma^{12}$ are respectively the attractive and repulsive constants, where ϵ and σ are the energy well depth and the van der Waals diameter, respectively. Using a continuum approach, where the atoms at discrete locations on the molecule are averaged over a surface or a volume, the molecular interatomic energy is obtained by calculating integrals over the surface or the volume of each molecule, given by

$$E = \eta_1\eta_2 \int_{\Sigma_1}\int_{\Sigma_2} \left(-\frac{A}{\rho^6} + \frac{B}{\rho^{12}}\right) d\Sigma_2 d\Sigma_1, \tag{1}$$

where η_1 and η_2 represent the mean surface or the mean volume density of atoms on each molecule. Further, by writing

$$I_n = \int_{\Sigma_1}\int_{\Sigma_2} \rho^{-2n} d\Sigma_2 d\Sigma_1, \quad n = 3, 6, \tag{2}$$

Eq. (1) can be written as

$$E = \eta_1\eta_2(-AI_3 + BI_6). \tag{3}$$

To determine the radius of the pore which will maximise the suction force, we utilize the suction energy concept proposed in [14]. The suction energy W is defined as the total energy or work generated by the van der Waals interactions acquired by a particular molecule to enter the pore,

$$W = \int_{-\infty}^{\infty} F(Z)dZ = \int_{-\infty}^{\infty} -\frac{\partial E}{\partial Z} dZ, \tag{4}$$

where E is as given in Eq. (3). Note that due to the symmetry of the systems studied here we only need to consider the axial force.

The numerical values of the Lennard-Jones parameters used for nonbonded interaction for each atomic element are taken from the work of Mayo et al. [16], and are presented in Table 1. In the system of two atomic species, the Lennard-Jones constants can be obtained using the empirical combining laws or mixing rules [17], which are given by $\epsilon_{12} = \sqrt{\epsilon_1\epsilon_2}$ and $\sigma_{12} = (\sigma_1 + \sigma_2)/2$, where the subscripts 1 and 2 refer to the respective individual atoms. Both trypsin and lysozyme molecules are modelled as spheres with hydrodynamic diameters of 38 Å and 30 Å, respectively [12]. The mean volume density for the spherical protein can be calculated by $3N/(4\pi a^3)$ where N is a total number of atoms in a protein and a denotes a radius of the protein. The value of the mean atomic surface density for the silica, SiO_2, pore is 0.386 Å^{-2} [18].

Table 1
Numerical values for the Lennard-Jones constants ($A = 2D_0R_0^6$ and $B = D_0R_0^{12}$, and units are converted to Å and eV).

site-site	R_0 (Å)	D_0 (kcal/mol)	A (eVÅ^6)	B (eVÅ^{12})
C	3.8983	0.0951	28.9469	50795.2337
H	3.1950	0.0152	1.4023	745.8187
N	3.6621	0.0774	16.1917	19527.3227
O	3.4046	0.0957	12.9264	10065.7103
Si	4.2700	0.3100	162.9665	493896.0409

Since there are two different interactions from SiO_2 pore, namely a silicon site and an oxygen site, the total energy may be obtained as a proportional ratio of the mean surface density, one-third silicon and two-third oxygen [18]. Trypsin and lysozyme comprise four different types of atoms, namely carbon, hydrogen, nitrogen and oxygen. Similar to the approach for SiO_2 [18], the total potential energy can be determined based on the ratio of the different atoms.

3. Interaction energy

In this paper, we assume that the adsorption of protein molecules into silica pores is attributed to a process generally referred to as physical adsorption, or physisorption, caused by van der Waals forces. To model the van der Waals interaction between a protein molecule and a pore of silica we use the Lennard-Jones potential and a continuum approach. Here, we study proteins trypsin and lysozyme to compare our results with Kisler et al. [12]; they are modelled as hard dense spheres [6–11]. For simplicity, we also assume the isoelectric point (pH associated with zero net charge) for both proteins [19], so that we may neglect the electrostatic energy in the present model. Further, to compare our results with those of Kleestorfer et al. [20] we assume the silica pore to have a cylindrical structure with free space inside.

In the following subsection, we determine the interaction energy between a spherical protein and a single atom. By assuming that the single atom is now located on another spherical protein or on a surface of cylindrical pore, the interaction between two spheres and the interaction between a sphere and a cylindrical pore are determined as shown in Subsections 3.2 and 3.3, respectively.

3.1. Interaction between a spherical protein and an atom

Here, we determine the interaction energy between a dense sphere and an atom, a configuration shown in Fig. 1. In the

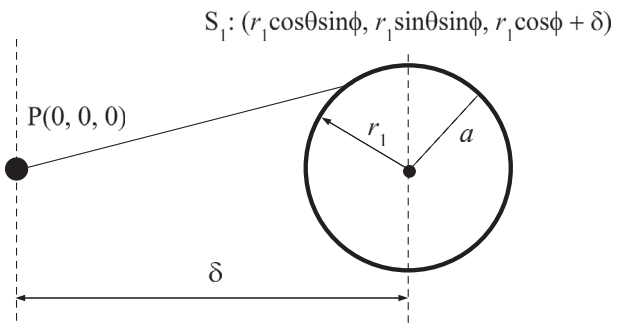

Fig. 1. Interaction between a sphere and an atom.