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Kinetics of adsorption of lipase onto different mesoporous materials: Evaluation of Avrami model and leaching studies

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ABSTRACT

Mesoporous silica materials were synthesized by hydrothermal route and covalently modified with glutaraldehyde. The adsorption (chemical adsorption and physical adsorption) of enzyme *Candida rugosa* lipase onto pure and functionalized mesoporous silica materials having different pore diameter were studied. Kinetics of adsorption was evaluated by Avrami model and specific kinetic parameters *n* and *k* were determined experimentally. Leaching studies revealed that chemical adsorption via covalent binding was less prone to leaching. Maximum loading capacity of each mesoporous material was analyzed and the result showed that MCF-25G has highest adsorption capacity. It was also observed that MCF-25G has highest stability towards enzyme binding.

1. Introduction

The adsorption of a protein molecule on solid support is a worthy scientific problem due to their applications. Mainly it is adapted in biorelated technologies such as medical implant, bio-sensing and drug delivery. It is used to understand protein surface interaction. The structure of support should influence adsorption. The first step to an efficient adsorption process is the search for an adsorbent with good capacity and long lifetime and availability at economical cost. Adsorption process is widely used for dye removal, waste water treatments and enzyme immobilization [1].

Different supports are used for this process. The use of naturally occurring easily biodegradable materials having low cost are preferred as supports. Among them most important is chitosan and clay. A porous material having high surface area should be good adsorbent and the time taken for attaining adsorption equilibrium is less. Hata et al. first reported the effect of pore size and influence of solvent on the loading [2]. Vallet-Regi and co-workers confirmed the role of pore size as an important factor determining the adsorption and release of biomolecules [3]. There are other pieces of evidence where pore size has effectively altered the loading and release of protein. Hartono et al. describes that, for enzyme immobilization inside mesoporous silica pore size is a crucial factor [4]. Due to the small pore size of the MCM-type (Mobil Composition of Matter) materials, the molecular size that could be incorporated and loaded inside the pores was limited to 2–3 nm. Therefore, larger organic molecules, proteins, enzymes and DNA could not be used. Rate of adsorption influences several experimental variables such as solution pH, temperature, ionic strength, protein size and bulk and surface concentration [5]. Small pores tend to limit the diffusion of enzyme through the pore, while large pores increase the possibility for enzyme immobilization. The pore diameter of mesoporous materials is large enough to allow penetration of the protein molecule into the large internal surface area/pores of these materials. A more of protein molecule is adsorbed in modified silicate materials than MCM-41 (Mobil Composition of Matter No. 41) as shown by Deere et al. [6]. Therefore mesostructure has a significant effect on bio-adsorption. Variety of mesoporous materials have been synthesized with varying sizes, shape and morphology. They provide easy and direct access to host large molecules mesoporous silica material therefore hold great promise for use as support to immobilize enzymes [7–9]. Cytochrome C is a small (12,384 Da) redox protein, with an approximate spherical diameter of 40 Å immobilized on MPS as explained by Diaz and Balkus [6,10]. The various adsorption protocols used with lipases have been extensively reviewed by Malcata et al. [11] and Bajaj et al. [12]. A great number of synthetic or natural carriers with different shapes/sizes, porous/non-porous structures, different aquaphilicities and binding capacity have been used for lipase immobilization.

Most of the supports usually bind from 2 to 50 mg protein per gram of support. While some supports are claimed to bind as high as 170 mg protein per gram of support, such high binding capacity

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may result in steric interference problems and loss of enzyme activity [13]. In general, the maximum adsorption is observed at pH close to the isoelectric point of the lipase. In addition, porous supports are superior to nonporous supports for immobilization of lipases due to their greater surface area. However, porous supports must have an internal morphology that allows not only the lipase binding but also an easy accessibility to substrate molecules in order to minimize diffusional limitation. The pore sizes best suited for lipase adsorption are at least 100 Å in diameter as identified by Mojovic et al. [14,15]. Ji et al. observed that the adsorption capacity and rate of adsorption is dependent on the solution pH, protein, pore size, pore volume, morphology and protein properties [16,17]. Ajitha et al. also studied influence of pore diameter and solution pH on adsorption of enzymes. The highest equilibrium capacity of 220 mg amylase/g supports was achieved for mesoporous silica material having 84 Å pore diameter. The main factors for protein adsorption on pure silica supports are strong interactions between the surface silanol groups and the surface charge of protein molecules. The interactions include hydrophobic interactions, electrostatic attraction, the intermolecular cohesive attraction and repulsion. The hydrated silica surface includes hydrogen bonded structure [18]. These results revealed that interaction between protein and mesoporous material is an important parameter which controls the amount of protein adsorption.

Surface modified silica shows higher protein adsorption capacities compared to the unfunctionalized one. Pore surface may be functionalized with chemical species to modify their adsorption properties. This makes them suitable to host different species and to protect them for long time, under appropriate conditions. In aqueous solution, the amino groups of amino functionalized silica are much easier to cationize and they adsorb enzyme anions strongly by electrostatic attraction. The ionic strength was found to strongly influence the amount of protein adsorbed, with little absorption occurring at high ionic strengths [6].

A simple kinetic analysis of adsorption of protein shows the pseudo-first-order. The kinetics of protein binding on silica was proven by KEKAM model. A group of authors developed a general kinetic equation known as a Kolmogorov–Erofeev–Kazeeva–Avrami–Mampel (abbreviated KEKAM) equation [14,15]. This model implies that the reaction is located on the surface active sites of the solid support. The physical meaning of k and n parameters is derived from the KEKAM equation this is the main advantage of KEKAM equation. The most important factor in the Avrami kinetic model is the constant n, the values of which can be used to verify possible alterations of the adsorption mechanisms in relation to the contact time and the temperature. Generally, n may be considered as the criterion that determines the area where these heterogeneous reactions occur. The value of determines whether the adsorption process may be limited by surface reaction ($n > 1$) or not. The main disadvantage of this model is sometimes it shows the presence of two and/or three linearized regions, in relation to the time and adsorption temperatures. In this manner, two and/or three independent sets of values of n ($n1$–$n3$) and k ($k1$–$k3$) can also be considered.

Previous work done in this field with zeolite shows low binding capacity due to low surface area and pore diameter compared to mesoporous silica material. They also possess hydrophobic nature. Pure silica materials are hydrophilic in nature and glutaraldehyde modified silica materials are hydrophobic. Kinetics of lipase binding on mesoporous silica materials was not studied in detail. The kinetics of enzyme binding on mesoporous silica having different morphology and pore size has not been reported. Catalysis by immobilized enzyme depends on the binding of enzyme to the support and it necessitates the study of kinetics of enzyme adsorption.

In present study, the dependence of time and temperature in adsorption and specific kinetic parameters were evaluated by Avrami model. The mechanism of lipase binding to mesoporous silica materials and dependence of pore size were estimated. The binding capacity of the support can affect maximum loading behaviour as well as leaching of enzyme. MCF like support has strong affinity for the protein and the process happens fairly quickly and it can afford large amount of enzyme. The activity of immobilized enzyme was monitored by the hydrolysis of *p*-nitrophenyl palmitate. Its stability was checked by repeated batchwise experiments.

2. Experimental

2.1. Materials

Tetraethyl orthosilicate (98%), Pluronic P123, n-decane (98%), 1,3,5-trimethyl benzene (TMB), 3-aminopropyl triethoxysilane (3-APTES 98%), glutaraldehyde (25%) and *Candida rugosa* lipase were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., Bangalore. All other chemicals were of the highest purity available commercially.

2.2. Synthesis of different mesoporous materials

The mesoporous silica materials were prepared in aqueous hydrochloric acid using triblock co-polymer surfactant Pluronic P123 (poly (ethylene oxide)-block-poly (propylene oxide)-block-poly (ethylene oxide), EO_{20}-PO_{70}-EO_{20}, $M_{av} = 5800$) [19]. Two different organic swelling agent agents were used to increase the pore diameter. P123 (2 g, 0.3 mmol) was dissolved in 2 M HCl containing n-decane [20] or TMB at 45 °C. The resultant solution was rapidly mixed with silica precursor under vigorous stirring to form a reactive gel having composition 1 g P123: 1 g auxiliary chemical: 2 g TEOS: 8 g HCl. pH of the resulting mixture was maintained below 2 (pH < 2). The synthesis was carried out under conventional hydrothermal conditions by treating the precursor gel at 130 °C for 48 h in Teflon lined autoclave. The solid samples were separated by filtration, washed thoroughly with deionized water, 1% ammonium nitrate and finally with 5% aqueous ethanol. It was dried at ambient temperature calcined at 600 °C for 12 h. Silica material synthesized without organic swelling agent designated as MS-9 and with n-decane and TMB are designated MS-13 and MCF-25 (Meso-cellular silica foams) respectively.

2.3. Functionalization of silica support

The synthesized silica was amino functionalized by condensing 0.5 g solid with 1–5 mmol 3-APTES solution in 100 mL acetone at 40 °C for 8 h with constant stirring under an inert nitrogen atmosphere. Products were separated by filtration washed with soxhlet extraction, dried at ambient temperature. This amino functionalized silica sample was treated with 1–5 mmol solution of glutaraldehyde in distilled water for 8 h at 40 °C. The products were cooled, filtered and washed with distilled water till excess glutaraldehyde was removed which was tested by Tollens reagent then dried at 60 °C for 6 h [21]. Glutaraldehyde functionalized samples were designated as MS-9G, MS-13G and MCF-25G. Abbreviations of different mesoporous silica nanoparticles prepared were listed and described in a Table 1.

2.4. Characterization of pure, functionalized and immobilized silica materials

The X-ray diffraction measurements of the samples were taken on a Panalytical Xpert PRO MPD model with Ni filtered Cu Kα radiation ($\lambda = 1.5406$ Å) within the 2θ range 0.1–5° at a speed of 0.25°/min at room temperature. Nitrogen physisorption measurements were done in a Micromeritics Tri-Star 3000 surface area and