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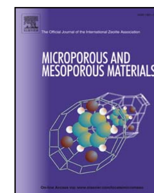

Batch adsorption/desorption for purification of scFv antibodies using nanozeolite microspheres

Ali Mesgari-Shadi[a,b], Mohammad-Hossein Sarrafzadeh[a,**], Baharak Divband[c], Jaleh Barar[b,d], Yadollah Omidi[b,d,*]

[a] Biotechnology Group, School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran
[b] Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Inorganic Chemistry Department, Faculty of Chemistry, University of Tabriz, Tabriz, Iran
[d] Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Zeolites are known as good adsorbents with ion exchange capacity because of their unique physiochemical properties. Their capability of metal ion immobilization makes them a good candidate as an alternative to the chromatography resins. In this study, two types of nanozeolite microspheres including ZSM-5 (Si/Al ∼ 27) and 13X (Si/Al ∼ 1.3) were synthesized. The Ni^{+2} ions were immobilized on the synthesized zeolites along with a local clinoptilolite (Si/Al ∼ 7.53). Batch adsorption/desorption experiments were performed for the purification of the scFv antibodies using these zeolites. All the zeolites showed a high-speed adsorption kinetics with the shortest time for ZSM-5-Ni^{+2}, reaching the maximum adsorption (100%) in less than 3 min in the pHs ranging from 6.5 to 7.5. In addition to the metal affinity, the hydrophobic property showed to be one of the most effective factors on the adsorption of scFvs. As a result, the less hydrophilic zeolite ZSM-5 was found to be the best adsorbent of scFvs. The mixed mode performance of zeolites by the combination of the metal affinity and the cation exchange properties were analyzed. The Ni^{+2}-immobilized natural clinoptilolite was the only one that displayed a mixedmode capability. In the elution step, the Ni^{+2}-immobilized ZSM-5 provided a markedly higher scFv purity (∼90%) with an acceptable purification yield (60%) just by the metal affinity mode. Taken all, relatively short desorption times (∼10 min) of the scFv antibodies along with the high adsorption/desorption yields make the ZSM-5-Ni^{+2} as a promising metal affinity chromatography media for the low concentration scFv solutions.

1. Introduction

Zeolites are crystalline aluminosilicates with a well-defined structure. Although zeolites are known as microporous nanomaterials, their mesoporous and macroporous structures could be synthesized [1,2]. The plethora of pores and cavities of zeolites make them useful materials for the separation of different molecules and accommodation of the organic and inorganic compounds.

Most of the adsorption capacity of the zeolites are due to the presence of micropores, joining of which makes pores with different sizes ranging from 0.1 to 1 (μm). Such cavities and micro-ducts may function as the passageway for the traverse of the molecules and also entrap macromolecules such as antibodies (Abs). Mechanistically, molecules can be adsorbed to the entrance of the pores and then diffuse into deeper spaces of the cavities of particles [3]. Due to the zeolite's substantial adsorption capacity, much more applications have been developed for the utilization of zeolites as catalysts, adsorbents and ion exchangers. There are several physical properties that can influence the adsorption performance of zeolites, including surface hydrophilicity (based on Si/Al ratio), surface charge density, particle size and pore diameter.

Protein adsorption on a surface is a complex process affected by several factors such as surface properties of protein and adsorbent, protein type, pH, and ionic strength [4,5]. Zeolites, as well-known adsorbents, could be used as the protein sorption surfaces. Zeolite morphology and its surface characteristics were shown to have a great potential for the selective adsorption of proteins [6].

Electrostatic interactions are considered as the main mechanisms that may influence the protein adsorption on a surface. Charge intensity on the surface could affect the protein adsorption along with the protein

* Corresponding author. Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran.
** Corresponding author. School of Chemical Engineering, College of Engineering, University of Tehran, Tehran, Iran.
E-mail addresses: sarrafzdh@ut.ac.ir (M.-H. Sarrafzadeh), yomidi@tbzmed.ac.ir (Y. Omidi).

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structure and functionality. Zeolites possess a negative charge distribution in their framework that causes the cations and positively charged proteins to be adsorbed. The charge intensity on the surface of the protein and zeolite cavities can directly influence the quality and magnitude of the adsorption [7,8].

Wu et al. [9] showed that surface and protein charges were not the unique governing mechanisms in the protein adsorption to the zeolite surface. They showed that, in addition to the zeolite types, the pore size could significantly influence the protein adsorption and even its activity. Accordingly, The zeolite's pore sizes are the main controlling factors that might alter the protein binding capacity [10]. Thus, in the case of the microporous zeolites, the main fraction of the proteins might bind to their outer surface, while the large mesopores of the zeolites could efficiently adsorb the macromolecules [11].

Negative charge distribution in the structure of zeolites gives them the cation exchange potential, which could be exploited for the adsorption and desorption of proteins [12]. This capacity may rationalize the zeolites utilization as the cation exchange chromatography resins for the separation of proteins. Since more than two decades ago, different types of zeolites have been tested for the separation and purification of proteins such as Abs [10,13–16].

Controlling the Si/Al ratio during the synthesis can produce different types of zeolites with distinct hydrophilic properties. Generally, the higher the Si/Al ratio is, the less the hydrophilicity of zeolites will be. This can consequently result in the lower cation exchange capacity of zeolites [17].

The immobilized-metal affinity chromatography (IMAC) is considered as one of the most common methods used for the purification of the recombinant proteins containing a histidine (His) tag. This technology was developed based on the affinity of the transition metal ions to the histidine in the protein structure. Despite some limitations, this method has widely been used for the purification of a number of commercial protein-based pharmaceuticals [18].

Owing to the different characteristics and capabilities of zeolites tailored during various synthesis and development methods, they have found many versatile applications [19,20]. By the immobilization of desired metal ions (e.g., Ni^{+2}) on the zeolite structure, a carrier might be established for the IMAC-based separation of different types of the macromolecules such as Abs and biotech drugs [21]. Xu et al. [22] immobilized Co^{+2} on the cation sites of a nanozeolite to separate the His-tagged proteins. Zhang et al. [21] showed that, in addition to the protein structure, the type of the transitional metal ions used in the zeolite crystals could significantly affect the protein separation performance.

Single chain variable fragments (scFvs) are Ab fragments used as valuable tools for the diagnostic and therapeutic purposes [23–26]. In comparison with the whole antibodies, these small-size Ab show some advantages, including high tissue penetration capability, easy handling and expression in the microorganisms, and targeted delivery of various drugs [27,28].

Downstream processing of the biopharmaceuticals could significantly and directly influence the quality and the cost of the production of biotech drugs. Chromatographic methods, as the heart of the protein purification process, play a major role in the downstream processing of protein drugs. Despite the high performance of the affinity chromatography resins in the purification of protein-based drugs, cost limitations have enforced many researchers to investigate on their replacement with new chromatography media [29,30]. For this purpose, the cation exchangers and IMAC methods epitomize the alternative approaches [31].

In this current study, the adsorption/desorption capability of three different synthesized and natural types of zeolites modified with Ni^{+2} immobilization were investigated. Further, the potential of these structures was evaluated in terms of the purification of scFvs. The kinetics of adsorption and desorption and the effects of pH were also studied. Having capitalized on the zeolite-based chromatography media, the effects of morphological factors (e.g., zeolite surface area and pore diameter) on the adsorption capacity of scFvs were evaluated. Further, the capability of modified zeolites as IMAC resins along with the mixed mode applications, by combination of the metal affinity and cationic exchange properties, were evaluated.

2. Experimental

2.1. Materials

Tryptone was purchased from Quelab Laboratories Inc. (Montreal, Canada). Yeast extract was obtained from HiMedia Laboratories Pv.t Ltd (Mumbai, India). Glycine and Tetramethylethylenediamine (TEMED) for SDS-PAGE were purchased from Sigma-Aldrich Corporation (Steinheim, Germany). All other chemicals used in this study were from Merck Company (Darmstadt, Germany).

For the synthesis of zeolites, all of the chemicals were directly used without further purification. The local clinoptilolite ($Si/Al \sim 7.53$) was obtained and treated by an acid washing process, which was named as zeolite type C.

2.2. Fabrication of the template–free nanozeolite microspheres

Template–free nanozeolites (NaZSM-5 and 13X) microspheres were hydrothermally synthesized according to the following procedures.

For the synthesis of the template–free NaZSM-5, about 50 mmol sodium hydroxide and 6 mmol sodium aluminate solutions were mixed with the silica sol (25 wt. %). The mixture was vigorously stirred for 12 h. Then, the obtained gel was transferred to a homemade autoclave reactor (100 mL) and treated hydrothermally at 180 °C for 32 h. Finally, the product was filtered and washed with the distilled water and dried at 90 °C. The prepared powder sample was named Z.

For the preparation of the template–free nano 13X microspheres, 10 mmol sodium hydroxide and 25 mmol sodium aluminate were dissolved in deionized water and mixed with the proper amount of silica sol (25 wt. %) under vigorous stirring for 8 h. Then, the slurry was transferred to the autoclave reactor and treated hydrothermally for 12 h at 90 °C. Finally, the product was filtered and washed with distilled water and dried at 80 °C. This prepared powder sample was named X.

2.3. Immobilization of the Ni^{+2} on the zeolites

The immobilization of Ni^{+2} on the two synthesized zeolites along with the type C were performed by the liquid phase ion exchange (LPIE) method using saturated solutions of Ni $(NO_3)_2.6H_2O$ at the ambient temperature for 72 h. After the exchange of the Ni^{+2} the zeolite suspensions were filtered and washed with the copious amounts of deionized water and finally were dried. These Ni^{+2} immobilized zeolite samples were named Z-Ni^{+2}, X-Ni^{+2}, and C-Ni^{+2}.

2.4. Zeolite characterization

The particle sizes and morphology, metal dispersion, and crystalline structures of the nanozeolite microspherses were determined by the scanning electron microscopy (SEM), MIRA3 TESCAN, equipped with the energy dispersive X-ray (EDX) facility and by the X-ray diffraction (XRD) using a Siemens D500 diffractometer with Cu kα radiation ($\lambda = 1.5418$ A and $2\theta = 4–80°$) at room temperature. Single point surface area estimated for the zeolites using Brunauer–Emett–Teller (BET) was measured by N2 adsorption-desorption isotherm at liquid nitrogen temperature using NOVA2000 (Quantachrome, USA).

2.5. The scFv antibody analysis

The soluble scFv concentration and the adsorption/desorption portion determinations were based on the densitometry analysis using

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