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Synthesis and chromatographic characteristics of iminodisuccinic acid-functionalized silica stationary phase

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ABSTRACT

In this study, we developed a multifunctional chromatographic medium by using iminodisuccinic acid (IDS) as ligand, γ-glycidoxypropyltrimethoxysilane (γ-GLDP) as spacer arm and silica as matrix. The medium was characterized by fourier transform infrared spectrometry (FT-IR). Binding capacity of IDS on chromatographic medium was determined by potentiometric titration. The effect of mass ratios (w/w) between silica and IDS on the medium synthesis was discussed. The optimal ratio of matrix, spacer arm and ligand was 1.5 g: 2.0 mL: 4.6 g. Based on different chromatographic modes, protein mixtures of bovine serum albumin, ribonuclease and lysozyme were successfully separated with IDS-Silica column and IDS-Cu(II)-Silica column, respectively. To examine metal chelating behavior of IDS-Silica stationary phase, optimal geometries and related parameters of complexes formed by IDS with Fe^{3+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+} and Ca^{2+} were achieved by quantum computing. The stabilities of the complexes were predicted according to the results of theoretical calculation. Sorption capacities of IDS-Silica stationary phase for metal ions were determined by using offline frontal chromatography (FC) combined with inductively coupled plasma atomic emission spectrometry (ICP-AES). A good agreement was found between simulating results and experimental observations. The order of chelating strength of IDS-Silica medium for metal ions was $Fe^{3+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+} > Ca^{2+}$. Compared with IDA-Silica, Asp-Silica and Glu-Silica stationary phases, IDS-Silica displayed a stronger chelating property for metal ion.

1. Introduction

In recent years, chromatographic stationary phase synthesized by using aminocarboxyl chelators as ligands have become powerful tools for the separation and purification of biological macromolecules [1–3].

This kind of ligands has the characteristics of cation exchange for protein and chelating property for metal ion. Ligands immobilized with metal ion can be used as immobilized metal affinity chromatography (IMAC) column, which can be applied for separating and purifying the proteins with affinity for metal ion. These ligands include tricarboxylic acid ethylenediamine (TED) [4], L-glutamic acid (L-Glu) [5], L-aspartate (L-Asp) [6] and iminodiacetic acid (IDA) [1,7], etc. Among them, IDA is the most widely used. Although these chelators have good cation exchange features, the chelating strengths of the chelators for metal ion are not strong enough, which limits applications of IMAC column in separation and purification of proteins.

As illustrated in Fig. 1, IDS [8] is a pentadentate aminocarboxyl chelator with five coordination atoms, which can provide lone pair electrons for metal ion. On the basis of the strong coordination of IDS for metal ion, the complex formed by IDS with metal ion should be more stable. At present, the researches about metal chelating property of IDS were mainly focused on using IDS as a chelator in aqueous solution. In comparison with EDTA, IDS in aqueous solution showed approximate and even better chelating performance for most metal ions [9]. IDS has become a preferred reagent to remove heavy metal ions from industrial wastewater due to its strong chelating property for metal ion, and environment friendly characteristics [8,9].

Based on the strong metal chelating property of IDS, if it can be chemically bonded to the silica support, the obtained IDS-Silica stationary phase will have the following advantages: 1) Column operation of the stationary phase also can remove the metal ion from the solution. Compared with IDS in aqueous solution, IDS-Silica stationary phase has the economy of repeated use and the convenience of continuous operation. Multiple distribution-equilibriums of metal ion in solid-liquid two phases can meet increasingly stringent environmental requirements. 2) Using IDS in aqueous solution, the function of IDS is single and only as a chelator

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to remove the metal ion from the solution. If IDS is bonded on to the silica monomer, IDS-Silica stationary phase itself is a typical weak acidic chromatographic packing with cation-exchange property. Furthermore, the stationary phase immobilized metal ion can be used as IMAC packing for purifying protein. This chromatographic mode with dual functions can greatly enrich the applications of IDS. (3) Compared with other aminocarboxyl chelators with low-dentate, IDS with five coordination atoms should have stronger chelating ability for metal ion. Through the systematic study of this novel-type IMAC packing, a method to relieve the leakage of metal ions from IMAC column in the process of protein purification can be proposed.

Currently, the preparation and applications of IDS-Silica stationary phase have never been reported. In this paper, a new type of IDS-Silica stationary phase was synthesized according to an indirect method by putting forward to use IDS as ligand, silica gel as matrix and γ-GLDP as spacer arm for the first time. Multifunctional characteristics of novel separation medium were discussed in details.

2. Experimental

2.1. Materials and reagents

Silica gel (7 μm, 300 Å) was obtained from Lanzhou Institute of Chemical Physics (LICP) of the Chinese Academy of Sciences (Lanzhou, China). γ-GLDP was purchased from Gaixian Chemical Engineering Institute (Liaoning, China). IDS were kindly provided by Desai Chemical Engineering Co., Ltd. (Hebei, China). EDTA was purchased from East China Reagent Factory (Tianjin, China). $CuSO_4.5H_2O$, $FeCl_3.6H_2O$, $NiCl_2.6H_2O$, $ZnCl_2$, $CoCl_2.6H_2O$ and $Ca(NO_3)_2.4H_2O$ of analytical regent (AR) grade were all purchased from Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Other reagents were all from Xi'an Chemical Reagent Factory (Xi'an, China). All the solutions were prepared with ultra-pure water.

Bovine serum albumin (BSA), Ribonuclease (RNase) and Lysozyme (Lys) were purchased from Sigma Company (St. Louis. Mo., USA). 2.0 mg/mL protein mixtures of BSA, RNase and Lys were prepared with 20 mM phosphate buffer (PB) (pH 6.0).

2.2. Apparatus

ÄKTA purifier 10 (Amersham Biosciences, Sweden) was used for chromatographic experiments. Thermostatic water bath HHS21-4 (Shanghai, China) was for the synthesis of stationary phase. Automatic potentiometric titration instrument ZDJ–4B (Shanghai, China) was for determining the binding capacity of IDS ligand. Fourier transform infrared spectrometer (FT-IR VERTEX70, Bruker Co., Germany) was used for the characterization of silica and IDS-Silica stationary phase. Inductively coupled plasma atomic emission spectrometer (ICP-AES ULTIMA 2, HORIBA Jobin Yvon Co., France) was used for determining the sorption capacities of metal ions on IDS-Silica column. Potentiometer TitraMate 20 (Mettler Toledo, Switzerland) was used for adjusting the pH of the solution. Slurry packing apparatus 124PP (Chemico, Japan) was used for column packing. Pure-water instrument UPD-II-107 was used for preparing ultra-pure water (Xi'an, China). Quantum computing was performed with Gaussian 09 packages (Revision D.01, Fox, D. J. Gaussian, Inc).

2.3. Synthesis of IDS-Silica stationary phase

4.6 g of IDS was dissolved in 40 mL H_2O. The pH of solution was adjusted to 10.0–11.0 with 1 M NaOH followed by the addition of 2 mL γ-GLDP. The reaction was carried out at 65 °C for 12 h with stirring. Then the pH of reaction solution was adjusted to 4.0 with 6 M HCl. 1.0 g, 1.5 g, 2.0 g, 3.0 g and 4.0 g of silica were added to the reaction solution, respectively. The reaction was performed at 90–95 °C for 2 h with stirring. The obtained IDS-Silica stationary phases with different ratios of silica and IDS (Silica:IDS = 1.0:4.6, 1.5:4.6, 2.0:4.6, 3.0:4.6, 4.0:4.6, w/w) were washed successively with water, 10% acetic acid and water again.

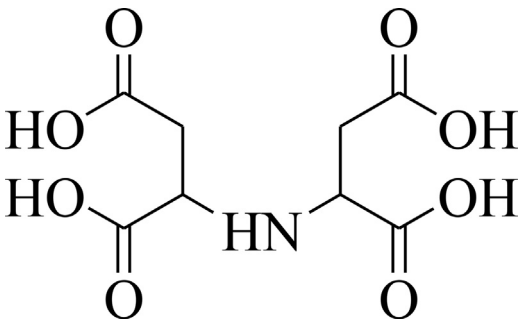

Fig. 1. Molecular structure of IDS.

2.4. Infrared characterizations of silica and IDS-Silica stationary phase

The dry silica and IDS-Silica stationary phase were added with 0.1 g of KBr separately, then the mixture samples were ground and characterized by FT-IR using the KBr pellet (pressed-disk) technique. The spectra of the samples were recorded in the range of 4000–400 cm^{-1} with an average of 16 scans at a spectral resolution of 4 cm^{-1}.

2.5. Column packing

IDS-Silica slurry was prepared by using isopropyl alcohol (99.7%) as slurry solvent. At the pressure of 40 MPa, 20 mL slurry was packed into a stainless-steel column (50 × 4.6 mm I.D.) with isopropyl alcohol for 10 min, then with ultra-pure water for 10 min. Performance testing of packed columns was made with 0.1 mol/L $NaNO_2$. The plate number and asymmetry factor (A_S) for all the columns were similar, about 4100 N/m and 1.7, respectively.

2.6. Determination of binding capacity for IDS

1 g of IDS-Silica stationary phases with different ratios (Silica:IDS = 1.0:4.6, 1.5:4.6, 2.0:4.6, 3.0:4.6, 4.0:4.6, w/w) were immersed in 10 mM HCl for 12 h, respectively. After filtering the solutions, 20 mL H_2O and 5 mL of 1 M NaCl were added. The pH values of the solutions were adjusted to 2.0 with 0.1 M HCl. The acid-base potentiometric titration was performed with 0.0100 M NaOH. The binding capacities of IDS were determined by the titration volume of NaOH using the titration curve of γ-GLDP-Silica as a blank.

The binding capacity of IDS was calculated according to the Eq. (1):

$$Q = \frac{C \times (V_2 - V_1)}{3m} \quad (1)$$

where Q stands for the binding capacity of IDS ($\mu mol/g_{IDS\text{-}Silica}$), C is the concentration of NaOH standard solution (0.0100 mol/L), V_1 is blank volume (mL), V_2 is the titration volume of NaOH (mL), m is the amount of IDS-Silica stationary phase (g), the mole ratio of NaOH to IDS is 3:1.

2.7. Immobilization of metal ions

The packed IDS-silica columns (50 × 4.6 mm I.D.) was connected to the chromatographic system, then perfused with 50 mM $CuSO_4$

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