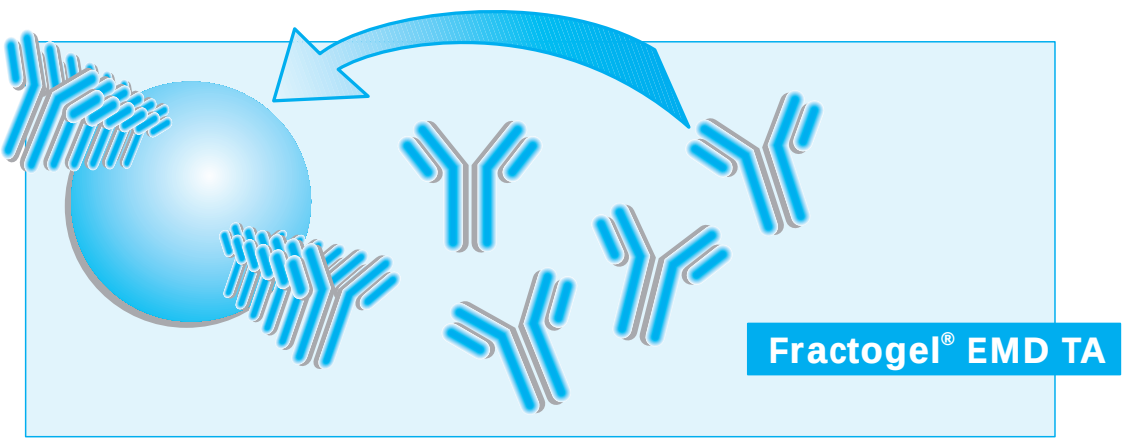
Thiophilic adsorption chromatography on Fractogel® EMD TA



Introduction

High performance affinity chromatography is one of the most powerful techniques for the isolation of biomolecules. The high efficiency of the method is based on the specific interaction of a covalently bound ligand with its counterpart immobilized on a chromatographic support. This adsorption is due to electrostatic forces between charged groups, hydrogen bonds and nonpolar or hydrophobic interactions. Apart from biospecific ligands such as receptors, substrates, cofactors and antibodies there is a wide range of applications using pseudo biospecific compounds like dyes, metal ions, amino acids or sulphur containing groups as ligands. The latter one is designed especially for the efficient isolation of antibodies. One important advantage of these pseudo biospecific ligands is their chemical stability.

Advantages of the tentacle mediated thiophilic adsorption

One disadvantage of conventional affinity chromatographic media is their relatively low protein binding capacity. In order to improve this, the amount of bound ligand should be increased. The new Fractogel® EMD TA for thiophilic adsorption chromatography is synthesized according to the tentacle technology, where the group specific ligands are present in a high density. Thus, the thiophilic tentacle material has a high protein binding capacity and is suitable to purify antibodies in an analytical as well as in a preparative scale.

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However, even more important than the absolute number of ligands, their spatial steric accessibility is related to a suitable capacity of an affinity gel. As known from the tentacle-type ion exchangers the linear polymer chains provide an appropriate spacing which results in minimized non-specific interactions with proteins combined with high protein binding capacities. The 3 S-type ligand which is present in Fractogel® EMD TA shows significantly increased thiophilic interactions compared to 2 S-type ligands which seem to resemble more closely to solely hydrophobic materials.

Application area

The main application area of the specific chromatographic support carrying a sulphur containing ligand is the isolation of proteins with thiophilic regions and peptides with aromatic amino acid residues. The corresponding technique is called thiophilic adsorption chromatography. This chromatographic method is based on a salt promoted adsorption of proteins to a sulfone and thioether containing heteroaliphatic ligand. The binding of the protein takes place mainly via accessible tryptophane and / or phenylalanine residues. Corresponding motifs can be observed within conserved regions of various antibodies.

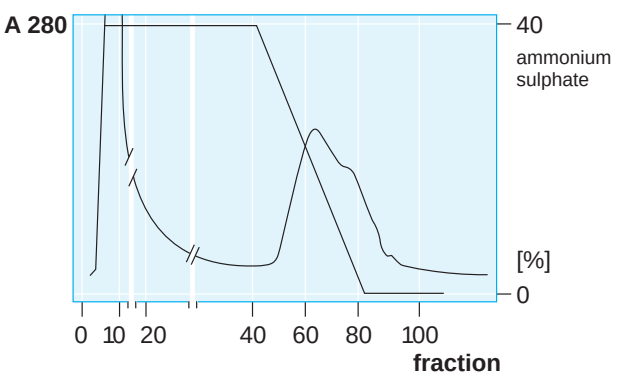

Fig. 1. Separation of polyclonal antibodies from human serum using Fractogel® EMD TA. The first peak contains albumin and other contaminating proteins. Immunoglobulines (IgG, IgM, IgA) can be eluted with a decreasing salt gradient.

Therefore, thiophilic adsorption chromatography is very useful for the purification of immunoglobulins (monoclonal and polyclonal antibodies). Albumins are not adsorbed on thiophilic media, which often simplifies the effective separation of antibodies (Fig. 1).

Antibodies of the IgM sub-class can also be bound on a Fractogel® EMD TA column. The binding of antibodies from different species to thiophilic adsorption supports offers advantages compared to the Protein A method. All antibodies tested so far bind to Fractogel® EMD TA (Tab. 1). Due to the gentle elution conditions at physiological pH values high recoveries of biological active antibodies can be obtained.

Certain other proteins and peptides carrying thiophilic areas located on their surface can also be isolated by this technique, using Fractogel® EMD TA. Operating at high concentrations of ammonium sulphate, Fractogel® EMD TA creates hydrophobic properties providing special selectivities for hydrophobic proteins.

Table 1: Antibody-binding characteristics of Protein A, Protein G and Fractogel® EMD TA

Source of antibody and subclass	Fractogel TA	Protein A	Protein G
Human IgG_1	+	+	+
Human IgG_2	+	+	+
Human IgG_3	+	-	+
Human IgG_4	+	+	+
Human IgM	+	+	-
Human IgA	+	+	-
Mouse IgG_1	+	weak	+
Mouse IgG_{2a}	+	+	+
Mouse IgG_{2b}	+	+	+
Mouse IgG_3	+	-	+
Rat IgG_1	+	weak	+
Rat IgG_{2a}	+	-	+
Horse IgG	+	-	+
Goat IgG	+	+	+
Chicken IgG	+	-	-
Bovine IgG	+	weak	+
Rabbit IgG	+	+	+
Sheep IgG	+	-	+
Dog IgG	+	+	-
Pig IgG	+	+	+
Cat IgG	+	+	-
Chicken (yolk) IgY	+	-	-
recombinant ab (scFv)	+	-	-

Stability of the gel

Fractogel® EMD TA is very stable and can be used for various applications. The sulfur containing ligand itself is covalently attached. Thus, no leakage of the ligand occurs. The gel is stable from pH 1 up to pH 14. However, prolonged exposure to extreme pH-values should be avoided. There is no restriction using the gel between pH 2 and pH 12 . Several hundred runs can be performed without loss of resolution or capacity. Due to the high pressure stability of the gel up to 20 bar high flow rates can be applied. The particle size of this high resolution gel is in the range of 20-40 µm.

Protein binding capacity

The protein binding capacity for γ–globulin is significantly higher for the tentacle-type thiophilic gel in comparison to conventional materials prepared by other surface modification techniques. For Fractogel® EMD TA a protein binding capacity of 30 mg γ-globulin per milliliter of gel can be achieved. Due to the hydrophilic properties of the tentacles, unspecific hydrophobic interactions are minimized and high mass recoveries can be obtained.

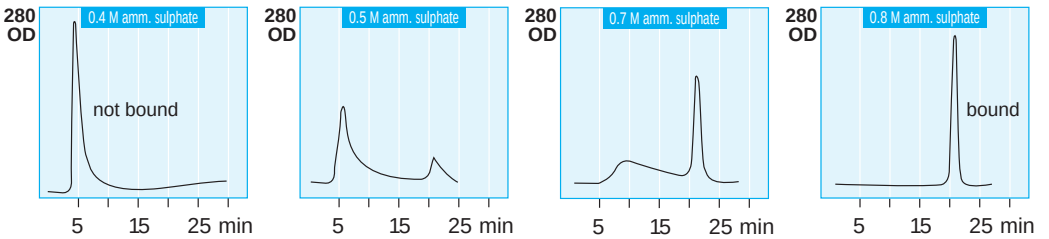

Fig. 2. Salt promoted adsorption of human IgG on Fractogel® EMD TA.

Separation method

Chromatography on Fractogel® EMD TA can be integrated easily into a purification scheme - for example subsequently to an ammonium sulphate precipitation step - since the thiophilic adsorption takes place in the presence of high salt concentrations. The influence of the salt concentration on the binding efficiency is shown in figure 2. The best results for antibody isolations are obtained at ammonium sulphate concentrations between 0.8 M and 1.5 M.

In general, the equilibration of the column should be performed with 20 mM phosphate buffer containing 0.8-1 M $(NH_4)_2SO_4$ at a pH value of 7.0-8.0. Gradient elution generated with buffers containing low salt concentrations are used for the elution of bound immunoglobulins. For example, the elution can be achieved applying a linear or step-wise gradient generated by 20 mM phosphate buffer (pH 7.0-8.0) without ammonium sulphate. Sometimes the addition of 0.5 up to 1 M NaCl in the elution buffer is suitable.

Regeneration:

Short term treatment with sodium hydroxide solution (0.1 to 0.5 M) is best suited for the regeneration of Fractogel® EMD TA. Regeneration can also be performed by rinsing with 50% ethylene glycol. Another successful method to remove tightly bound or denaturated material from the column is to rinse with 20% ethanol or 6 M urea. For regeneration of Fractogel media with organic solvents a linear flow rate of 1cm/min should not be exceeded.

Ordering information

Catalog No.	Content	Description	Particle size	Type of chromatography
1.16473	25, 250 ml	Fractogel® EMD TA (S)	20-40 µm	thiophilic adsorption

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