

SERVACEL®

Cellulose Ion Exchangers - Overview

In contrast to the above mentioned resin based exchangers and adsorbents, cellulose ion exchangers have a very loose hydrophilic network (no pores) with well separated charged groups. Ionic macro(bio)molecules are adsorbed to only a few sites which can be desorbed selectively under extremely mild conditions with high recoveries. pH stability is excellent between 3 and 12.

In order to provide greater stability and pressure resistance, the cellulose matrix can be produced in »regenerated« microcrystalline form. Here the particles are granular and the amorphous (fibrous) part of the cellulose fibers is removed. For additional stabilization the fibers are crosslinked.

SERVACEL® CM 52 and DEAE 52 allow a more dense bed packing: superior resolution can be obtained. The fibrous materials on the other hand can be operated at higher flow rates at somewhat reduced resolution. To facilitate the handling we offer the most common types as "preswollen" material: Thus time consuming preparations (see below) of the exchangers are not necessary.

■ Anion exchangers (basic types)

DEAE Cellulose:

DiEthylAminoEthyl-groups are covalently bound to the cellulose hydroxylic groups. This anion exchanger can be applied for the chromatography of proteins with neutral or acidic isoelectric points (IP), which can easily be determined by isoelectric focusing electrophoresis (IEF). Serum components, enzymes, peptides, polysaccharides, RNA-types, viruses, lipids and ribosomes have been separated. DEAE cellulose is also suitable for purification of nucleic acids.

■ Cation exchangers (acidic type)

CM Cellulose:

Cellulose is derivatized with CarboxyMethylchloride. The resulting weakly acidic high capacity exchanger can be used for neutral and basic proteins, peptides, lipids.

Chemical Properties

Our cellulose ion exchangers are supplied as analytical grade. They are carefully washed with caustic, acid, methanol and water. Further washings are usually not required prior to use. Flow rate, particle size and capacity are rigorously controlled.

Although carefully purified, it is advisable to examine whether elution with the strongest of the used buffers yields material which might interfere with the analysis. Cellulose ion exchangers, especially the high substituted grades, tend to bleed soluble polysaccharides into solution (up to 30 ppm). In this case exhaustive washing with this eluent is recommended. For detection, 1 ml of eluent is mixed with 2 ml of 0.2 % solution of anthrone in sulfuric acid without cooling. If positive, a green colour develops, which can be diluted with glacial acetic acid and compared with a standard. Cellulases can degrade the ion exchangers (see below).

Capacity

Capacity determination:

The exchangers differ mainly in their capacity. This is determined by titration. 4 g of the preswollen types are dried over night at 50 °C (122 °F). 1 g is stirred for 5 minutes with 20 ml 0.5 M NaOH (basic exchangers) resp. 20 ml 0.5 M HCl (acidic exchangers). The suspension is quantitatively transferred into a funnel and washed with water until the eluent is neutral (ca. 200 ml). Rinse into a beaker, add 20 ml 1 M NaCl solution and (under stirring) titrate with 0.1 M HCl to pH 3 (anion exchanger) and 0.1 M NaOH to pH 10 (cation exchanger). The values obtained are not representative for large biomolecules (otherwise, 1 g of an ion exchanger with a capacity of e.g. 1 mM /g would bind 1 mM BSA, that means 67 g BSA).

Capacity for proteins:

The preswollen types (CM 52 and DEAE 52) are delivered in moist form (methanol). Using higher salt concentrations than recommended below may produce precipitation. Keep the preswollen adsorbent in a tightly closed container to prevent it from drying out

Cation and anion exchangers (preswollen):

1. Suspend 200 g of the package (corresponding to 40 - 60 g dry ion exchanger) in 1 L buffer (at least 0.2 M or in the strongest eluent intended to be used during chromatography).
2. Adjust pH with acid or alkali to within 0.1 pH units of the desired value required for chromatography.
3. Allow the slurry to settle for about 30 - 60 minutes (depending on the nature of the buffer and the diameter of the settling vessel) and siphon off the supernatant and fines almost to the level of the settled ion exchanger.
4. Bring the slurry back to the initial volume by addition of the buffer required for the start of the chromatography (normally in the range of 10 - 50 mM).
5. After dispersion and settling remove supernatant by siphoning as described above (3.).
6. Disperse again in diluted buffer (see 4.).
7. After the ion exchanger has settled, remove the supernatant down to the settled exchanger plus about 20 %.

The ion exchanger is now ready for use in batch techniques or column chromatography.

Cleaning and regeneration

Cleaning in place (CIP):

CIP is a method especially beneficial to be applied to preparative columns. They can be cleaned up properly without emptying the packing. The adsorbent is percolated with 2 bed volumes (BV) of 2 M NaOH. For strongly adsorbed material the column temperature can be raised to 50 °C (122 °F). Then the surplus of caustic is removed by 2 BV of demineralized (sterile) water. For prolonged storage, 0.1 % sodiumazide can be added to the water for the cation exchangers and 0.1 % benzalkonium chloride for the anion exchangers. Alternatively, a 20 % ethanol/water mixture is recommended.

Sterilization:

For this purpose, the anion exchangers must be transformed into the chloride form, the cation exchangers into the sodium form and the surrounding medium be neutral. If possible the regenerated, sterilized adsorbent should be stored at 4 °C (40 °F) to prevent from degradation by cellulases.

SERVACEL® Cellulose Anion Exchangers

Cat.No.	Name	Main usage
45059	DEAE 52	Proteins, nucleic acids

SERVACEL® Cellulose Cation Exchangers

Cat.No.	Name	Main usage
45029	CM 52	Basic proteins, peptides

Dynamic binding capacities of cellulose ion exchangers for proteins

Type	Cat. No.	Properties	g/ml	Capacity mg/100 g	Capacity titr.(mM/g)
CM 52	45029	preswollen, microcrystalline, granular	1.0	1000 ¹⁾	1.0
DEAE 52	45059	preswollen, granular, microcrystalline, crosslinked	0.9	600 ²⁾	1.0

The values were determined by column chromatography. If batch adsorption is used, the protein binding capacities are nearly the half.

1) In 0.01 m/l Na₂HPO₄, pH 8.5, 10 mg/ml BSA.

2) In 0.01 m/l Na-Acetate, pH 5, 10 mg/ml Lysozyme

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