Sephadex ion exchange resins

ION EXCHANGE CHROMATOGRAPHY

Ion exchange is probably the most frequently used chromatographic technique for separating and purifying proteins, polypeptides, nucleic acids, and other charged biomolecules. In addition to its widespread usefulness, ion exchange is also easy to perform. Results are reliable and reproducible. Furthermore, ion exchange chromatography resins generally combine high capacity with high resolution.

The popularity and general applicability of the technique has resulted in a wide range of ion exchange resins being available today. One group of resins, Sephadex™ ion exchangers, has for many years held a key position in several important application areas (Fig 1).

- Based on well documented and well proven Sephadex base matrix
- Simple and economical to use
- Very low nonspecific adsorption
- Separate molecules over broad molecular weight and pH ranges

Fig 1. Sephadex ion exchange resins are widely used

Resin characteristics

Sephadex is a dry, bead-formed resin prepared by cross-linking dextran with epichlorohydrin. The resin swells in aqueous solutions. Sephadex ion exchangers are produced by introducing functional groups onto Sephadex. These groups are attached to glucose units in the matrix by stable ether linkages. Sephadex ion exchangers are derived from either Sephadex G-25 or Sephadex G-50. The G-25 matrix is more highly cross-linked than the G-50. Ion exchangers based on Sephadex G-25 have greater rigidity and thus swell less than those based on G-50, which are less rigid.

These properties mean that A-25 (A = anion exchanger) and C-25 (C = cation exchanger) type ion exchangers are good choices for small molecules up to a molecular weight of about 30 000 (e.g., peptides, oligosaccharides, and oligonucleotides) whereas A-50 and C-50 types are better suited to larger biomolecules, such as proteins in the molecular weight range 30 000 to 100 000. High molecular weight molecules, which are excluded from the bead, may be adsorbed to some extent on the outer surface.

As described later, Sephadex A-50 and C-50 ion exchangers are suitable for batch separations.

The terms strong and weak refer to the extent of variation of ionization with pH and not to the strength of binding. Strong exchangers (QAE and SP Sephadex) are completely ionized over a wide pH range whereas the charge of weak exchangers is pH dependent. Weak anion exchangers lose charge when pH increases whereas weak cation exchangers gain charge.

Tables 1 and 2 summarizes the main characteristics of Sephadex ion exchangers.

Table 1. Resin characteristics

	DEAE Sephadex A-25	DEAE Sephadex A-50	QAE Sephadex A-25	QAE Sephadex A-50
Matrix	Cross-linked dextran, spherical			
Type of ion exchanger	Weak anion	Weak anion	Strong anion	Strong anion
Ionic capacity (mmol/g dry resin)	3.0–4.0	3.0–4.0	2.6–3.4	2.6–3.4
Available capacity[1] (mg/mL resin)				
Thyroglobulin (M_r 669 000)	1	2	1.5	1.2
HAS (M_r 68 000)	30	110	10	80
α-lactalbumin (M_r 14 300)	140	50	110	30
Particle size distribution, dry (µm)[2]	40–100	40–100	40–100	40–100
Recommended operating flow velocity[3]	≥ 120 cm/h	≥ 60 cm/h	≥ 100 cm/h	≥ 60 cm/h
pH stability, operational[4]	2–13	2–12	2–13	2–12
pH stability, CIP[5]	2–13	2–12	2–13	2–12
pH ligand fully charged[6]	Below 9	Below 9	Entire pH range	Entire pH range
Chemical stability	Stable to commonly used aqueous buffers			
Physical stability	Negligible volume variation due to changes in pH or ionic strength	Volume changes due to changes in pH or ionic strength	Negligible volume variation due to changes in pH or ionic strength	Volume changes due to changes in pH or ionic strength
Autoclavability	30 min at 121°C in 0.1 M sodium chloride			

[1] The available binding capacity was estimated in 0.05 M Tris-HCl, pH 8.3

[2] ≥ 80% volume share within given range

[3] 5 cm diameter, 10 cm bed height, at room temperature using 0.02 M Sodium chloride

[4] pH range where resin can be operated without significant change in function

[5] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function

[6] pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges

Table 2. Resin characteristics

	CM Sephadex C-25	CM Sephadex C-50	SP Sephadex C-25	SP Sephadex C-50
Matrix	Cross-linked dextran, spherical			
Type of ion exchanger	Weak cation	Weak cation	Strong cation	Strong cation
Ionic capacity (mmol/g dry resin)	4.0–5.0	4.0–5.0	2.0–2.6	2.0–2.6
Available capacity[1] (mg/mL resin)				
IgG (M_r 160 000)	1.6	7	1.1	8
Bovine COHb (M_r 69 000)	70	140	70	110
Ribonuclease (M_r 13 700)	190	120	230	100
Particle size distribution, dry (µm)[2]	40–100	40–100	40–100	40–100
Recommended operating flow velocity[3]	≥ 120 cm/h	≥ 100 cm/h	≥ 100 cm/h	≥ 100 cm/h
pH stability, operational[4]	2–13	2–12	2–13	2–12
pH stability, CIP[5]	2–13	2–12	2–13	2–12
pH ligand fully charged[6]	Above 6	Above 6	Entire pH range	Entire pH range
Chemical stability	Stable to commonly used aqueous buffers			
Physical stability	Negligible volume variation due to changes in pH or ionic strength	Volume changes due to changes in pH or ionic strength	Negligible volume variation due to changes in pH or ionic strength	Volume changes due to changes in pH or ionic strength
Autoclavability	30 min at 121°C in 0.1 M sodium chloride			

[1] The available binding capacity was estimated in 0.1 M Acetate buffer, pH 5.0

[2] ≥ 80% volume share within given range

[3] 5 cm diameter, 10 cm bed height, at room temperature using 0.02 M Sodium chloride

[4] pH range where resin can be operated without significant change in function

[5] pH range where resin can be subjected to cleaning- or sanitization-in-place without significant change in function

[6] pH range where ligand is fully charged; although the ligand is fully charged throughout the range stated, only use the resin within the stated stability ranges

Capacity

Due to the differences in swelling, ion exchangers based on Sephadex G-25 have a higher ionic capacity per mL swollen resin than those based on G-50. Dynamic capacity also depends on the pH, ionic strength, and the nature of the sample (Tables 3 and 4).

Table 3. Dynamic capacity (mg/mL swollen resin) data for DEAE and QAE Sephadex ion exchangers

		Thyroglobulin (M_r 669 000)	HSA (M_r 68 000)	α-lactalbumin (M_r 14 300)
DEAE Sephadex	A-25	1.0	1.0	140.0
	A-50	2.0	110.0	50.0
QAE Sephadex	A-25	1.5	10.0	110.0
	A-50	1.2	80.0	30.0

Capacities were determined at a flow velocity of 75 cm/h. The buffer was 0.05 M Tris, pH 8.3.

Table 4. Dynamic capacity (mg/mL swollen resin) data for CM and SP Sephadex ion exchangers

		IgG (M_r 160 000)	Bovine COHb (M_r 69 000)
CM Sephadex	C-25	1.6	70.0
	C-50	7.0	140.0
SP Sephadex	C-25	1.1	70.0
	C-50	8.0	110.0

Capacities were determined at a flow velocity of 75 cm/h. The buffer was 0.05 M Tris, pH 8.3.

Stability

Sephadex resins are stable in water, salt solutions, organic solvents, and alkaline and weakly acidic solutions in the pH range of 2 to 10 (details see Tables 1 and 2). The resins can be autoclaved as a slurry in the salt form for 30 min at 121°C and pH 7. Exposure to strong oxidising agents or dextranases should be avoided. When resins are stored in the swollen state, a nonionic antimicrobial agent should be included in the buffer.

The pressure/flow curve shown in Figure 2 illustrates the relationship between the pressure drop over a packed bed of DEAE Sephadex A-25 and the flow velocity in a 5 cm i.d. column with a bed height of approximately 10 cm. The curve is essentially linear in the typical operating range up to 0.15 bar where the flow velocity is about 380 cm/h. The maximum pressure drop (i.e., the pressure drop where the flow velocity levels off) was not determined in this case.

Sephadex A-50 and C-50 resins, which are less rigid and therefore more prone to volume changes, are better suited to the batch technique. Batch separation is simple to perform and no technical difficulties are caused by the swelling or shrinkage of the ion exchanger.

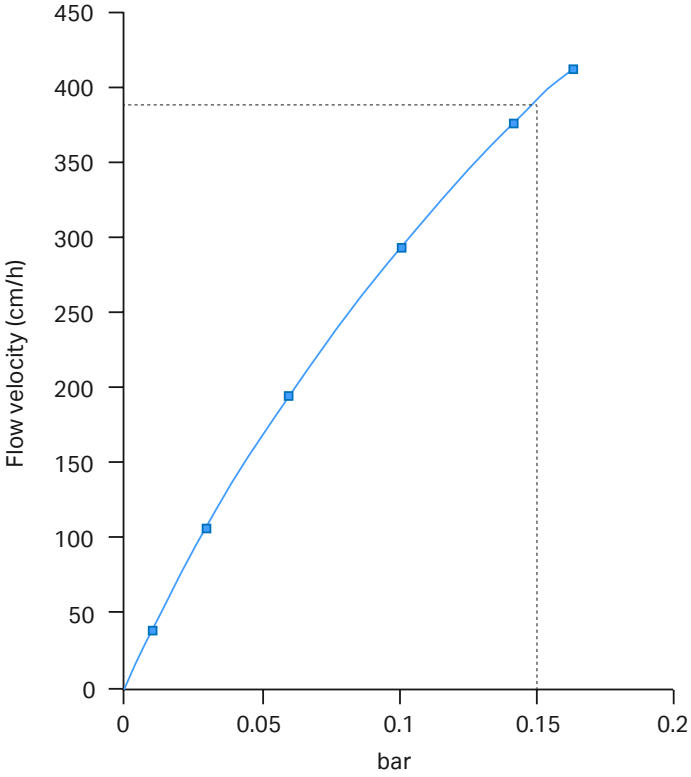

Fig 2. The pressure/flow curve for a 10 cm bed of DEAE Sephadex A-25 in a 5 cm i.d. column is essentially linear, which demonstrates the suitability of Sephadex A-25 and C-25 ion exchangers for column use

Swelling varies with ionic strength and pH

Ion exchangers based on Sephadex swell in aqueous solutions. As an example, Table 5 shows dry and hydrated bead sizes of Sephadex cation and anion exchangers. As dry resins are swollen before use, it is the wet bead diameter that is of practical importance when choosing the correct type of equipment to use, for example mesh size of the column nets.

Table 5. Hydrated bead sizes of Sephadex ion exchangers. Note that lower ionic strengths will give higher hydrated/dry diameter ratios

Product	Medium	Hydrated median diameter µm*	Diameter ratio hydrated/dry*
DEAE Sephadex A-25	0.15 M NaCl	127	1.83
	0.50 M NaCl	121	1.75
DEAE Sephadex A-50	0.15 M NaCl	214	1.75
	0.50 M NaCl	182	2.69
	0.15 M NaCl	214	1.75
CM Sephadex C-25	0.15 M NaCl	131	1.94
	0.50 M NaCl	125	1.86
CM Sephadex C-50	0.15 M NaCl	250	3.41
	0.50 M NaCl	221	3.02

* Average values from three product batches of each ion exchanger

Initial swelling of dry Sephadex ion exchangers should be performed in 0.2 M salt. In distilled water the beads will swell too much, too quickly, which may cause breakage. Swelling varies with ionic strength and pH. A-25 and C-25 exchangers have a fairly rigid bead structure and size variations, due to changes in pH or ionic strength, are therefore small. A-50 and C-50 exchangers, on the other hand, swell more than their A-25 and C-25 equivalents because of their less rigid structure. In addition, swelling at a given pH is highest at low ionic strengths, as repulsion between similarly charged groups on the matrix then is maximal (see Fig 3). Fluctuations in swelling will be minimized in buffers with ionic strengths above 0.1 M.

A similar pattern can be seen in Figure 4, which shows how pH affects the degree of swelling. Note, however, that the swelling of the strong ion exchangers QAE and SP Sephadex is virtually independent of pH because, as strong exchangers, they remain charged over a wide pH range.

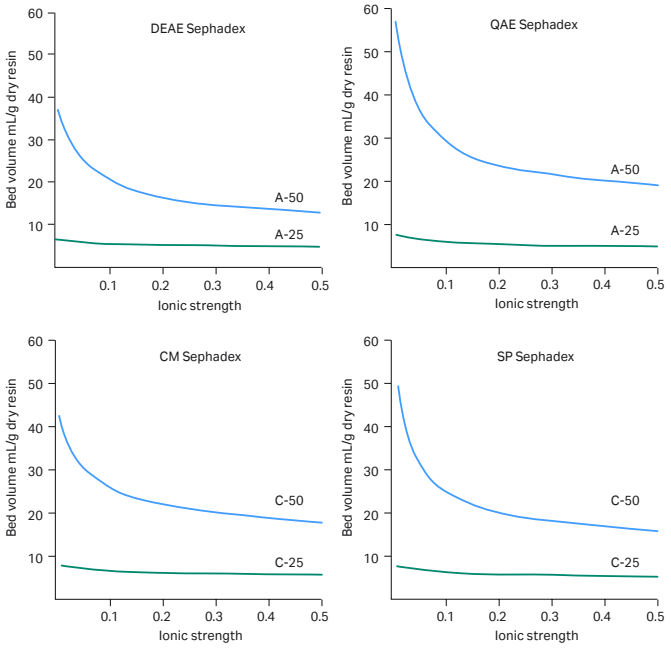

Fig 3. Effect of ionic strength on swelling. Bed volumes obtained from 1 gram dry resin as a function of ionic strength. DEAE and QAE Sephadex were measured in Tris-HCl buffer, pH 7.6 with varying NaCl concentration. CM and SP Sephadex were measured in acetate buffer, pH 4.3 with varying NaCl concentration.

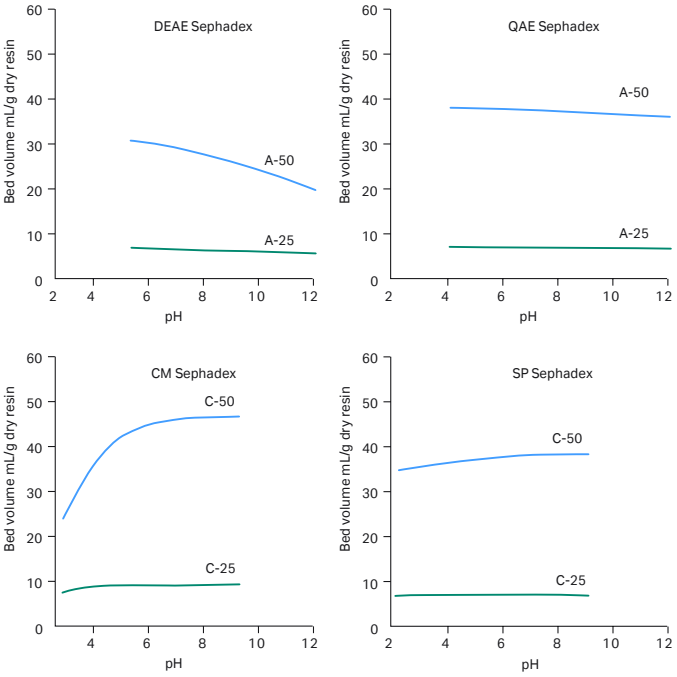

Fig 4. Effect of pH on swelling. Bed volumes obtained from 1 gram dry resin as a function of pH. DEAE and QAE Sephadex were measured in imidazole and ethylenediamine buffers of varying pH at a constant ionic strength of 0.05. CM and SP Sephadex were measured in phosphate buffers of varying pH at a constant ionic strength of 0.05.

Column packing

As with any other column chromatographic technique, packing is a critical step in the experiment. A well-packed column gives even flow, minimizes band broadening and promotes high resolution.

Cytiva has columns available for ion exchange chromatography at laboratory, pilot and full production scales.

Prior to packing, Sephadex ion exchangers should be swollen at the pH to be used in the experiment. Complete swelling takes 1 to 2 d at room temperature. The required amount of ion exchanger should be stirred into an excess of starting buffer. The supernatant should be removed and replaced with fresh buffer several times during the swelling period. To avoid generating fines, the ionic strength during swelling should be 0.2 M salt.

Further information is available in the handbook “Ion Exchange Chromatography — Principles and Methods” (Product code 11000421), which is a practical guide to the technique, its applications, and experimental procedures.

Storage

Dry powders of DEAE Sephadex A-25, DEAE Sephadex A-50, QAE Sephadex A-25, QAE Sephadex A-50, CM Sephadex C-25, CM Sephadex C-50, SP Sephadex C-25 and SP Sephadex C-50 should be stored between 4°C to 25°C.

Store swollen medium in the presence of a suitable bacteriostat, e.g., 20% ethanol at 4°C to 30°C.

Ordering Information

Product	Quantity	Product code
DEAE Sephadex A-25	100 g	17017001
DEAE Sephadex A-25	500 g	17017002
DEAE Sephadex A-25	5 kg	17017003
DEAE Sephadex A-50	100 g	17018001
DEAE Sephadex A-50	500 g	17018002
DEAE Sephadex A-50	5 kg	17018003
DEAE Sephadex A-50	40 kg*	17018007
QAE Sephadex A-25	100 g	17019001
QAE Sephadex A-25	5 kg	17019003
QAE Sephadex A-50	100 g	17020001
QAE Sephadex A-50	5 kg	17020003
CM Sephadex C-25	100 g	17021001
CM Sephadex C-25	500 g	17021002
CM Sephadex C-25	5 kg	17021003
CM Sephadex C-50	100 g	17022001
CM Sephadex C-50	500 g	17022002
CM Sephadex C-50	5 kg	17022003

* Pack size available upon request

Related literature

Handbook	
Ion Exchange Chromatography — Principles and Methods	11000421
Selection guides	
Ion exchange columns and media	18112731
Prepacked chromatography columns for ÄKTA™ systems	28931778

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