

## Sephadex® ion exchange media

## Data File

### *Ion exchange chromatography*

- Based on well documented and well proven Sephadex base matrix
- Simple and economical to use
- Very high capacities even at elevated ionic strengths
- Very low non-specific adsorption
- Separate molecules over broad molecular weight and pH ranges
- Wide range of applications at laboratory and process scales
- Suitable for column or batch techniques

### Introduction

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 media generally combine high capacity with high resolving power.

The popularity and general applicability of the technique has resulted in a wide range of ion exchange media being available today. One group of media, Sephadex ion exchangers, has for many years held a key position in several important application areas, especially at process scale. Typical process scale applications include the fractionation of plasma components such as prothrombin complex using the batch ion exchange technique, and the purification of insulin by traditional column chromatography.

### Sephadex ion exchangers

#### *General description*

Sephadex ion exchangers are produced by introducing functional groups onto Sephadex, a cross-linked dextran matrix. These groups are attached to glucose units in the matrix by stable ether linkages

**18-1117-58**

Edition AA



Sephadex ion exchange media are widely used for process scale applications. Their separation power, ease of use and overall economy make them suitable for both column and batch techniques.

Table 1 shows the full range of eight Sephadex ion exchangers. Anion and cation exchangers are designated A-25 or A-50 and C-25 or C-50 respectively.

**Table 1.** The range of Sephadex ion exchangers. See Table 2 for more detailed information.

Type		Description
DEAE Sephadex	A-25 A-50	Mixed weak and strong anion exchanger*
QAE Sephadex	A-25 A-50	Strong anion exchanger
CM Sephadex	C-25 C-50	Weak cation exchanger
SP Sephadex	C-25 C-50	Strong cation exchanger

\* DEAE Sephadex contains functional groups that display both strong and weak ion exchange groups (1/3 strong, 2/3 weak).

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.

Table 2 summarizes the main characteristics of Sephadex ion exchangers.

## Bead form

Sephadex is a dry, bead-formed gel prepared by cross-linking dextran with epichlorohydrin. The gel swells in aqueous solutions. Different types of Sephadex belonging to the G-series differ in their degree of cross-linking and hence in their degree of swelling.

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 (see Table 6).

These properties mean that A-25 and C-25 type ion exchangers are a good choice for small molecules up to about 30 000 MW (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 ideal for batch separations.

**Table 2.** General characteristics of Sephadex ion exchange media.

	DEAE Sephadex	QAE Sephadex	CM Sephadex	SP Sephadex
Type of exchanger	Mixed weak and strong* anion exchanger	Strong anion exchanger	Weak cation exchanger	Strong cation exchanger
Main functional group	Diethylaminoethyl	Diethyl-(2-hydroxy-propyl)aminoethyl	Carboxymethyl	Sulphopropyl
Counter ion	Chloride	Chloride	Sodium	Sodium
Rec. pH working range	2–9	2–10	6–10	2–10
Dynamic capacity	See Tables 3 and 4.			
Mol wt range	A-25 and C-25 types: up to 30 000. A-50 and C-50 types: 30 000–100 000.			
Bead form	Spherical, 40–125 µm in dry form			
Bead structure	Cross-linked dextran			
Chemical stability	Stable in water, salt solutions, organic solvents, alkaline and weakly acidic solutions and denaturing solvents			
Physical stability	A-25 and C-25 types: robust – have good flow properties and negligible volume changes due to changes in pH and ionic strength. A-50 and C-50 types: volume may vary due to changes in pH and ionic strength.			
Autoclavable	Swollen, in salt form for 30 min at 120 °C.			
Availability	Supplied dry in a range of laboratory and process scale pack sizes. See ordering information			

\* See Table 1.

**Table 3.** Dynamic capacity (mg/ml swollen gel) data for DEAE and QAE Sephadex ion exchangers.

Protein (MW)		Thyroglobulin (669 000)	HSA (68 000)	$\alpha$ -lactalbumin (14 300)
DEAE Sephadex	A-25	1.0	30.0	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 rate of 75 cm/h. The buffer was 0.05 M Tris, pH 8.3.

**Table 4.** Dynamic capacity (mg/ml swollen gel) data for CM and SP Sephadex ion exchangers.

Protein (MW)		IgG (160 000)	Bovine COHb (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 rate of 75 cm/h. The buffer was 0.1 M acetate buffer, pH 5.0.

## Capacity

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

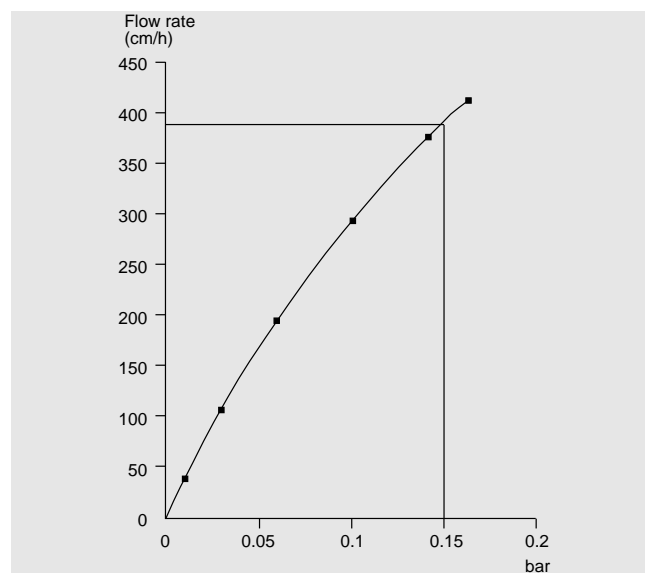
## Stability

Sephadex ion exchangers are insoluble in all solvents commonly used in liquid chromatography. They are stable in water, salt solutions, organic solvents, and alkaline and weakly acidic solutions in the pH range of 2–10. The media can be autoclaved as a slurry in the salt form for 30 minutes at 120 °C and pH 7. Exposure to strong oxidising agents or dextranases should be avoided. When media are stored in the swollen state, a non-ionic antimicrobial agent should be included in the buffer.

The high mechanical stability of Sephadex A-25 and C-25 media gives outstanding flow properties, which makes them ideal for packing and operating in large scale chromatography columns.

The pressure/flow rate curve shown in Figure 1 illustrates the relationship between the pressure drop over a packed bed of DEAE Sephadex A-25 and the linear flow rate 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 fluid velocity is about 380 cm/h for this batch. The maximum pressure drop i.e. that pressure drop where the flow rate levels off was not determined in this case.



**Fig. 1.** The pressure/flow rate 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.

Table 5. Batch to batch reproducibility is demonstrated by some typical data from the quality control of DEAE Sephadex A-50.

Lot. No.	Flow rate at $\Delta p/L=10$ [cm/h]	Function; salt conc. in elution peak; M NaCl	CO Hb1	CO Hb2	HSA	Total capacity mmolCl <sup>-</sup> /g
(Limits)	–	(min. 60)	(0.04–0.07)	(0.06–0.10)	(0.16–0.22)	(3.0–4.0)
5042	1972	–	0.06	0.09	0.20	3.40
18896	1981	97	0.06	0.09	0.19	3.30
2783	1986	83	0.05	0.07	0.16	3.40
13799	1991	103	0.05	0.07	0.17	3.42
226550	1994	90	0.05	0.07	0.17	3.29

Table 6. Hydrated bead sizes of Sephadex ion exchangers. Note that lower ionic strengths will give higher hydrated/dry diameter ratios.

Product	Medium	Hydrated median diameter $\mu\text{m}^*$	Diameter ratio hydrated/dry*
DEAE Sephadex A-25	0.15 M NaCl	127	1.83
DEAE Sephadex A-25	0.50 M NaCl	121	1.75
DEAE Sephadex A-50	0.15 M NaCl	214	3.17
DEAE Sephadex A-50	0.50 M NaCl	182	2.69
CM Sephadex C-25	0.15 M NaCl	131	1.94
CM Sephadex C-25	0.50 M NaCl	125	1.86
CM Sephadex C-50	0.15 M NaCl	250	3.41
CM Sephadex C-50	0.50 M NaCl	221	3.02

\* Average values from three product batches of each ion exchanger.

Sephadex A-50 and C-50 media, 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.

### Batch to batch reproducibility

Consistent quality and performance from batch-to-batch is important for all separation media, but it assumes extra significance when the media are routinely used in industrial processes, as is the case with Sephadex ion exchangers. Some randomly selected quality control data gathered from over 30 years prove beyond doubt that these media have been produced with outstanding batch-to-batch consistency (see Table 5).

### Swelling varies with ionic strength and pH

Ion exchangers based on Sephadex swell in aqueous solutions. As an example, Table 6 shows dry and hydrated bead sizes of Sephadex cation and anion exchangers.

As dry media are swollen before use, it is the wet bead diameter that is of practical importance when choosing the correct type of equipment to use, e.g. mesh size of the column nets.

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 due to 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 is then maximal (see Fig 2). Fluctuations in swelling will be minimized in buffers with ionic strengths above 0.1.

A similar pattern can be seen in Figure 3, 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 since, as strong exchangers, they remain charged over a wide pH range.

### Separation principle

The separation effect of ion exchange chromatography depends on the reversible adsorption of charged solute molecules to immobilized groups of opposite charge. Most separations can be described in five stages.

## Effect of ionic strength on swelling

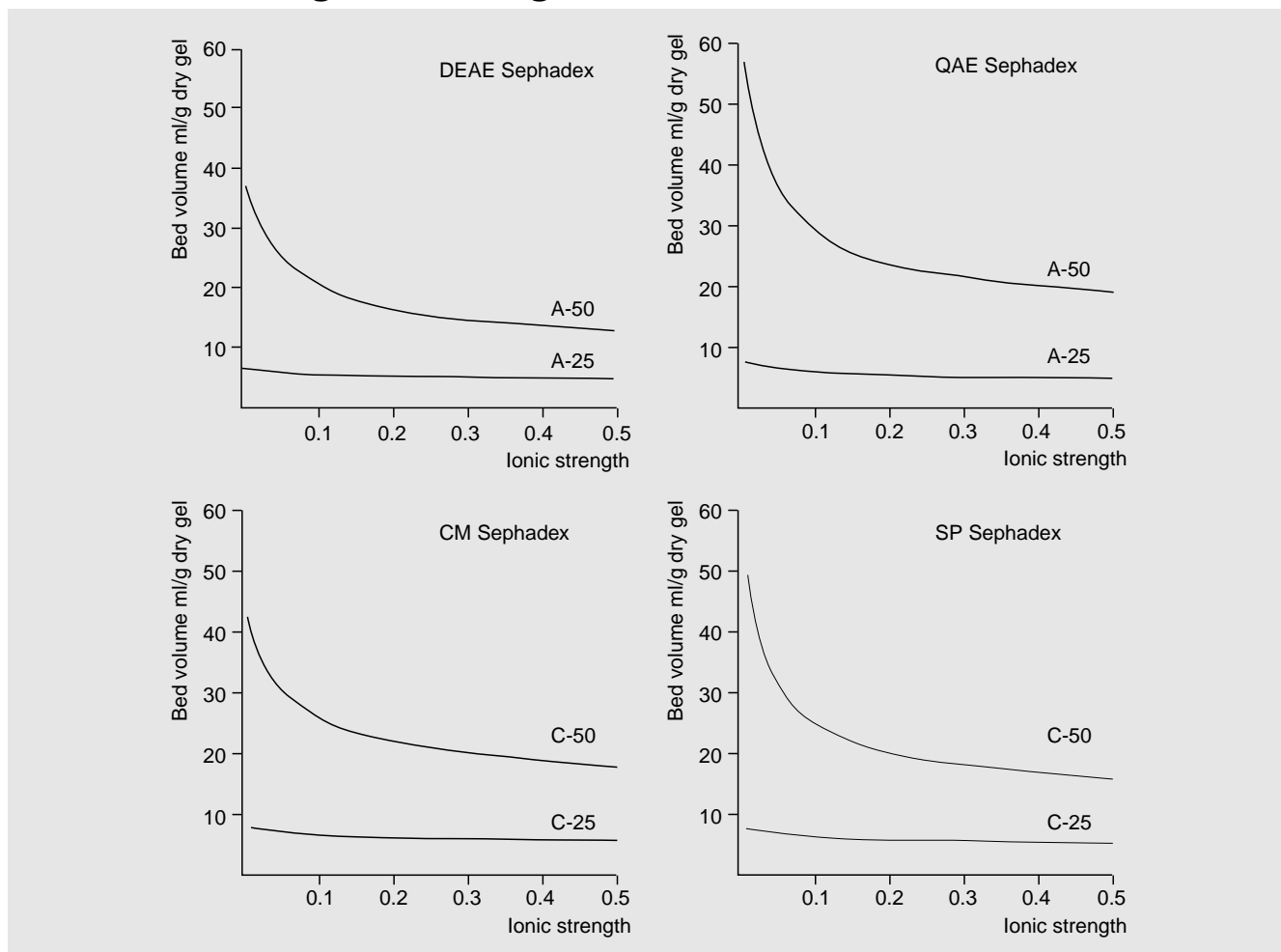


Fig. 2. Bed volumes obtained from 1 gram dry gel as a function of ionic strength. DEAE and QAE Sephadex were measured in Tris HCl buffer pH 7.6 with varying NaCl concentration. CM and SP Sephadex were measured in acetate buffer pH 4.3 with varying NaCl concentration.

Stage one is equilibration. The pH and ionic strength of the equilibration buffer are adjusted to favour binding of the molecules of interest in the solute. Weak ion exchangers have their own buffering capacities and need longer equilibration times. At this stage the immobilized groups are associated with exchangeable counter ions.

Stage two is sample application and adsorption. Solute molecules of interest displace the counter ions and bind reversibly to the gel. Unbound substances can be washed out using starting buffer.

(An alternative strategy sometimes used is to bind the contaminants at this stage, in which case, it is the substance of interest that is washed out of the gel.)

Note that contaminants may compete with the substance of interest and also bind at stage two, thus reducing the capacity for binding the intended

product. If this occurs, the experimental conditions and/or the chosen ion exchanger should be changed to favour binding the substance of interest.

Stage three involves increasing the ionic strength of the eluting buffer or changing its pH to conditions that favour the elution of the bound substance of interest.

The fourth and fifth stages are the removal from the column of substances not eluted under the previous experimental conditions followed by re-equilibration to the starting conditions for the next separation cycle.

Ion exchange separations can be carried out in a column or by a batch procedure. The basic principle outlined in the five stages described above applies to both methods.

## Effect of pH on swelling

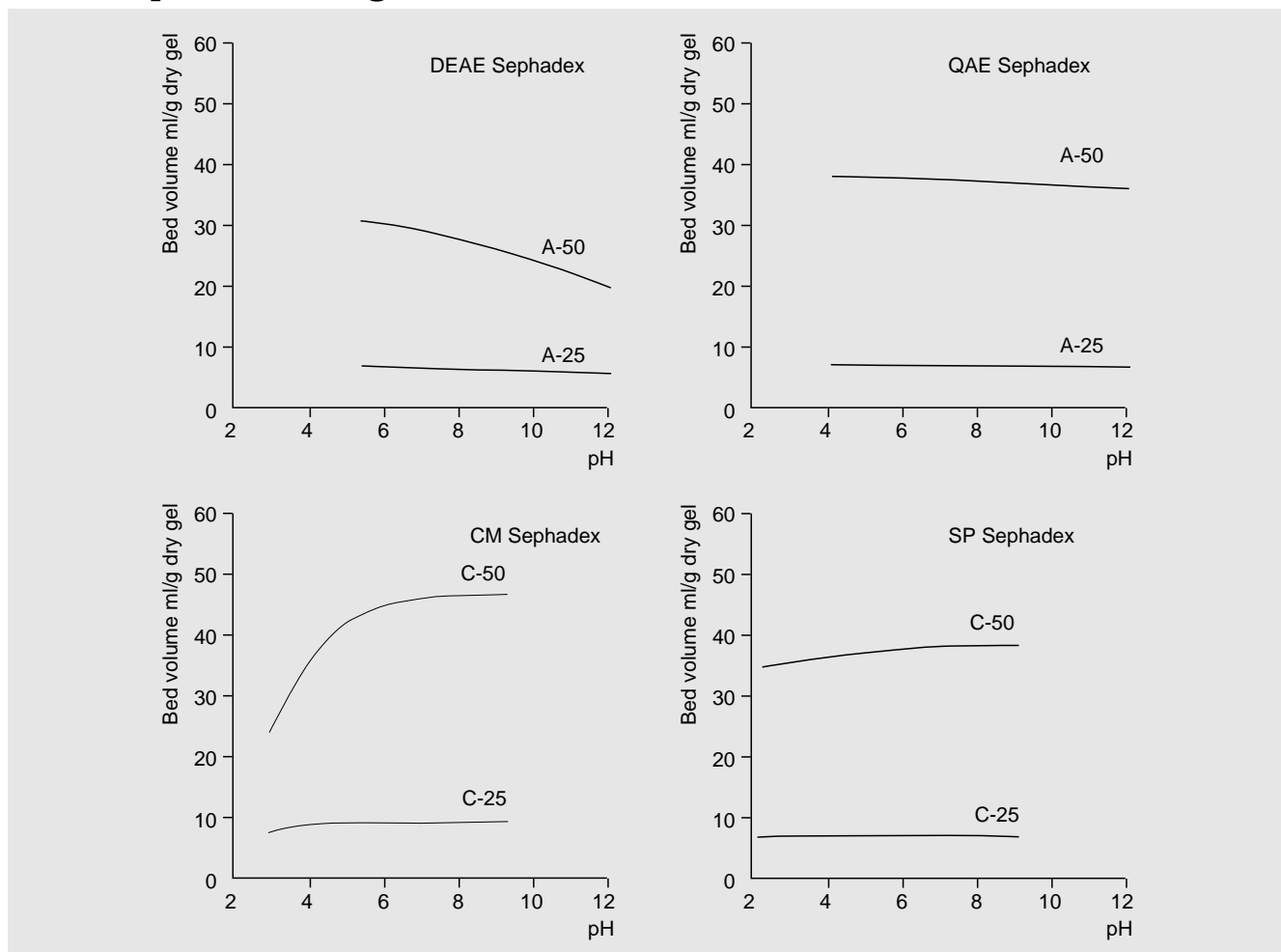


Fig. 3. Bed volumes obtained from 1 gram dry gel as a function of pH. DEAE and QAE Sephadex were measured in imidazole and ethylenediamine buffers of varying pH at a constant ionic strength of 0.05. CM and SP Sephadex were measured in phosphate buffers of varying pH at a constant ionic strength of 0.05.

### Working with Sephadex ion exchangers

Sephadex A-25 and C-25 ion exchangers are highly suitable for column chromatography and Sephadex A-50 and C-50 for batch techniques. This versatility combined with low non-specific interaction, consistent quality, batch to batch reliability and excellent price performance, makes Sephadex ion exchangers an obvious choice for large scale applications.

### Column chromatography

Good column design and good packing techniques are important in realising the full separation potential of ion exchange chromatography.

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

Our range of process columns covers all stages from process development to full scale production

and includes INdEX™ columns, BPG™ columns, Process Stack columns (PS370), BioProcess™ Stainless Steel (BPSS) columns and CHROMAFLOW™ columns. BPG, BPSS and CHROMAFLOW are designed for applications that demand the highest standards of hygiene, such as in pharmaceutical production.

As in most adsorptive techniques, ion exchange is carried out in short columns. Bed heights are typically 5–15 cm. Once the separation has been optimized, scale up is achieved by increasing the column diameter.

### 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.



Fig. 4. CHROMAFLOW columns are designed and constructed to meet the stringent demands of process scale chromatography.

Prior to packing, Sephadex ion exchangers should be swollen at the pH to be used in the experiment. Complete swelling takes 1–2 days at room temperature or 2 hours in pH 7 buffer at approximately 100 °C. 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.

### *Applications of process scale column techniques*

The excellent chemical and physical stability plus the ease of packing and cleaning have made Sephadex A-25 and C-25 types popular choices for a wide range of applications. They are used especially for separating small proteins, peptides and carbohydrates because of the low non-specific adsorption of these types of biomolecules.

DEAE and QAE Sephadex A-25 have been used extensively for the large scale production of peptide hormones as, to a lesser extent, has SP Sephadex C-25. CM Sephadex C-25 is e.g. used for the large scale purification of oligosaccharides.

### *Batch separation*

There is essentially no difference in separation principle between a column desorbed by stepwise

elution and a batch procedure. As described earlier, either the substance of interest or contaminants may be attached to the ion exchanger.

Although batch procedures are less efficient than column techniques they may offer advantages in particular cases. When very large sample volumes with low protein concentration have to be processed, the sample application time on a column can be very long and filtration of such a large sample can also be rather difficult to perform. Binding the sample in batch mode will be much quicker and there will be no need to remove particulate matter.

A batch procedure can also be an attractive approach if high sample viscosity generates high back-pressure in a column procedure or if high back-pressure is generated by contaminants such as lipids, which may cause severe fouling and clogging of the column.

When working with batch ion exchange, the starting conditions are selected in the same way as in column chromatography, i.e. choose buffer pH and ionic strength to bind the substance of interest but to prevent as many contaminants as possible from binding.

To maximize recovery, the starting conditions should be selected so that the protein of interest binds much stronger than is necessary in column chromatography. Unless the proteins adsorb to 100%, losses during subsequent washing will be inevitable, especially if the volume of liquid is large compared to the volume of adsorbent. To keep recovery high, the pH in a batch experiment may have to be several units away from the isoelectric point of the protein.

Batch separation is carried out by stirring the ion exchanger previously equilibrated in the appropriate buffer with the solution to be treated until the mixture has reached equilibrium. This usually takes about one hour. The slurry is then separated by filtration and washed with the buffer solution. In cases of incomplete adsorption, this procedure should be repeated on the filtrate with fresh ion exchanger. Then elution buffer is added (1–2 times the volume of the sedimented gel) and stirred until desorption is complete, which can take 30 minutes or more. Finally, suction is used to filter the buffer containing the desorbed product of interest from the adsorbent.

Figure 5 shows a batch tank suitable for use with Sephadex A-50 and C-50 ion exchangers.



Fig. 5. A stainless steel batch tank equipped with a stirring system.

The gel can also be packed in a column after the washing step and be eluted stepwise in the same way as during normal column chromatography. Resolution will however be lower for such a combined batch and column procedure compared with a normal column procedure, since the sample is bound uniformly throughout the gel slurry and the subsequent chromatographic bed.

Under these conditions stepwise elution is recommended since gradient elution will give broader bands and often poorer resolution. Also the amount of eluent will be lower in stepwise elution.

Batch chromatography is useful for concentrating dilute solutions and separating the substances of interest from gross contaminants during the initial capture stage of a purification scheme.

Ion exchangers used in batch processes are discarded after use, so the operator does not have to be concerned with regeneration or cleaning-in-place procedures. The overall economy of Sephadex ion exchangers also favours their one time use.

### *Applications of the batch technique*

Batch ion exchange with Sephadex A-50 and C-50 is ideal for the simple and economical Capture of proteins and complexes from large volumes of feedstocks. They are particularly useful for processing crude feedstocks that are not suitable for loading onto a column.

## Ordering information

Product	Quantity/Pack size	Code No.
DEAE Sephadex A-25	100 g	17-0170-01
DEAE Sephadex A-25	500 g	17-0170-02
DEAE Sephadex A-25	5 kg	17-0170-03
DEAE Sephadex A-25	40 kg	17-0170-07
DEAE Sephadex A-50	100 g	17-0180-01
DEAE Sephadex A-50	500 g	17-0180-02
DEAE Sephadex A-50	5 kg	17-0180-03
DEAE Sephadex A-50	40 kg	17-0180-07
QAE Sephadex A-25	100 g	17-0190-01
QAE Sephadex A-25	500 g	17-0190-02
QAE Sephadex A-25	5 kg	17-0190-03
QAE Sephadex A-50	100 g	17-0200-01
QAE Sephadex A-50	500 g	17-0200-02
QAE Sephadex A-50	5 kg	17-0200-03
CM Sephadex C-25	100 g	17-0210-01
CM Sephadex C-25	500 g	17-0210-02
CM Sephadex C-25	5 kg	17-0210-03
CM Sephadex C-50	100 g	17-0220-01
CM Sephadex C-50	500 g	17-0220-02
CM Sephadex C-50	5 kg	17-0220-03
SP Sephadex C-25	100 g	17-0230-01
SP Sephadex C-25	500 g	17-0230-02
SP Sephadex C-25	5 kg	17-0230-03
SP Sephadex C-25	40 kg	on request
SP Sephadex C-50	100 g	17-0240-01
SP Sephadex C-50	500 g	17-0240-02
SP Sephadex C-50	5 kg	17-0240-03

IgG and prothrombin complex are representative of a number of plasma components purified on DEAE Sephadex A-50. Removal of detergents and the purification of whey proteins are common applications of CM Sephadex C-50.

Further information is available in the Amersham Biosciences handbook "Ion Exchange Chromatography – Principles and Methods" (article no. 18-1114-21), which is a practical guide to the technique, its applications and experimental procedures. Ask your local representative of Amersham Biosciences for details.

### *Columns*

For more information about the range of process scale columns, please contact your local sales office of Amersham Biosciences.