# Ultrogel® AcA

## Size Exclusion Chromatography Sorbents

- High resolution
- Semi-rigid particles
- Narrow particle size distribution
- Narrow pore size distribution

BIOSEPRA® ULTROGEL® ACA

**Table 1. Main Properties of Ultrogel AcA Sorbents.**

| | AcA 22 | AcA 34 | AcA 44 | AcA 54 | AcA 202 |
|---|---|---|---|---|---|
| Particle size (µm) | 60-140 | 60-140 | 60-140 | 60-140 | 60-140 |
| Acrylamide (%) | 2 | 3 | 4 | 5 | 20 |
| Agarose (%) | 2 | 4 | 4 | 4 | 2 |
| Exclusion limit (dt)* | 3,000,000 | 750,000 | 200,000 | 90,000 | 22,000 |
| Linear fractionation range (dt) | 100,000-1,200,000 | 20,000-350,000 | 10,000-130,000 | 5,000-70,000 | 1,000-15,000 |
| Resolution power (plates/m) | 1,500 | 1,500 | 1,500 | 1,500 | 3,000 |
| Desalting capacity | - | - | - | - | 45% gel vol. |
| Working pH | 3-10 | 3-10 | 3-10 | 3-10 | 3-10 |

BioSepra Ultrogel AcA sorbents from Pall® are a range of composite sorbents for size exclusion of biological macromolecules. They consist of polyacrylamide and agarose gel matrix and possess good mechanical and chromatographic properties when compared to classical size exclusion sorbents. They are suitable for medium and large scale applications. The principal characteristics of Ultrogel AcA are a narrow particle size distribution and a narrow pore size distribution. The particles are semi-rigid.

These characteristics combine to provide high resolution at high flow rates. In addition, Ultrogel sorbents can be simply and rapidly packed. Ultrogel AcA sorbents offer a choice between maximum resolution at recommended flow rates or rapid separations at higher flow rates with minimal loss of resolution. Five different types of Ultrogel AcA are available allowing the fractionation of molecules with molecular weights ranging from 1,000 and 1,200,000. The sorbent is mainly dedicated for fractionation, purification and molecular weight purification.

The concentration of polyacrylamide and agarose in the Ultrogel AcA beads vary with the sorbent designation. For instance, Ultrogel AcA 34 sorbent contains 3% polyacrylamide (Ac) and 4% agarose (A). The particle size is comprised between 60 and 140 µm.

In addition to the superior physicochemical properties of Ultrogel AcA, this sorbent provides excellent separation efficiency, demonstrated by the low HETP (Height Equivalent to a Theoretical Plate). The HETP of approximately 0.67 mm corresponds to over 1,500 theoretical plates per meter; excepted for Ultrogel AcA 202 the HETP is 0.33 mm (eq. 3,000 plates per meter). There are only moderate HETP variations as a function of flow rate.

In order to obtain optimum separation of the sample components, Ultrogel AcA sorbent should be chosen so that the molecular weights of all sample solutes to be purified fall within its fractionation range. If the molecular weights are unknown, the optimum sorbent type can be determined on an analytical scale empirically.

The sorbents are shipped in 1 M sodium chloride containing 20% ethanol* and are available in a range of package sizes. Special packaging to meet specific manufacturing requirements is available on request.

** Ultrogel AcA 202 is supplied in 1 M sodium chloride, 20% ethanol and 5 mM EDTA.*

## Stability

Ultrogel AcA sorbents are semi-rigid particle sorbents, which possess good mechanical properties. They can be used at high flow rate while maintaining a good resolution.

Ultrogel AcA sorbents are chemically stable in buffer solutions between pH 3 and 10. Any conventional buffer for biochemical separation purposes can be used. A high concentration of denaturing agents such as urea or guanidine hydrochloride, or detergents such as SDS, should be avoided. As with all gel fractionation sorbent, the disruptive influence of such additives should be checked in each case. The buffers used should have an ionic strength of at least 0.05 M to minimize non-specific adsorption or ion exchange effects between the sample proteins and the sorbent matrix.

Detergents and denaturing agents may be used as eluants only in restricted conditions: guanidine hydrochloride should be used at a maximum concentration of 2 M. Urea is a denaturing agent comparable to guanidine. It can be currently used at 2 M. SDS, Triton X-100, DOC, Rennex and other detergents can be used without significant modifications of the sorbent properties.

Ultrogel AcA sorbent should be used between 2°C and 36°C; a temperature below 0°C is not advised. Ultrogel AcA sorbent cannot be autoclaved, as this may decompose the agarose.

## Applications

The main applications of Ultrogel AcA are:

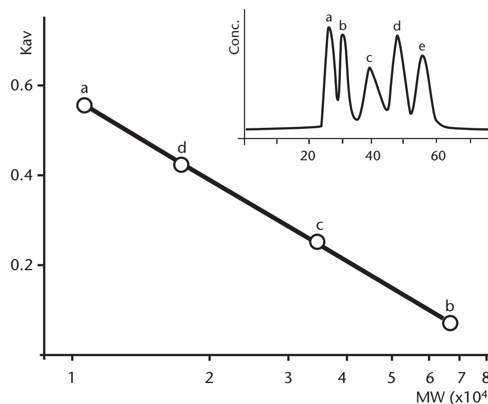
- Fractionation and purification of biological molecules by size exclusion,
- Determination of molecular weights,
- Desalting.

Ultrogel AcA sorbents allow the separation of components of a complex mixture by size exclusion. The fractionation range is comprised between 1,000 and 1,200,000 daltons. Each type of sorbent has a specific molecular weight/fractionation range.

### • *Determination of molecular weights*

Molecular weights of proteins may be reliably determined by choosing an Ultrogel AcA sorbent which has the required fractionation range. The first step in the process consists in the determination of a selectivity curve with known standard proteins. The second step consists in the size exclusion of the studied protein and the comparison of its elution volume or its Kav with the selectivity curve (see figure 1).

*Figure 1. Determination of a selectivity curve for Ultrogel AcA 54 using a mixture of known proteins.*




Column: 1.6 x 40 cm; Buffer: 0.05 M Tris-HCl, pH 7.4 containing 0.17 M sodium chloride: sample constituted of Blue-Dextran 2000 (a) for the determination of the void volume; Bovine serum albumin (b) (MW 68,000); ß-lactoglobulin (c) (MW 35,000); Myoglobin (d) (MW 17,800); And cytochrome c (e) (MW 12,400) ; Sample volume: 0.6 ml; Flow rate: 4.8 cm/h. The insert represents the elution curve.

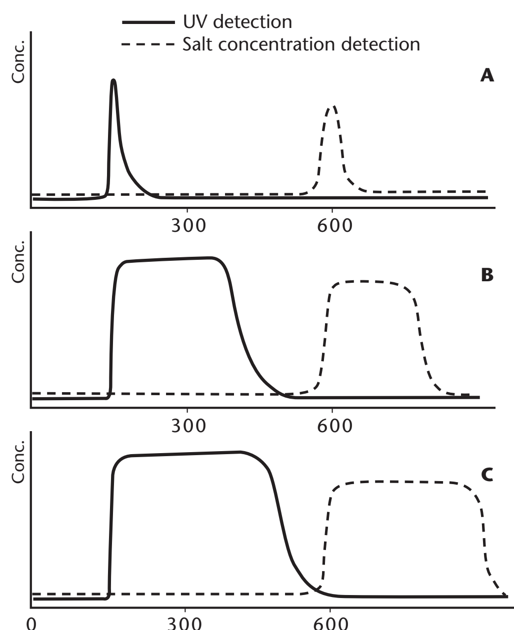
### • *Desalting*

The term is used to describe the separation of substances present in a solution into 2 groups : one consisting of the macromolecules which are totally excluded from the sorbent, and the other consisting of the low molecular weight components which diffuse into the sorbent network. Ultrogel AcA 202 sorbent is dedicated to this application (see figure 2).

#### 1. Separation of salts

If the separation of proteins from salts is optimized, very large volumes may be processed. The sample volume must theoretically correspond to the difference between the total sorbent volume and the void volume (generally 60% of the sorbent volume). For sorbents traditionally used for desalting, the recommended sample volume is only 30% of the sorbent

*Figure 2. Example of desalting using Ultrogel AcA 202.*




Column: 5 x 37 cm; volume: 730 ml; Sample: bovine serum albumin (5 mg/ml) containing NaCl (6.5 mg/ml); Flow rate: 7 cm/h (i.e. 140 ml/h). Sample volumes: A = 36 ml (5 % of sorbent volume); B = 220 ml (30 % of sorbent volume); C = 327 ml (45 % of sorbent volume); 280 nm U.V. detection.

volume. Because the peak broadening is limited with Ultrogel AcA 202 sorbent, as a result of the regularity of the particles and the pores, the sample volume on AcA 202 sorbent may be as high as 40% of the sorbent volume, or even 45% under certain circumstances. Similarly, the concentration of each component in the sample may be as high as 40 g/L without affecting the desalting quality.

### 2. Removal of various reagents

In addition to the removal of salts, desalting can be extended to :

- Removal of low molecular weight sugars (e.g. lactose from whey).
- Removal of small aromatic compounds (e.g. phenol during the purification of nucleic acids).
- Removal of fluorescent or radioactive compounds (e.g. fluorescein, rhodamine and $I^{125}$ during the general labelling of proteins).
- Removal of certain detergents used for the solubilizing proteins (SDS, urea, guanidine salts).

In all cases, desalting with Ultrogel AcA 202 sorbent is superior to classical dialysis because of the considerable savings in time, the very low degree of dilution, and high recovery.

Since it is a mild process, size exclusion desalting is sometimes the only technique which can be employed for the total removal of salts from highly unstable biological macromolecules. It should also be noted that desalting is the only technique which can be used for rapid buffer exchange of a protein solution. This is done simply by passing the protein solution through an Ultrogel AcA 202 column reequilibrated with the new buffer.

• *Affinity*

Ultrogel AcA also provide excellent supports for the preparation of derivatives for use in affinity chromatography. The quality of the agarose used in the manufacture of these sorbents results in an almost total absence of the non-specific adsorption's which are frequently encountered with competitive sorbents of this type. The regular size distribution of Ultrogel AcA particles, coupled with their mechanical rigidity, allows their use for extended periods of time with no significant decrease in flow rate. The large pore sizes also allow high coupling capacities to be achieved. The Ultrogel AcA sorbent most commonly used in affinity chromatography is Ultrogel AcA 22. The principal activation methods used for attaching a spacer arm or for immobilizing a ligand directly onto the sorbent involve the following reagents: glutaraldehyde, cyanogen bromide, epichlorohydrin, bis-epoxiranes, benzoquinone, divinylsulfone or periodate.

## References

1. Gomi, K. and Jajiyama, N., *J. Biol. Chem. 276/33* (2001) 36508.
2. Sehgal, N. and Goswami, S., *Indian J. Biochem. Biophys. 38/4* (2001) 263.
3. Knutson, V.P. et al., *J. Immunol. Meth. 145* (1991) 263.
4. Diesterhaft, M., *Biochem. Biophys. Res. Commun. 125/3* (1984) 888.
5. Boschetti, E. et al., *Biochimie 54* (1972) 439.

## Ordering Information

| Product | Cat. No. | Size |
|---|---|---|
| Ultrogel AcA 22 | 23013-014 | 1 L |
| Ultrogel AcA 22 | 23013-030 | 10 L |
| Ultrogel AcA 34 | 23015-019 | 1 L |
| Ultrogel AcA 34 | 23015-035 | 10 L |
| Ultrogel AcA 44 | 23022-015 | 1 L |
| Ultrogel AcA 44 | 23022-031 | 10 L |
| Ultrogel AcA 54 | 23019-011 | 1 L |
| Ultrogel AcA 54 | 23019-045 | 10 L |
| Ultrogel AcA 202 | 24892-010 | 1 L |
| Ultrogel AcA 202 | 24892-036 | 10 L |

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