



A New Cost-Effective Adsorbent for the Capture and Purification of Antibiotics

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Abstract

The purification of antibiotics such as Cephalosporin C from complex fermentation broths can be accomplished effectively using chromatographic adsorbents. A new chromatographic adsorbent, Amberlite™ XAD™18, has recently been introduced for the purification of antibiotics. Amberlite XAD18 is a highly cross linked, polystyrenic adsorbent that has high capacity and excellent selectivity. Additionally the particle size distribution of this new adsorbent allows the use of either fixed bed column mode or simulated moving bed (SMB) operation. This work will demonstrate the utility of Amberlite XAD18 for the purification of antibiotics.

Figure 1 - Cephalosporin Separation Challenge is to resolve very closely related molecules

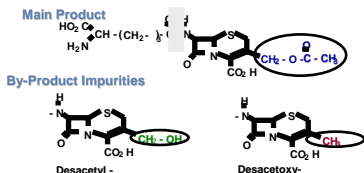
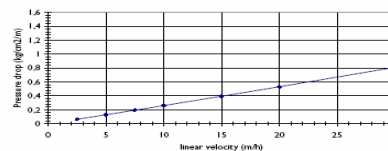


Figure 4 - Amberlite XAD18 Pressure Drop



Applications

Amberlite XAD18 was designed to combine both higher capacity and improved selectivity for antibiotic purifications. Higher capacity translates to improved plant productivity and better process economics. Improved selectivity translates to higher purity, even at high loading levels.

Selectivity for Cephalosporin C and separation from the closely related species desacetyl Cephalosporin C and desacetoxy Cephalosporin C in fermentation broth were determined using both Amberlite XAD16 and Amberlite XAD18. The commercial broth had initial concentrations of 79% Cephalosporin C, 17% desacetyl Cephalosporin C, and 4% desacetoxy Cephalosporin C. The broth was loaded onto the columns to the desired concentration (35-55 g/L). A two BV water rinse was performed, followed by elution with three BV of 0.05M NaOAc, and finally three BV of water. An example elution profile for Amberlite XAD18 is shown in Figure 5. The elution profile and purity-yield were determined by fraction analysis of the eluent. As shown in Figure 6 below, the yields at either 80% or 90% purity were much higher with Amberlite XAD18 versus Amberlite XAD16.

Figure 5 - Cephalosporin C Purification Chromatogram

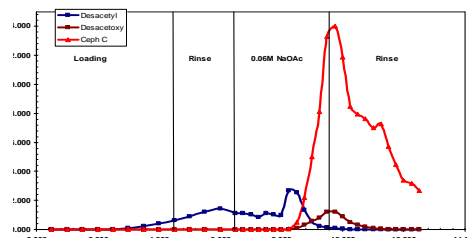


Figure 6 - Cephalosporin C Yield/Purity Curves

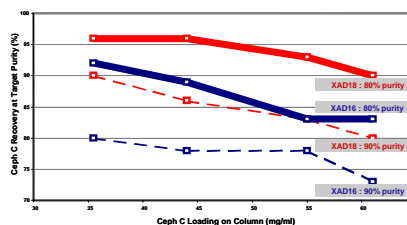
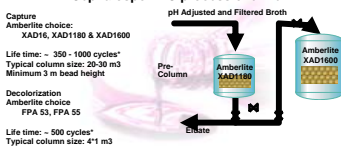


Figure 2 Cephalosporin C process overview



Product Properties

Amberlite XAD18 is a macroreticular cross-linked polystyrene/divinylbenzene polymer intended for large-scale biopharmaceutical production. The product properties are listed below in Table 2.

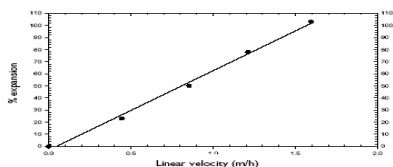
Table 2 - Amberlite XAD18 Product Properties

Particle Size	425 ± 50µm
Harmonic Mean Size	< 1.7
Uniformity Coefficient	< 2%
Fines Content (≥12µm)	
Surface Area	≥ 800 m ² /g
Mean Pore Size	150 angstroms
True Wet Density	1.03 g/mL
pH Range	1 to 14
Temperature Range	4 to 150°C
Swelling Properties	Volume Increase
Methanol	15 - 20%
2-Propanol	15 - 20%
Acetone	10 - 15%

Hydraulic Characteristics

Figure 3 shows the bed expansion of Amberlite XAD18 as a function of backwash flow rate at a temperature of 20°C. Figure 4 shows the pressure drop for Amberlite XAD18, as a function of service flow rate at 20°C. Pressure drop data are valid at the start of the service run with water and a correctly classified, settled, drained bed.

Figure 3 - Amberlite XAD18 Bed Expansion



Cephalosporin C Purification

The derivatives of Cephalosporin C, based from 7-ACA, form the second most important group of antibiotics prescribed today throughout the world. The principal method of producing the base product for all of the cephalosporin drugs is via fermentation of the Cephalosporin C and subsequent cleavage of the amide bond to produce 7-ACA. However, during the fermentation which can take several days, some very similar products are formed with the Cephalosporin C which must be removed by the downstream recovery process. The structure of the two principal impurities are shown in Figure 1 and it can be seen that they differ only very slightly from that of Cephalosporin C itself. Figure 2 shows schematically what a typical Cephalosporin C process might look like.

Regeneration of Amberlite XAD18

Amberlite XAD18 is compatible with a number of regenerants in cleaning protocols. Water-miscible organic solvents, such as methanol, ethanol, acetone, isopropanol, etc. can be used for removing hydrophobic compounds, oils, and antifoulers. Alkaline solutions (e.g. 1N NaOH) can be used for regenerating resins fouled with proteins, peptides, and other biological materials. Dilute acids (0.1 - 0.5% HCl) can be used to remove weakly basic compounds. Dilute oxidizing agents (<0.5%) such as hydrogen peroxide, can be used to enhance the removal of proteins. Hot nitrogen or steam (<150C) can be used for removing volatile compounds.

Pretreatment of Amberlite XAD18

Amberlite XAD18 is supplied as a water wettable product imbedded with sodium chloride and sodium carbonate salts to inhibit bacterial growth. These salts must be washed from the adsorbent prior to use. It is recommended that this be done by washing the resin in a column with deionized water at a flow rate of two to three bed volumes (BV) per hour until the salt levels are reduced to a sufficiently low level.

After the salts have been removed, it is recommended that Amberlite XAD18 be equilibrated with organic solvent in order to ensure optimal performance. The following equilibration procedure is recommended:

1. Connect the column outlet to a suitable waste container or vessel. Do not connect the column to a detector at this stage.
2. Equilibrate the column with a total of eight BV of either 100% acetonitrile, acetone, ethanol, propanol or methanol. Use a maximum flow rate of two BV/hr.
3. Equilibrate the column with five BV of a 50% solution of either acetonitrile, acetone, ethanol, propanol, or methanol in water. Use a maximum flow rate of one BV/hr.
4. The column is now ready for operation.

Conclusions

- Amberlite XAD18 is an improved polymeric resin for the adsorption and purification of antibiotics.
- Amberlite XAD18 demonstrates excellent physical stability.
- Amberlite XAD18 provides superior capacity and selectivity for antibiotics versus other commercially available adsorbents.

References

1. US464466, Process of producing inosinycin nucleotides, The Upjohn Company Inventor(s) Argoudelis, Alexander D.; Stroman, David W.
2. US3997662, Antibiotics and their preparation, Rhône-Poulenc S.A. Inventor(s) Pirenet, Sylvie; Jinet, Leon; Piret-Horme, Jean
3. US4279997, Process for production of aminoglycoside antibiotics, Yamanouchi Pharmaceutical Co., Ltd.
4. WO9950271, Novel process for the fermentative production of cephalosporin, DSM N.V. Inventor(s) BOOGERS, Ivo; Adriaens, Lambertus; Antonius; VAN DE SANDT, Emilius; Johannes, Albertus; Xavierus; SCHIPPER, Dick
5. US3914157, Preparation of antibiotics by fermentation, Merck & Co., Inc. Inventor(s) Stapley, Edward O.; Mata, Justo M.
6. GB2137201, Process for the isolation of tylosin, Etila S.p.A./T, Farmalab Carlo Inventor(s) Coppo, Ernesto; Giaccone Rosa, Onorino; Varesco, Carlo; Lazzari, Giovanni; Fabris, Danilo
7. US3629233, Process for purifying erythromycin, Kaken Chemical Co., Ltd.
8. US5223413, Process for the preparation of vancomycin, Biochin Biotechnologie Leanyvallat Inventor(s) NAOY, Masaru; JIKAI, et al.
9. US5574135, Process for making vancomycin, Abbott Laboratories Inventor(s) Chu, Alexander H. T.

