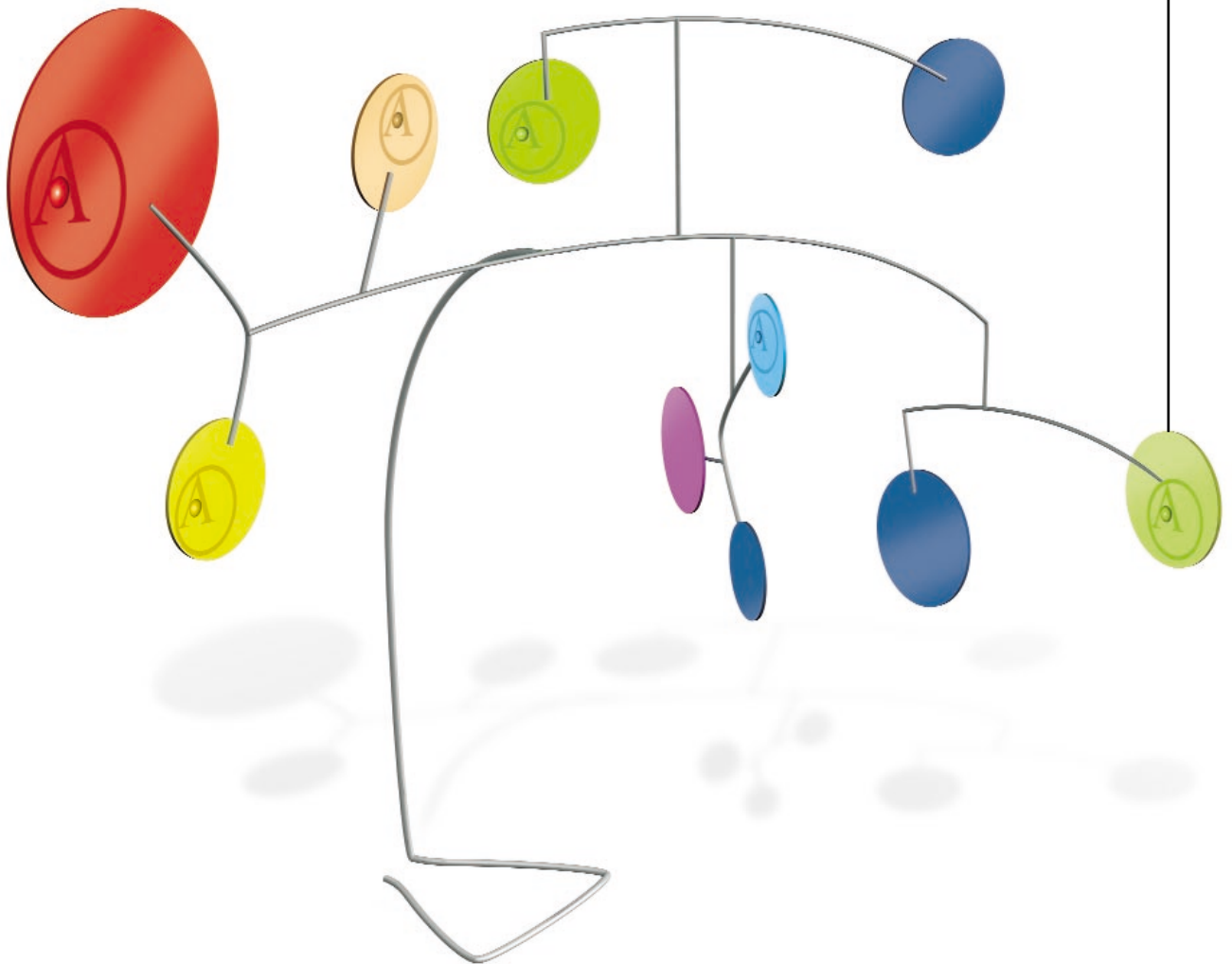




ADVANCED BIOSCIENCES

Enhancing biopharmaceutical production and purification

● AMBERCHROM™ XT Reversed phase chromatographic resins



Enhancing the art of protein, peptide and oligonucleotide polishing

AMBERCHROM™ XT chromatographic resins were developed to solve the problems associated with large scale biomolecule purification utilizing silica based media or standard polymeric reversed phase chromatographic media. Customers have reported short resin life for silica based media and low pressure stability for polymeric reversed phase media. Amberchrom™ XT reversed phase chromatographic media address these market needs:

- Improved performance in hydrophobic/polar peptide purification
- CIP
- Higher performances
 - Improved mechanical stability
 - Unique selectivity
- Provides the required chemical stability, selectivity, and high capacity.

Amberchrom™ Product Line

Amberchrom™ XT chromatographic media are an extension of the existing Amberchrom™ CG line which you might be already familiar with. They are targeted at large scale biomolecule purification and address the needs of the customer for a pressure stable, selective, high capacity polymeric reversed phase chromatographic media.

Table 1 provides the physical characteristics of the Amberchrom™ XT media.

The polystyrene/DVB based media offer excellent pressure stability and broad chemical compatibility for unparalleled efficiency and economy in large scale operation.

Table 1: AMBERCHROM™ XT resin properties

Matrix	Polystyrene/divinylbenzene
Bead Form	spherical, macroporous
Pore Size	300 Å
Particle Size	Amberchrom™ XT20 – 20 µm Amberchrom™ XT30 – 30 µm
Maximum Pressure	60 bar
pH Stability	1 – 14
Operating Temperature	4 – 60° C

CIP Stability

Manufacturing today's biomolecules requires rigorous, validated cleaning methods to derive the highest value out of the purification resins used. The most common accepted cleaning procedure is the use of caustic cleaning methods to assure the hydrolysis and removal of peptide and protein based residuals in the purification system.

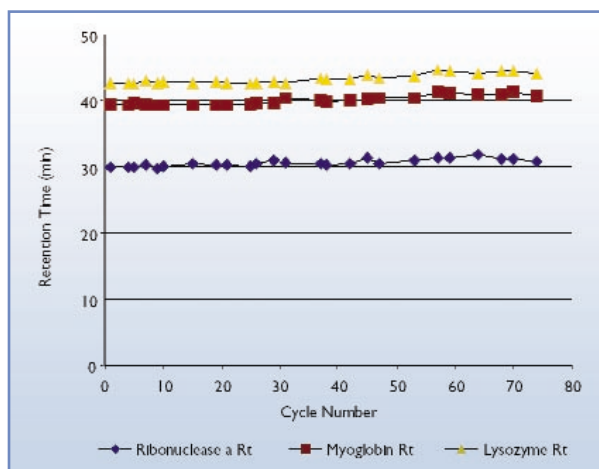
The polymeric resins used in the manufacture of Amberchrom™ XT chromatographic resins are extremely stable to CIP protocols based on caustic hydrolysis.

Customers reported short resin life when using silica based media and CIP cleaning procedures for therapeutic biopharmaceutical production.

Amberchrom™ XT media provide sustained capacity for biomolecules after repeated cycles of cleaning with caustic solutions as high as 1.0 M NaOH for over 80 cycles (Figure 1).

The media retain their original capacity throughout the cycling regimen with various protein probe molecules.

Figure 1: AMBERCHROM™ XT - CIP stability



A column of Amberchrom™ XT was subjected to caustic cycling study to demonstrate stability in CIP environments. A mixture of ribonuclease a, myoglobin, and lysozyme was separated using a linear gradient of acetonitrile with 0.1% TFA on a 1.0 cm I.D. x 10 cm L column. The column was then cleaned with ten column volumes of 1.0M sodium hydroxide solution at a linear velocity of 38 cm/h. The retention times of the three proteins were monitored over the course of the exposure.

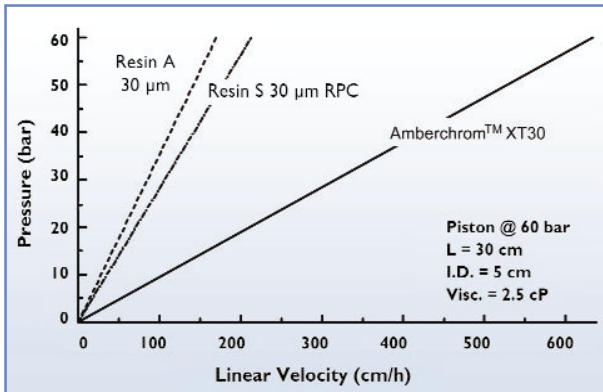
High Performance Purification

To achieve the high purity required for biopharmaceutical entities, manufacturers have adopted high performance chromatography procedures in the final steps of their purification trains.

The combination of high pressure and small diameter resins provides extremely high purity in polishing applications. Many manufacturers have implemented silica based polishing systems to achieve the required purity level in their products. This grew from the use of silica based analytical columns used in the discovery laboratories. The manufacturers were not able to use the familiar polymeric reversed phase media in these high pressure systems due to a lack of pressure stability. Amberchrom™ XT resins were developed to address the needs of the customer for a pressure stable, high capacity polymeric reversed phase chromatographic media.

Amberchrom™ XT media provide improved pressure stability when compared to currently marketed polymeric reversed phase media (Figure 2). Polymeric reversed phase media had developed a reputation for low pressure stability. Amberchrom™ XT resins were developed specifically to address this performance issue.

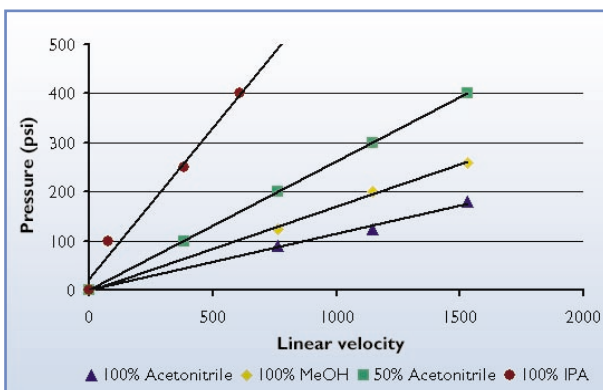
Figure 2: AMBERCHROM™ XT Pressure stability



Three 30 µm polystyrene divinylbenzene polymers were packed in a 5 cm I.D. x 30 cm L dynamic axial compression (DAC) column. Amberchrom™ XT30 provided superior pressure flow characteristics, which allows higher throughput.

Figure 3 demonstrates the pressure flow curves of the Amberchrom™ XT resin with various mobile phases of increasing viscosity. The data in Figure 2 show up to a 500% increase in flow rates for Amberchrom™ XT30 when compared to commercial 30 µm polymeric reversed phase media at a differential pressure of 60 bar. Amberchrom™ XT media are compatible with installed process scale HPLC equipment operating with differential pressures of up to 60 bar.

Figure 3: AMBERCHROM™ XT Pressure stability



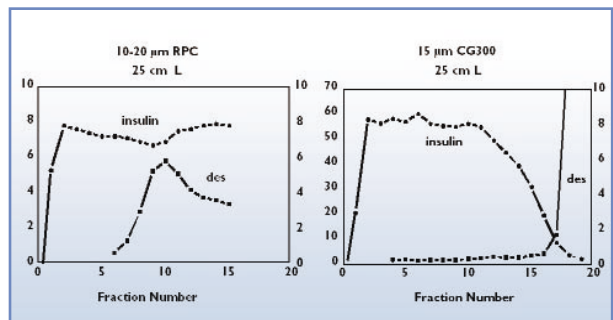
A 1 cm I.D. x 25 cm L column of Amberchrom™ XT20 was flow packed at a maximum linear velocity of 726 cm/h to a maximum pressure of 500 psi. It was then equilibrated with various solvents and solvent mixtures prior to measuring the pressure drop across the column. Amberchrom™ XT demonstrated a linear pressure / flow response with all solvents up to 500 psi.

Selectivity

One of the major advantages biopharmaceutical manufacturers have relied on polymeric reversed phase media for is their improved selectivity when compared to silica based media.

Like Amberchrom™ CG media, which have a demonstrated ability to resolve difficult to separate closely related biomolecular variants such as desamido - insulin from insulin (Figure 4), Amberchrom™ XT provide the biopharmaceutical development and manufacturing community with a new separation medium for their chromatographic tool kits.

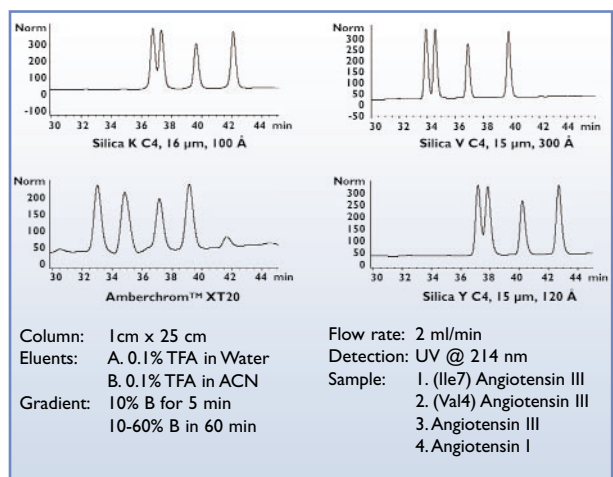
Figure 4: Resolving power of AMBERCHROM™ CG300



The two columns contained the same number of theoretical plates, so the resolving power is a function of the polymer selectivity.

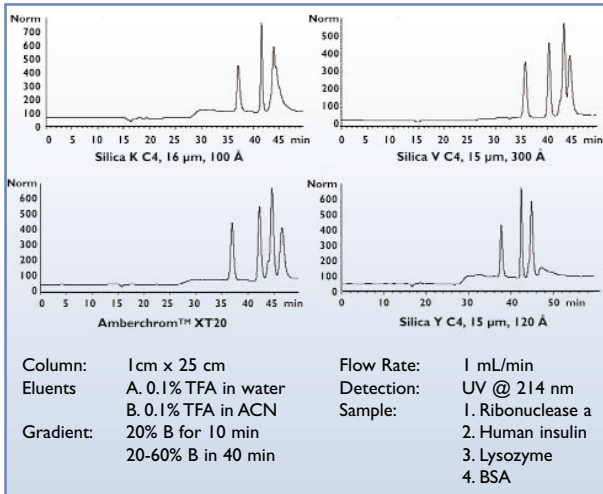
Amberchrom™ XT polymeric reversed phase media demonstrate superior selectivity for closely related biomolecules also. This is shown in the ability of the resin to resolve all four variants of Angiotensin, while 15-16 µm C4 silica media ranging from 100-300 Å pore sizes did not resolve the two variants of Angiotensin III (Figure 5).

Figure 5: Angiotensin separation Selectivity comparison



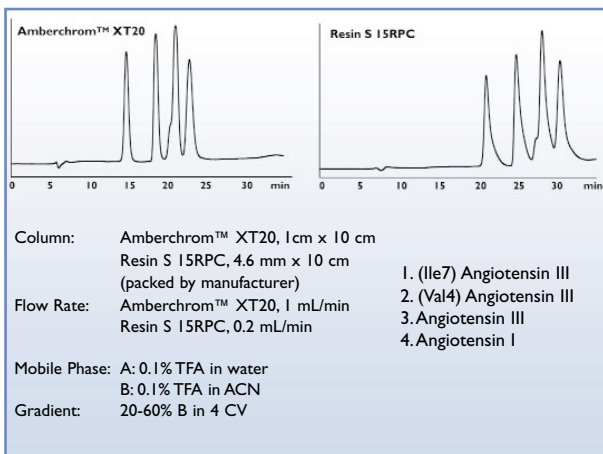
The resolving power of Amberchrom™ XT resin is further demonstrated in the separation of a mixture of protein of varying molecular weights (Figure 6). Amberchrom™ XT provides superior resolution when compared to common silica based chromatography columns.

**Figure 6: Protein separation
Selectivity comparison**



The improved selectivity and operating efficiency is also demonstrated over other polymeric reversed phase chromatographic media. Figure 7 demonstrates increased resolution and reduced peak tailing when compared to other polystyrene based polymeric media.

**Figure 7: AMBERCHROM™ XT20
and Media S 15RPC**



The above comparison demonstrates the sharp peak shape and resolution of Amberchrom™ XT resins for small peptides. Both columns were packed with polystyrene divinylbenzene resins, however the unique pore distribution of Amberchrom™ XT20 provided a better separation of the protein mixture.

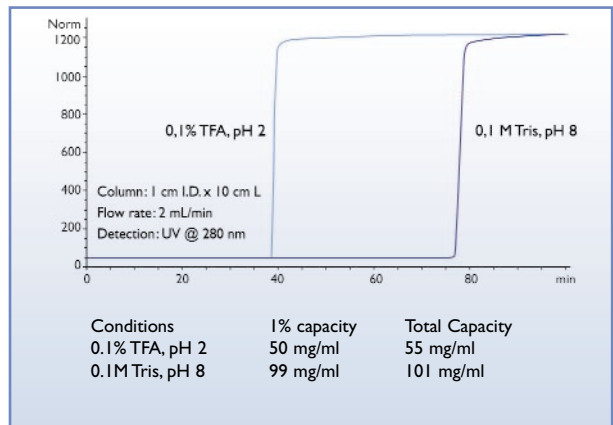
Improved Operating Range

Biopharmaceutical products are complex. The net effect of ionization of amino acids, side chains, hydrophobic areas arising from folding and the resulting tertiary structure of the molecule offer opportunities for unique separations strategies.

Polymeric reversed phase chromatographic resins like Amberchrom™ XT provide an expanded operating range to be exploited in designing your separations strategies. Peptide manufacturers have found this expanded pH operating range and the improved selectivity of the Amberchrom™ XT resins to meet their most difficult separations problems.

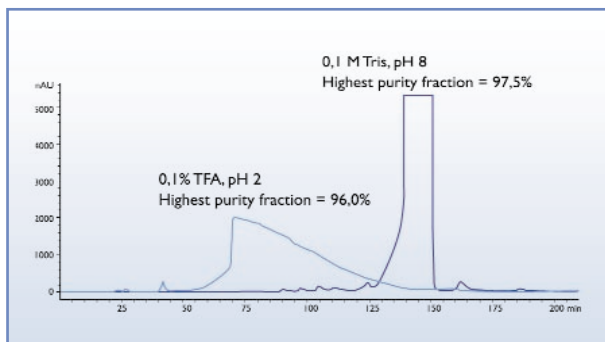
By utilizing the ability to operate in strongly basic conditions, it has been demonstrated that the capacity of Amberchrom™ XT resin for the glycopeptide based antibiotic Vancomycin was doubled (Figure 8) and the elution profile was significantly improved (Figure 9).

**Figure 8: Achieving high capacity
breakthrough curves for Vancomycin
on AMBERCHROM™ XT20**



Under basic conditions Vancomycin behaves as a more hydrophobic molecule, and the dynamic capacity on Amberchrom™ XT20 doubles. This demonstrates the advantage of a wider pH range with polymeric resins.

**Figure 9: Benefits of operating under basic conditions
Vancomycin purification on AMBERCHROM™ XT20**

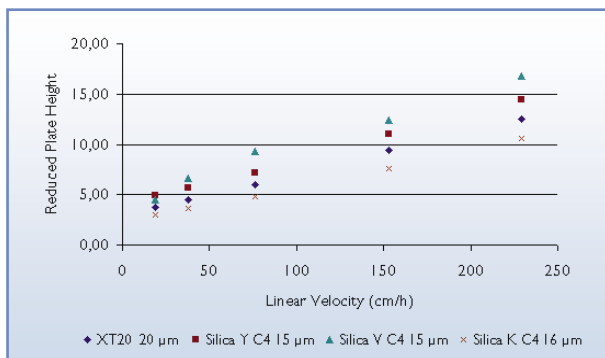


A chromatographic polishing step was developed for vancomycin, (Sigma USP grade (MW 1485)). The HPLC purity of the feedstock was around 89%. Preparative purification was performed on a 1cm I.D. x 25 cm L Amberchrom™ XT20 column under two different pH conditions. An equivalent ACN linear gradient of 3 to 25% over 10 CV was used for each. The purification was improved by increasing the pH. The elution was accomplished in a much tighter band and a higher product purity was achieved. This purification also demonstrates the advantage of having a wide pH stability range for developing a purification scheme.

Improved Capacity

Amberchrom™ XT reversed phase media have been optimized to provide the benefits of caustic stability, selectivity, and high pressure resistance while delivering high capacity for biomolecules such as insulin. Figure 10 demonstrates that the capacity for human insulin is directly in line with existing silica based media.

**Figure 10: AMBERCHROM™ XT20
Efficiency comparisons using human insulin**



Column efficiency was determined using human insulin for Amberchrom™ XT20 and three competitive silica products. As shown above, Amberchrom™ XT can be packed to yield high efficiency for demanding separations.

Table 2 demonstrates the total capacity of the Amberchrom™ XT resins for a variety of biomolecules as well as the 1 % capacity breakthrough values for these representative molecules.

**Table 2: AMBERCHROM™ XT20
Capacity vs molecular weight**

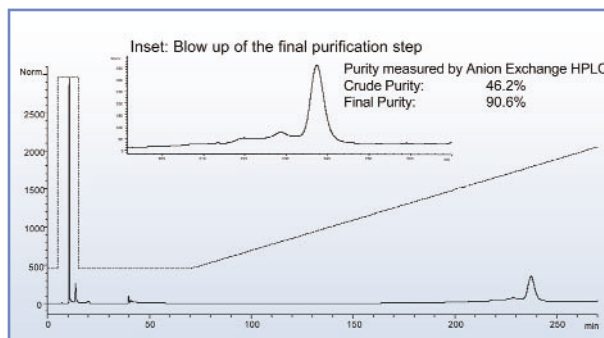
Molecular Weight	Probe	1% Capacity (mg/mL)	Total Capacity (mg/mL)	% of Capacity
1,485 MW	Vancomycin	99	101	98
5,600 MW	Insulin	76	94	81
14,000 MW	Lysozyme	40	53	75
43,000 MW	Ovalbumin	15	54	28
67,000 MW	BSA	3	20	15

Dynamic capacity measurements were determined on Amberchrom™ XT20 using five different molecular weight probes in a 0.1% TFA mobile phase at 152 cm/h. Vancomycin capacity was determined in 0.1M Tris, pH 8.0. The data show that capacity is highest for molecules under 14,000 MW, and 1% breakthrough capacity drops as a percentage of total dynamic capacity as the MW of the probe molecule increases. As shown above, Amberchrom™ XT resins are best-suited for peptides and small proteins.

Oligonucleotide Purification

Amberchrom™ XT reversed phase media have also been demonstrated to be excellent for the purification of crude oligonucleotide preparations in a single step (Figure 11).

**Figure 11: AMBERCHROM™ XT20
20-mer oligonucleotide purification**



A single-step purification of a 20 mer phosphodiester oligonucleotide was performed using a 1cm I.D. x 25 cm L column of Amberchrom™ XT20. The crude DMT on oligonucleotide, which was not fully deprotected, was loaded onto the column, detritylated, and eluted. Purity was assessed using anion exchange HPLC. This unoptimized separation demonstrated the ability of Amberchrom™ XT resins to purify crude oligonucleotides in a single step.

Conclusions

Amberchrom™ XT reversed phase chromatographic resins provide:

- High capacity for biomolecules
- Mechanical stability / more rigidity
- A wider range of pH stability than silica products
- High efficiency and excellent selectivity for superior chromatographic performance
- Excellent separation capabilities for peptides and oligonucleotides
- Ideal for hydrophobic and polar peptides.

Please call Rohm and Haas at the numbers on the back of this brochure for additional information about AMBERCHROM™ chromatographic resins or visit us at: <http://www.amberchrom.com>.

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Please visit our website : www.amberchrom.com
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Ion exchange resins and polymeric adsorbents, as produced, contain by-products resulting from the manufacturing process. The user must determine the extent to which organic by-products must be removed for any particular use and establish techniques to assure that the appropriate level of purity is achieved for that use. The user must ensure compliance with all prudent safety standards and regulatory requirements governing the application. Except where specifically otherwise stated, Rohm and Haas Company does not recommend its ion exchange resins or polymeric adsorbents, as supplied, as being suitable or appropriately pure for any particular use. Consult your Rohm and Haas technical representative for further information.

Acidic and basic regenerant solutions are corrosive and should be handled in a manner that will prevent eye and skin contact. Nitric acid and other strong oxidising agents can cause explosive type reactions when mixed with Ion Exchange resins.

Proper design of process equipment to prevent rapid buildup of pressure is necessary if use of an oxidising agent such as nitric acid is contemplated. Before using strong oxidising agents in contact with Ion Exchange Resins, consult sources knowledgeable in the handling of these materials.

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