AMBERCHROM® XT

Chromatographic Grade Resin

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For Biomolecule Purification and Polishing

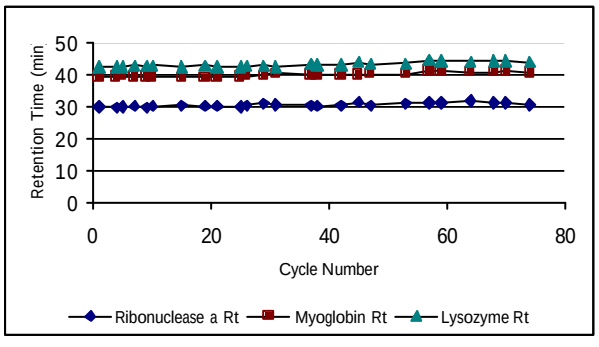
PRODUCT DATA SHEET

AMBERCHROM XT polishing grade resins are insoluble polystyrene divinylbenzene polymers manufactured for high value reversed phase chromatographic applications. AMBERCHROM XT resins' high surface area, unique pore size and pore volume distribution make them ideally suited for separation of proteins, peptides and nucleic acids. They are an excellent technical and economical alternative to RPC silica, and can be used in high resolution, high pressure chromatography. AMBERCHROM XT resins are available in two different particle size ranges (20 and 30 microns), and are supplied as a dry resin. Rehydration recommendations are provide in the product specifications area of this data sheet.

EXCELLENT pH STABILITY

Unlike RPC silica, AMBERCHROM XT chromatographic resins, due to their polymeric nature and lack of bonded phase, can be operated and cleaned over a pH range of 1-14. As the graph below demonstrates, there is no change in retention properties after more than seventy cycles using 1.0M NaOH as a CIP agent.

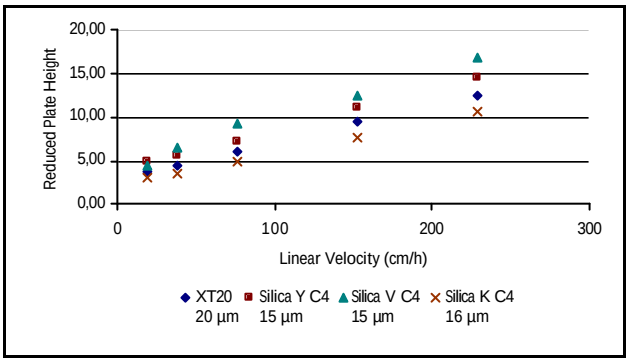
Figure 1: Caustic Stability of AMBERCHROM XT



HIGH EFFICIENCY

As shown in Figure 2, AMBERCHROM XT chromatographic resins provide similar efficiencies to silica products. Polymeric AMBERCHROM XT chromatographic resins can be packed to high efficiencies for demanding separations.

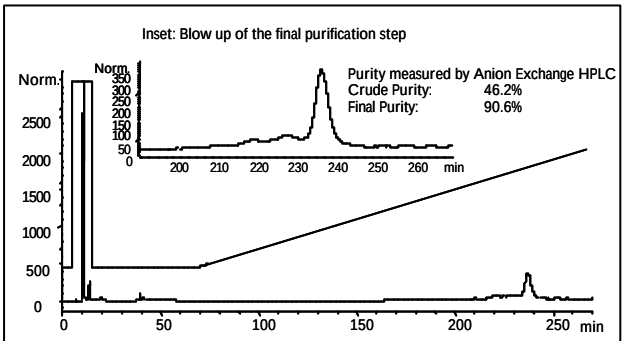
Figure 2: Efficiency Measurements with Human Insulin



AMBERCHROM XT chromatographic resins provide excellent separations platforms for oligonucleotides. Table 1 contains the conditions used for a crude oligonucleotide purification.
A single-step purification of a 20mer phosphordiester oligonucleotide was performed using a 1 cm 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.

Figure 3 presents the results from the separation which demonstrates the ability of AMBERCHROM XT resins to purify crude oligonucleotides in a single step.

Figure 3: AMBERCHROM XT20
20-mer Oligonucleotide Purification



Conditions

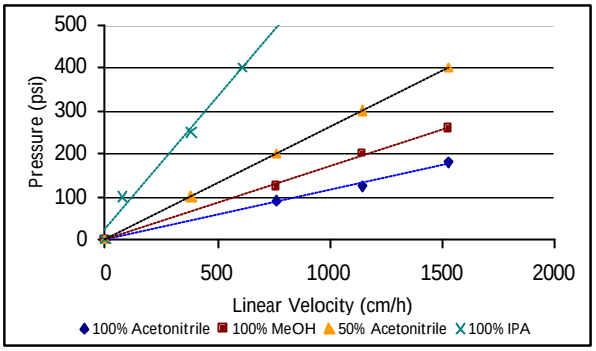
Column:	AMBERCHROM XT20, 1cm I.D. x 25cm L	
Flow rate	Loading and Equilibration:	4 mL / minute
	Detritylation:	4 mL / minute
	Elution:	1 mL / minute
Mobile Phase	A: 0.1M TEAA, pH 7.0 B: 20% acetonitrile in 0.1M TEAA, pH 7.0 C: 0.5% TFA (for detritylation)	
Gradient	20% - 55% B in 10 column volumes (200 mL)	
Sample	20-mer phosphodiester oligonucleotide (5'-AGC TAG CTA GCT AGC TAG CT-3') DMT-on, not fully deprotected	
Loading	100 µl of 1.0 µmol	
Detection	UV @ 260 nm	
Fractions	1 ml fractions collected	

EXCELLENT MECHANICAL STABILITY

Polymeric chromatographic resins are not always rigid enough to handle high pressures and are incompatible with HPLC equipment. AMBERCHROM XT chromatographic resins overcome these limitations and are designed to handle pressures up to several hundred psi.

Figure 4 demonstrates the linearity of the pressure / flow relationship in several different solvents and solvent mixtures.

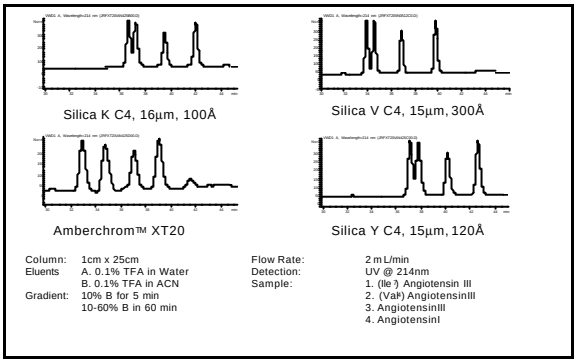
Figure 4: Pressure/Flow for AMBERCHROM XT
1cm I.D. x 25cm L Column containing 20 µm Resin



UNIQUE SELECTIVITY

Another advantage of AMBERCHROM XT chromatographic resins is their unique selectivity. As shown below, angiotensins with one amino acid difference can be separated on Amberchrom XT while conventional silica matrices have difficulty separating all four angiotensin variants.

Figure 5 : Selectivity – Angiotensin Separation

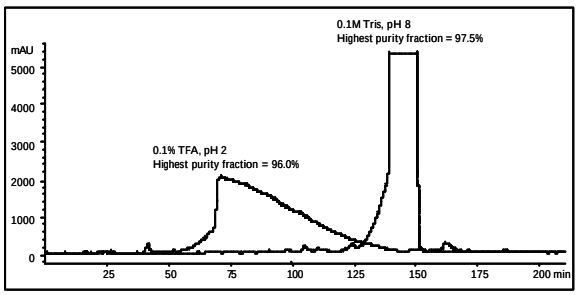


IMPROVED RESOLUTION FOR HYDROPHOBIC AND POLAR PEPTIDES

Vancomycin, a hydrophobic cyclic peptide was used as a model system to demonstrate the improved resolution under basic pH operating conditions. When purification is conducted under pH 2.0 conditions which are consistent with current protocols for silica based media, Amberchrom XT demonstrates typical capacities and elution profiles.

However, when the physical capabilities of Amberchrom XT are utilized and the purification is conducted using a pH 8.0 Tris buffer, the capacity of Amberchrom XT doubles to 100 mg/ml resin (Figure 6) and the elution profile and purity (Figure 7) are significantly improved over traditional procedures.

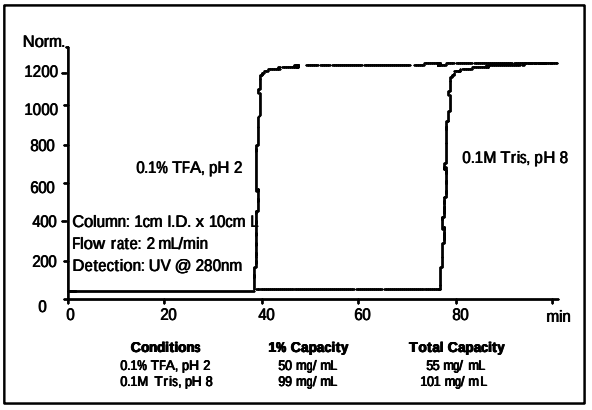
Figure 6: Benefits of operating under basic conditions Vancomycin purification on Amberchrom XT20



A chromatographic polishing step was developed for vancomycin, *(Sigma USP grade (MW 1485))*. The HPLC purity of the feedstock was ~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.

Figure 7: Breakthrough curves for vancomycin on Amberchrom XT20



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

SUPERIOR DYNAMIC CAPACITY

AMBERCHROM XT resins provide superior dynamic capacities for molecules over a wide range of molecular weights when compared to competitive products. Table 2 shows the dynamic capacities for vancomycin (MW 1,485), bovine insulin (MW 5,600), and lysozyme (MW 14,000). Capacities were measured at 153 cm/h using 0.1% TFA as a loading buffer.

Table 1: Dynamic Capacity

Packing Material	Probe	1% Capacity (mg/mL)	Total Capacity (mg/mL)
XT20	Vancomycin	50	55
XT30	Vancomycin	44	nd
Resin S 15RPC	Vancomycin	28	30
Resin P, 100Å	Vancomycin	37	44
XT20	Insulin	76	94
XT30	Insulin	80	98
Resin S 15RPC	Insulin	76	nd
Resin P, 300Å	Insulin	53	61
Silica Y, 300Å	Insulin	44	46
XT20	Lysozyme	40	53
XT30	Lysozyme	38	nd
Resin P, 100Å	Lysozyme	27	55
Resin P, 300Å	Lysozyme	26	38
Silica V C4	Lysozyme	23	24

REGULATORY STATUS

A Material Regulatory Support (MRS) package is maintained for Amberchrom™ XT resins. It is available upon request under CDA for users of this product. This material is manufactured under strict controls, and plant audits by potential customers are welcomed.

TABLE 2: AMBERCHROM XT

Matrix	Polystyrene divinylbenzene
Pore size	300 Å
Particle size	AMBERCHROM XT20 : 20 µm AMBERCHROM XT30 : 30 µm
pH range	1 - 14
Operating temperature	4 – 40 °C
Maximum pressure	60 bar
Recommended mobile phases	Mixtures of ACN, EtOH, MeOH, propanol, isopropanol and acetone
Rehydration and packing	60% n-propanol or 60% n-isopropanol in water
Chemical resistance	Insoluble in dilute solutions of acids or bases and common solvents : IPA, ACN, MeOH, EtOH.

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