Water Solutions

Separation and Purification for Bioprocessing Applications

DuPont™ AmberChrom™, DuPont™ AmberLite™ and DuPont™ AmberChrom™ XAD™ Ion Exchange Resins and Adsorbents

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Bioprocessing

Bioprocessing is a broad term encompassing the research, development, manufacturing, and commercialization of products prepared from or used by biological systems, including food, feed, biopharmaceuticals and cosmetics.

Although the pharmaceutical industry has been based on the synthesis of small organic molecules from the beginning, biopharmaceuticals have emerged as the next generation of therapeutics. Insulin, the first biologic drug, has been implemented in the treatment of diabetes for nearly a century.

Biologics are drugs made from complex molecules manufactured using living microorganisms, plants, or animal cells. Many biologics are produced using recombinant DNA technology. They are significantly larger and more complex than their small molecule counterparts and therefore require new techniques with sophisticated media for separation, concentration and purification.

DuPont is one of the largest manufacturers of ion exchange resins and polymeric adsorbents worldwide. Our comprehensive product line, technical expertise, and global reach follow for optimized performance of even the most complex manufacturing processes.

Continuous investment in manufacturing excellence and innovation combined with DuPont's global R&D capabilities and expertise allow us to address specific conditions and requirements of the bioprocessing industry. Our high quality brands, such as **AmberLite™, AmberLite™ XAD™** and **AmberChrom™** are well established and recognized in the biopharma industry.

Biomolecules: capture & purification

Biomolecules manufacturing can be divided into two steps:
- Upstream processing consisting of microbiological or chemical synthesis
- Downstream processing aimed at capturing, separating, purifying and polishing selected proteins, peptides or active molecules

DuPont, as global market leader in ion exchange resins, has a broad range of products with hydrophobic (polystyrene-DVB) or medium hydrophilic (polyacrylic) matrices, specifically designed to be used in downstream processing for small biomolecules such as peptides, oligonucleotides, antibiotics or natural extracts.

Peptides

There are more than 60 US Food and Drug Administration (FDA) approved peptide medicines currently on the market. This is expected to grow significantly, with approximately 140 peptide drugs in clinical trials and more than 500 therapeutic peptides in preclinical development. Peptides can be produced by chemical synthesis (solid-phase or solution) or by recombinant procedures. In both cases, polymeric adsorbents such as **AmberChrom™ CG** and **XT series** can be used for capture and polishing steps. **AmberChrom™ CG** and **XT** products have a definitive advantage on silica chromatographic products because they can afford CIP and SIP (Cleaning and Sanitization In Place) with caustic (1M NaOH at 60°C). It also means that manufacturing equipment can be fully cleaned and sanitized to prevent product carryover or endotoxin and viral contamination. **AmberChrom™ CG** and **XT** products are stable in any solvent at any pH and exhibit an outstanding mechanical stability. This can in turn enable certain types of compounds to be produced with much better productivity, economic viability and decreased environmental impact.

For the extraction and purification of biomolecules, DuPont offers different types of adsorbent media characterized by their particle size distribution, surface area, porosity, hydrophobicity and stability to a wide variety of conditions:
- Acids/Caustic – pH range of 1-14
- Peracetic acid, weak oxidizer
- Organic Solvents
- Steam sterilization

These products are stable to caustic cleaning in place procedures and are valid alternatives to silica chromatographic products.

Oligonucleotides

Although the oligonucleotides market remains relatively small, the rising need for synthesized oligonucleotides for the fast growing field of molecular diagnostics will boost the demand. Oligonucleotides are DNA or RNA polymers that are used for research, gene therapeutic drugs and probes for detecting DNA or RNA for molecular diagnostics and forensics uses. Oligonucleotides can be manufactured by chemical or enzymatic synthesis.

- In solid-phase and solution synthesis, each nucleotide is coupled to the chain until the desired sequence is assembled. The synthesis typically goes between 18 to 40 cycles with around 21 mers being the most common length. In each coupling cycle, a small percentage of the oligo chain will not be extended, resulting in a mixture of full-length product (n) and truncated sequences.
- In enzymatic synthesis, the techniques consist of copying small sequences of DNA with the help of a polymerase enzyme which is capable of synthesizing complementary sequences. This method leads to a higher purity than with chemical synthesis.

Oligonucleotides purification is the process of removing these shorter sequences from the desired full-length sequences. Reverse Phase Chromatography is the separation method of choice because it capitalizes on the difference in hydrophobicity between full-length product which contains a 5'-DMT group (DMT=4,4'- dimethoxytrityl) and truncated sequences (without DMT groups). While the full-length DMT oligo is retained on the column, the truncated sequences are washed off.

AmberChrom™ CG and **XT** resins can be used in the purification of **Lipid conjugated oligos** and **pegylated aptamers.**

Antibiotics

Antibiotics are drugs used to prevent or treat bacterial infections. They appeared at the beginning of the 20th century as one of the first therapies. However, their overuse in the past 30 years led to a reduced effectiveness of some of them. Antibiotics are classified according to their chemical structure, their mechanism of action and their spectrum activity. They can be produced by chemical synthesis or by fermentation. 15 years ago the trends were to lower research efforts into antibiotics; however, some companies still look for new developments on molecules capable of overcoming bacteria resistance. DuPont resins can be used at various steps of the antibiotic manufacturing process including exctraction, capture and concentration, decolorization and desalting, and final purification.

Vitamins

Vitamin B12

Vitamin B12, also known as cyano-cobalamin, is manufactured by fermentation using Propionibacterium freudenrechii and Pseudomonas denitrificans strains. The isolation of Vitamin B12 from fermentation broth is performed by treating the heated broth with cyanide orthiocyanate and sodium nitrite to obtain cyano-cobalamin. The separation can be then accomplished by a weak cationic resin such as **AmberLite™ FPC3500**.

Vitamin C

Vitamin C or L-ascorbic acid was first isolated in 1928 and subsequently identified as the long-sought antiscorbutic factor. Industrially produced L-ascorbic acid is widely used in the feed, food, and pharmaceutical sector as a nutritional supplement and preservative, making use of its antioxidative properties. Until recently, the Reichstein-Grüssner process, designed in 1933, was the main industrial route.
Here, D-sorbitol is converted to L-ascorbic acid via 2-keto-L-gulonic acid (2KGA) as key intermediate. Using a bio-oxidation with Gluconobacter oxydans and bio-oxidation steps with Ketogulonicigenium vulgare as a biocatalyst, D-sorbitol is converted to the intermediate 2KGA without chemical steps. In both production processes, both the ketogluconic acid and the ascorbate are obtained as the sodium salts. These sodium salts are acidified using a strong acidic cationic resin such as **AmberLite™ FPC23H** resulting in ketogluconic acid (2-KGA) and ascorbic acid. The cationic exchange resin is then regenerated with hydrochloric acid, or other strong inorganic acid. Following the ion exchange process, the purified ascorbic acid is crystallized.

Vaccines

Detergent removal

Vaccines are derived from a variety of sources including tissue extracts, bacterial cells, virus particles, and recombinant mammalian, yeast and insect cell-produced proteins and nucleic acids. The most common method of vaccine production is based on an initial fermentation process followed by purification. Production of vaccines is a complex process involving many different steps.
Detergent (e.g. Triton X100) promotes the liberation of host cell lipids into the process stream enabling the vaccine proteins to be removed from the cells. The use of detergents and homogenization by micro fluidization or osmotic shock has been successfully adopted in many large scale manufacturing processes.
Detergent removal is required to avoid the fouling of the high-resolution chromatography resins with lipids and host cell proteins in subsequent steps. This can be achieved with a hydrophobic polymeric adsorbent like **AmberLite™ XAD™4** and **AmberLite™ XAD™16N** as well as with **AmberChrom™ CG161M** or **AmberChrom™ CG300M**.

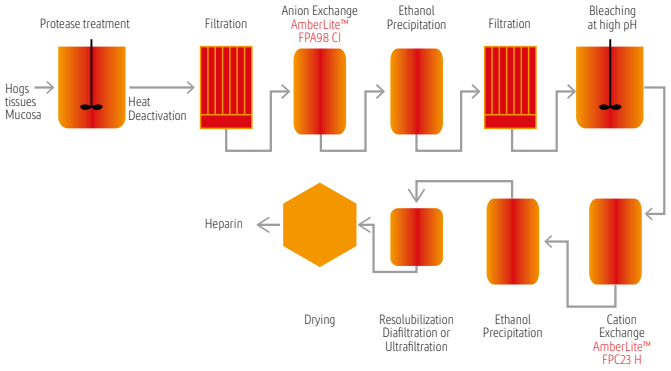
Diagnostics

Adsorbents and ion exchange resins can be used in Antimicrobial Removal Devices (ARD). These devices are used when patients have been administrated antibiotics that could prevent from the correct identification of bacteria in the blood analysis. The devices consist of a rubber capped vial loaded with culture media and a polymeric adsorbent such as **AmberLite™ XAD™** resins or **AmberLite™ CG50 Type 1** weak acid cation. In addition, ion exchange resins are commonly used in the manufacturing of radio-contrast agents like iodine, barium sulfate or gadolidium based compounds.

Natural Extracts

Heparin

Heparin is sulfated glycosaminoglycan (GAG) with the anticoagulant property of binding to antithrombin III to form a heparin-antithrombin III complex. This complex binds to and irreversibly inactivates thrombin and other activated clotting factors, such as factors IX, X, XI, and XII, thereby preventing the polymerization of fibrinogen to fibrin and the subsequent formation of clots. Nowadays, heparin is extracted from porcine intestinal mucosa by caustic brine at 50–55°C. After enzymatic digestion, the filtered solution is then contacted in batch or in column with an acrylic strong base macroreticular resin, **AmberLite™ FPA98 CL**. Crude heparin is then eluted from the resin with 3 to 5% concentrated brine, purified over a strong cationic resin such as **AmberLite™ FPC23H** and finally precipitated with ethanol before being dried or lyophilized.



Chondroitin

Chondroitin sulfate is also a sulfated glycosaminoglycan (GAG) composed of a chain of alternating sugars (N-acetyl-galactosamine and glucuronic acid). It is mainly extracted from bovine trachea, pig nasal septa, chicken keel, shark fins and fish cartilage. It is made of N-Acetylgalactosamine and glucuronic acid that can be attached to proteins. Its main application is treatment of osteoporitisis. The chondroitin purification process is similar to that of heparin, employing **AmberLite™ FPA98 CL**.

Plant extracts

Polyphenols are compounds found in natural foods that provide color and flavor and are highly valued for their antioxidant and health properties. These can be derived from many different sources including plants, fruits and oils. Polymeric adsorbents from the **AmberLite™ XAD™** product range are cutting edge products to recover and purify polyphenols extracted from natural products. Ion exchange resins can also be used to recover alkaloid compounds like morphine or natural drug extracts like taxol.

Enzyme immobilization

Biocatalysis can be defined as utilization of natural catalysts, such as protein enzymes, to perform chemical transformations on organic compounds. Reusability and maintenance of their structural stability during any biochemical reaction is mandatory for the commercialization of these bio-derived catalysts. To enhance their functional efficiency and reproducibility it is worth immobilizing enzymes to a phase (matrix/support) different from the one for substrates.

Enzyme	Application	Resin for enzyme immobilization
Lipases	Hydrolysis of carboxylic ester bonds, esterification, interesterification, and transesterification reactions	**DuPont™ Duolite™ A568**
Phospholipases	To tailor phospholipids (PLs) with defined fatty acid composition at the sn-1 and sn-2 positions (example: triglycerides (TAGs) with defined distribution of fatty acids)	**AmberLite™ XAD™ 7HP**
Glucose Isomerase	To convert glucose to fructose for High Fructose Corn Syrup (HFCS) production	**Duolite™ A568**
D-Hydantoinase	Large-scale synthesis of enantio-pure D-amino acids (for instance production of D-(p-hydroxyphenyl)glycine (HPG))	**Duolite™ A568**

Plasma detoxification

Extracorporeal blood filtration

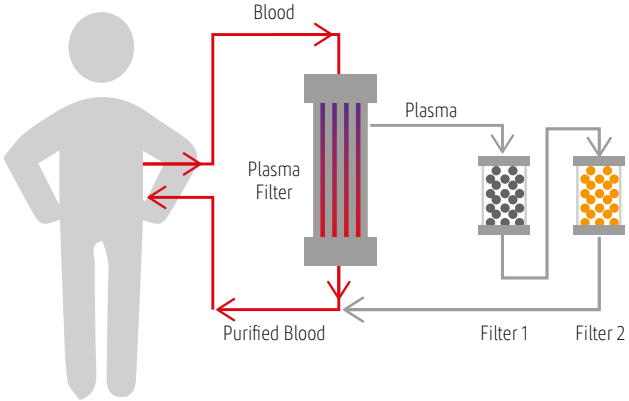
In acute liver or renal failure, a range of potentially toxic substances accumulates in the blood stream of the patient. Based on their large size, the protein-bound toxins are not able to cross the typical dialysis membranes. Consequently, the removal of protein-bound liver toxins by conventional dialysis techniques is inefficient.
Extracorporeal blood purification systems based on combined membrane/adsorption technologies are used in acute liver or renal failure to detoxify the plasma from albumin-bound toxins as well as to remove inflammatory mediators in sepsis patients. The plasma is separated from blood cells with a special membrane permeable to protein-bound toxins (albumin). It can be then filtered on one or several filters which can be filled with steam sterilized polymeric adsorbents such as **AmberChrom™ CG** products standing alone or in combination with an anion-exchanger for removal (for instance of bilirubin). The characteristics of the adsorbent in terms of particle size, pore size and surface activation are critical for the performance of the system. Depending of the therapeutic target, the adsorbent can be selected among the **AmberChrom™ CG** product range to achieve high performance.

Sepsis

Sepsis, also referred to as blood poisoning or septicaemia, is a potentially life- threatening condition, triggered by an infection or injury. During sepsis, triacylated peptides, diacylated peptides, or lipopolysaccharides (LPS) are released by pathogens, and are recognized by the Toll-like receptors located on the surface of antigen-presenting cells. Toll-like receptors also recognize locally produced damage- associated molecular patterns (DAMPs) from ischaemic renal tissue and circulating DAMPs released from extensive extra renal tissue damage in sepsis. This triggers the activation of leukocytes, endothelial cells, and epithelial cells that release more inflammatory mediators such as tumor necrosis factor–alpha (TNF-α), interleukin-1 (IL-1), IL-6, IL-8 and IL-10, causing cellular and tissue damage. This is called a 'cytokine storm', and can also occur in non-infectious conditions such as severe trauma, extensive burns, acute necrotizing pancreatitis, and post-cardiac arrest.

A potential therapy is to remove the excess circulating mediators in order to re-establish homeostasis and restore a more physiologic immune response. This can be performed by combining a plasma filter cartridge filled with a hydrophobic polymeric adsorbent with high affinity for inflammatory mediators, and a high-flux haemofilter for convective solute removal. Only filtrated plasma has direct contact with the adsorbent, which avoids biocompatibility problems when compared with direct haemoperfusion.

Extracorporeal blood filtration



The resources to run optimally

DuPont provides the support you need to operate productively and minimize unscheduled outages. From the stability of manufacturing on a global scale to technical expertise in addressing challenging conditions and situations, we are here to help – with system design, field support, plant optimization and more.

Technical support

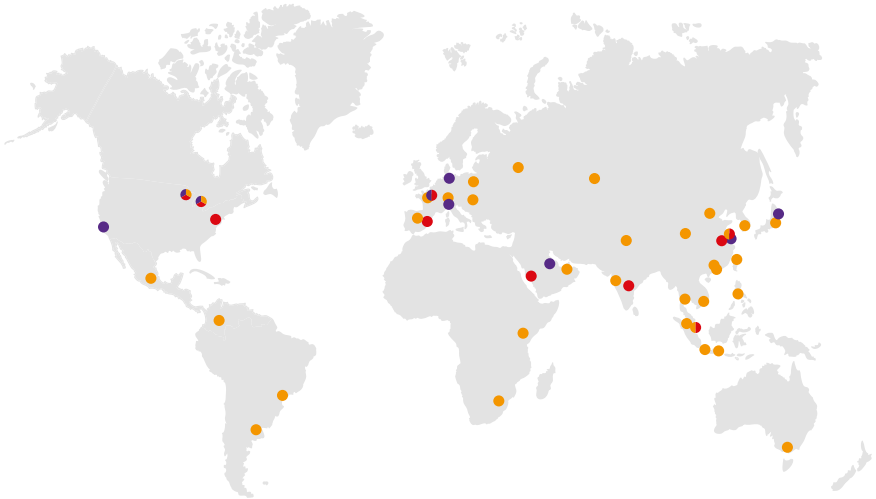
Our technical service and development specialists can work with you at any point in the design and operation of your plant in order to optimize performance; from water quality evaluation and system design consultation with our Water Application Value Engine (WAVE) design software, to monitoring and analyzing operational data, to troubleshooting and problem solving.

Research and development

Dow has been a partner to the water treatment industry for decades, with a history of innovations in ion exchange and membrane technologies driving key improvements in productivity and efficiency. Our global R&D capabilities allow us to address specific local water conditions and requirements, with a holistic focus on water quality and component-based design and research, providing improved performance.

Powering performance worldwide

With a large global manufacturing footprint, strong R&D expertise and technical support services and systems, we supply high market volumes with high quality. Dow partners with you, our customer, to understand unmet needs and develop tailored solutions.



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