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Patents and Literature

Affinity-Based Separations and Purifications

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ABSTRACT

The separation and purification of biologically functional molecules (e.g., proteins, antibodies, peptides, hormones, low molecular weight biologicals) is of fundamental importance to biotechnology. Affinity separations have become a particularly attractive method for bioseparations due to their high degree of selectivity. Numerous affinity ligands have been prepared in recent years including lectins, nucleic acids, inhibitors, and immunoresponse agents. Furthermore, a variety of novel supports have been synthesized to aid in the development of commercially useful affinity separation systems. Recent US patents and scientific literature on affinity separations and purifications are surveyed. Patent abstracts are summarized individually and a list of literature references are given.

Introduction

The objective of the Patents and Literature Section is to summarize and cite recent developments in industrial and academic research that are of general interest to the biotechnology community. These developments are portrayed within the scope of current US patents and world literature. The subject of this Patent and Literature review is Affinity-Based Separations and Purifications.

Patents

This section covers US patents concerning affinity-based separations and purifications from the period of January, 1989 to the present. The major search headings were affinity, immobilized, and bio with the cross-terms: separation, chromatography, purification, immuno, matrix, lectins, nucleic acids, adsorbents, and inhibitors. The major patents recovered under this search are described below. The abstracts have been edited for clarity. Copies of US patents can be obtained from the Commisioner of Patents and Trademarks, Washington, DC 20231.

Baird, A., Bohlen, P., and Gospodarowicz, D.
ISOLATION OF FIBROBLAST GROWTH FACTOR
US 4,785,079, Dec. 10, 1986 and US 4,902,782, Feb. 20, 1990
Assignee: Salk Institute for Biological Studies.

Basic Fibroblast Growth Factor (FGF) is substantially purified by the employment of affinity chromatography using heparin-linked support material. Described is a simplified three step procedure for extracting basic FGF from either mammalian brain or mammalian pituitary tissue. Salt precipitation, e.g., with ammonium sulfate is used to provide a partially purified precipitate that is then subjected to ion-exchange chromatography, e.g., using a Carboxymethyl-Sephadex column. Substantially pure basic FGF fractions are then obtained by fractionating the further partially purified fractions using affinity chromatography on a heparin-linked support e.g., Heparin-Sepharose.

Bargoot, F. G., Ferrari, C., Groopman, J. D., and Wogan, G. N.
AFFINITY COLUMN AND PROCESS FOR DETECTION OF LOW MOLECULAR WEIGHT TOXIC SUBSTANCES
US 4,859,611, Aug. 22, 1989
Assignee: Boston University and Massachusetts Institute of Technology.

An affinity matrix and a method for the detection of low molecular weight compositions such as aflatoxins are provided utilizing specific monoclonal IGM antibody having an affinity constant not less than about 10^9 L/mol. The detection is rapid, accurate, reproducible, and allows for quantitative recovery of the composition of interest.

Bassetti, A., Farneti, A., and Lagana, V.
PROCESS FOR THE BIOLOGICAL PURIFICATION OF WASTE WATERS
US 4,915,841, Apr. 10, 1990
Assignee: Snam Progetti SPA.

A process for the psychrophilic biological purification of waste waters having low concentrations of polluting substances is described. The process comprises an initial first anaerobic treatment step with an expanded mud bed of U.A.S.B. type at a low flow rate, a second treatment step comprising a fluid-bed treatment on a fine support at a high flow rate, and a final treatment step comprising anaerobic treatment for the nitrification and oxidation of the various substances reduced in the previous treatments. The flow is then recycled to the upstream steps for the removal of possible nutrients, such as nitrogen and phosphorus.

Buser, A. J. and Schutyser, J. A.
CARRIER MATERIAL FOR USE IN CHROMATOGRAPHY OR CARRYING OUT ENZYMATIC REACTIONS
US 4,882,226, Nov. 21, 1989
Assignee: Akzo N. V.

A carrier material usable in chromatographic separations or as a starting material is described. The material consists of a core obtained by addition polymerization of monomers of which at least 50 mole % consists of (meth)acrylic acid or a similar ester forming compound, and a hydrophilic coating material which is covalently bound to the core. Upon linkage of ligands or bioactive materials, the materials can be used as ion-exchangers, as a medium for affinity chromatography, or as selective clinical adsorbents.

Cabrera, K. and Wilchek, M.
SUBSTITUTED SILICA
US 4,894,468, Jan. 16, 1990
Assignee: Yeda Research & Development Co Ltd.

The invention relates to modified silica, substituted with a variety of substituents. The product contains primary hydroxyl silica. Furthermore a wide variety of derivatives of such hydroxysilica, which are of value in affinity chromatography and for various other uses, have been developed. The products combine the thermal and mechanical stability of the silica used as a carrier with the useful properties of the substituents.

Carrico, R. J., Hatch, R. P., and Patterson, W. L.
IMMOBILIZATION OF NUCLEIC ACIDS ON SOLVOLYZED NYLON SUPPORTS; POLYNUCLEOTIDE SEQUENCE DETECTION; SENSITIVITY
US 4,806,631, Feb. 21, 1989
Assignee: Miles,Inc.

Immobilization of nucleic acids by contact with a solid support comprising nylon whose amide groups have been partially solvated is described. Solvolysis of the nylon support can be accomplished by treatment with an agent such as trialkyloxonium salt under anhydrous conditions followed by addtion of water. The immobilized nucleic acid is particularly useful as an immobilized probe in hybridization assays to detect specific polynucleotide sequences in a test sample.

Chu, S.-C., Hsieh, Y.-S. and Lin, J. Y.
ISOLATION AND PURIFICATION OF PRE-S2 CONTAINING HEPATITIS B VIRUS SURFACE ANTIGEN BY CHEMICAL AFFINITY CHROMATOGRAPHY
US 4,855,055, Aug. 8, 1989
Assignee: National Science Council of Taiwan.

A Method for isolating and purifying pre-S2 containing HBSAG characterized by the use of polymerized human serum albumin (PHSA)-affinity column chromatography is presented.

Dwek, R. A., Feder, J., Howard, S. C., Parekh, R. B., Rademacher, T. W., and Wittwer, A. J.
TISSUE PLASMINOGEN ACTIVATOR FROM NORMAL HUMAN COLON CELLS
US 4,751,084, Nov. 11, 1986 and US 4,927,630, May 22, 1990
Assignee: Monsanto Co.

Normal human colon fibroblast tissue plasminogen activator is separated by lysine-Sepharose affinity chromatography into Types I and II glycoforms and characterized with respect to the relative incidence of each type of oligosaccharide comprising the respective Types I and II glycoforms.

Erion, J. L., Paul, E. M., Poutre, C. G., and Williamson, V. M.
PURIFICATION OF APASE-11 AND RETRIEVAL OF THE NEMATODE RESISTANCE GENE
US 4,933,286, Jun. 12, 1990
Assignee: Plant Cell Research Institute Inc.

An isoenzyme from tomato, acid phosphatase-1 isoenzyme (Apase11), has been purified to homogeneity and is subjected to amino acid sequencing and used to prepare anti-Apase-1 antibodies. The amino acid sequence permits design of probes to recover Apase-11 encoding cDNA; the antibodies are also useful for this purpose. The cDNA is useful to recover the genomic DNA encoding Apase-11, which can then be used in walking or jumping techniques to recover the genomic DNA which confers nematode resistance, since this DNA resides immediately adjacent to the Apase-11 gene on chromosome 6 of Lycopersicon esculentum.

Eveleigh, J. W. and Kobos R. K.
ENZYME IMMOBILIZATION AND BIOAFFINITY SEPARATIONS WITH PERFLUOROCARBON POLYMER-BASED SUPPORTS
US 4,885,250, Dec. 5, 1989
Assignee: Du Pont de Nemours, E I and Co..

A bioaffinity separation method is provided along with a solid affinity support. Additionally, immobilized enzyme systems are provided for use as enzyme electrode systems. The support is based on an inert perfluorocarbon polymer carrier with ligands or binders attached to its

surface. The ligand, binder or enzyme is preferably modified by attaching a perfluorocarbon anchor group, and the modified ligand, binder or enzyme is attached to the carrier through the anchor group. Methods for preparing such supports and their use in capturing target molecules from samples and in analytical applications are also provided.

Fuwa, T. and Miyoshi, K.
OLIGONUCLEOTIDE DERIVATIVES AND PRODUCTION THEREOF; FOR PURIFYING NUCLEIC ACIDS; AFFINITY CHROMATOGRAPHY
US 4,820,812, Apr. 11, 1989
Assignee: Wakunaga Seiyaku K. K.

An oligonucleotide derivative having an amino group protected with an eliminatable group is bonded through a phosphate group and a spacer with an appropriate length to the 5'-end of an oligonucleotide protected suitably at the 3' hydroxyl group. The production of these derivatives are disclosed.

Graatschus, M., Hunger, H.-D., Kagelmaker, H., and Rosenthal, A.
PROCESS FOR SOLID PHASE-SEQUENCING OF NUCLEIC ACID FRAGMENTS; IMMOBILIZATION, THEN DEGRADATION
US 4,849,077, Jul. 18, 1989
Assignee: Akademie der Wissenschaften der DDR.

A process for solid phase sequencing of nucleic acid fragments is disclosed . The object of the invention is to provide a sequencing process which enables the simultaneous sequencing of large amounts of long and short nucleic acid fragments and which is optimally automated. Thus, a solid support combining mechanical stability, anion exchange characteristics, and chemical elution of nucleic acids off the support is used. Immobilized nucleic acid fragments are chemically modified and subsequently the nucleic acid backbone is cleaved and eluted by chemical means. The present invention is applied to molecular biology and gene technolgy.

Hsia, J.-C.
PURIFICATION OF HEMOGLOBIN AND MODIFIED HEMOGLOBIN BY AFFINITY CHROMATOGRAPHY
US 4,925,574, May 15, 1990
Assignee: Canadian Patents & Development Ltd.

The invention disclosed relates to a method for the purification of hemoglobin by the technique of affinity chromatography. Contrary to current belief, the binding of oxygen and selected polyanions to hemoglobin is not mutually exclusive. This novel method comprises immobilizing a polyanion which specifically binds hemoglobin via its polyanion binding site, on a chromatographic gel and passing the hemoglobin containing solution or mixture through the gel. The

hemoglobin is thus retained in the gel, while impurities are eluted. This novel method is also applicable to the separation of unmodified hemoglobin from a liquid reaction mixture containing modified and unmodified hemoglobin.

Ishii, T., Kaiho, I., Moriguchi, S., Nakayama, Y., Shioda, S., and Suzuki, H.
ACTIVE SUPPORT SUBSTANCE AND ADSORBENT FOR CHROMATOGRAPHY
US 4,913,812, Apr. 3, 1990
Assignee: Showa Denko K K.

The active support substance and the adsorbent for chromatography, which have properties desired for affinity chromatography, can be constituted by a porous gel copolymer principally composed of (A) a glycidyl monovinylester or glycidyl monovinylether and (B) alkyleneglycol divinylester, and combination groups to be bound to ligands through a covalent bond. The (A) component is crosslinked by the (B) component and the combination groups being bound to epoxy groups of the (A) component.

Johnson, A. J. and Mathews, R. W.
METHOD FOR PURIFYING ANTIHEMOPHILIC FACTOR; SUGARS, AMINO ACIDS, POLYHYDRIC ALCOHOLS
US 4,847,362, Jul. 11, 1989
Assignee: New York University.

The present invention relates to a high-recovery, high-resolution method for purifying anti-hemophilic factor by using column chromatography techniques in the presence of sugars, polyhydric alcohols, amino acids, or salts.

Kato, K., Kawahara, K., and Yamada, T.
MUTUAL SEPARATION OF PROTEINS; HYDROXYAPATITE LIQUID AFFINITY CHROMATOGRAPHY
US 4,798,886, JAN. 17, 1989
Assignee: Takeda Chemical Industries Ltd.

A protein and a derivative thereof having a methionine residue added at the amino terminus are mutually separated with the utilization of the difference in affinity for hydroxyapatite between them. Typical separation is achieved on a hydroxyapatite column in liquid chromatography.

Klein, H.-U. and Kratzenstein, K.
PROCESS AND APPARATUS FOR THE BIOLOGICAL PURIFICATION OF WATER
US 4,931,183, Jun. 5, 1990
Assignee: Assigned To Individual

A process and apparatus for the biological purification of water by means of a closed percolating filter is described. The filter is operated with pure oxygen and/or air and an upward-flow filter disposed downstream of the percolating filter containing flotation filter material. Saturation of the gas cushion predominantly containing oxygen in the percolating filter is

controlled by selection of the operating pressure in such a fashion that the oxygen supply corresponds to the biochemical oxygen demand of the water on the one hand, while on the other hand a residual oxygen content dissolved in the water permits continuation with a biological fine purification operation in the upward-flow filter. The apparatus comprises a first chamber with the percolating filter, the water flowing downward and a second chamber with filter material through which the water flows upward. Water accumulating above the filter material is removed through a discharge conduit, with the waste water and oxygen-bearing gas being introduced into the top of the first chamber.

Kwiatek, M. S. and Sansone, M. J.
PREPARATION OF N-SUBSTITUTED PHENYL POLYBENZIMIDAZOLE POLYMERS
US 4,933,397, Jun. 6, 1990
Assignee: Hoechst Celanese Corp.

This invention discloses a unique process for the preparation of N-substituted phenyl polybenzimidazole polymers from unsubstituted polybenzimidazole polymers. An unsubstituted polybenzimidazole polymer is first reacted with an alkali hydride to produce a polybenzimidazole anion which is then reacted with a substituted or an unsubstituted phenyl fluoride to produce an N-substituted phenyl polybenzimidazole polymer. The N-substituted phenyl polybenzimidazole polymer produced by this process can be formed into a wide range of products, such as membranes, films resins or fibers. The product can be designed to meet special applications by the choice of the substitutent. These N-substituted phenyl polybenzimidazole polymers can be utilized for numerous applications including reverse osmosis, ultrafiltration, microfiltration, electrodialysis, ion exchange and affinity chromatography.

Margel, S.
METHOD FOR REMOVING COMPONENTS OF BIOLOGICAL FLUIDS
US 4,861,705, Aug. 29, 1989
Assignee: Yeda Research and Development C. Ltd.

Magnetic and non-magnetic agarose and agar polyaldehyde beads with diameters ranging from 40 microns up to 1 cm are provided for use in covalently binding compounds containing primary amino or thiol groups, such as proteins, antibodies, enzymes, and drugs. The beads are useful for various biological applications such as affinity chromatography, hemoperfusion, ion-exchange resins, cell labeling, diagnostic purposes, and cell separation.

Nathans, G. R. and Rosenstein, R. W.
PURIFICATION OF ANTIBODIES; STORAGE STABILITY
US 4,841,024, Jun. 20, 1989
Assignee: Becton Dickinson and Co.

IGG3 antibody is purified by affinity chromatography and collection of released antibody at a pH of 9.0 to 9.6 is described. The purification is

effected in a column containing both an affinity matrix and a desalting matrix, with the column being equilibrated to a pH from 9.0 to 9.6. IGG3 antibody may be stored in the same pH range.

Ohlson, S.
METHOD AND APPARATUS FOR ISOCRATIC AFFINITY CHROMATOGRAPHY
US 4,879,247, Nov. 7, 1989
Assignee: Perstorp Biolytica SE.

The invention relates to a high performance affinity chromatographic system characterized by an immobilized active substance interacting weakly ($K_{diss} = 10^{-3}$ M) with the desired structure of the type or types of molecule to be separated. The substance im covalently bound in its active state on a carrier of a solid, preferably porous, organic or inorganic material thereby making it suitable for use in an HPLC technique. Examples of useful inorganic materials include silica or glass, while organic materials include plastics or polysaccharides. Separation takes place by the mobile liquid being led through the column packed with the immobilized active substance of weak affinity for the complementary substances.

Rendleman, J. A. Jr.
SEPARATION OF CYCLODEXTRINS BY AFFINITY CHROMATOGRAPHY; HYDROPHOBIC LIGANDS
US 4,867,884, Sep. 19, 1989
Assignee: US Secretary of Agriculture.

Mixtures containing cyclodextrins are fractionated by affinity chromatography on matrices bearing hydrophobic ligands. The size and structure of these ligands can be altered to change the relative affinity of the ligands for different cyclodextrins and thus influence chromatographic fractionation.

Russell, F. E. and Sullivan, J. B.
ANTIBODY PURIFICATION PROCESS
US 4,849,352, Jul. 18, 1989
Assignee: Assigned to Individual

F(AB) fragments are isolated from an antibody containing source by contacting the antibody source with a papain-polyacrylamide matrix to produce F(AB) and F(C) fragments which are then passed through an antigen-polyacrylamide gel capable of attracting the F(AB) fragments. IGG antibodies are obtained by passing an antibody source through an antigen-polyacrylamide gel. These processes can be used to purify a wide variety of antibodies which can be used as therapeutic and diagnostic agents.

Wang, C. Y. and Wang, J. J.
RAPID IMMUNOAGGLUTINATION TEST FOR PRESENCE OF HIV IN BODY FLUIDS
US 4,879,211, Nov. 7, 1989
Assignee: United Biomedical Inc.

The present invention relates to a rapid immunoagglutination method for direct determination of the presence of HIV in body fluids to detect early infection by HIV. The method employs polyclonal anti-HIV IGG purified from the sera of known HIV infected individuals and adsorbed onto carboxylate modified latex beads.

Yamamoto, Y.
THIN-LAYER ROD FOR CHROMATOGRAPHY; PARTIAL CHEMICAL MODIFICATION OF STATIONARY PHASE
US 4,828,704, May 9, 1989
Assignee: Cosmo Oil Co. Ltd.

A thin-layer rod for thin-layer chromatography is described for use with a flame ionization detector. This system is comprised by a rodlike structure and a stationary phase made of an adsorbent which is superposed on the rod-like support with chemical modification made in at least part of the stationary phase.

Zawistowska ,U.
ALPHA-AMYLASE INHIBITOR
US 4,910,297, Mar. 20, 1990
Assignee: ABI Biotechnology Inc.

A novel procedure for the preparation of a protein which is an inhibitor of alpha-amylase II is described. The protein may be prepared by extracting barley meal with a Tris-HCl buffer and purifying the crude inhibitor thus obtained by a chromatographic procedure. Alternatively, the protein may be prepared by recombinant DNA techniques. The protein can be applied as an additive to sprout-damaged wheat flour which can then be used to provide improved quality bread.

Literature

This section surveys the literature in the area of affinity-based separations and purifications for the years 1988 and 1989. The major headings and cross-terms are the same as listed in the patent search. This section is not intended to be all encompassing and lists both review articles and research publications that highlight the varied nature of research in this field during the specified time period.

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