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PATENTS AND LITERATURE

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The objective of this section is to keep readers aware of significant inventions and trends in industrial research, as well as to highlight those areas of research that may lead to new biotechnological opportunities. In addition to immobilized biocatalysts, nucleic acid technology, applied immunology, and covered in earlier issues, two other subject areas will be surveyed in 1985: affinity separation and bioassays. The subject of this, the fourth Patent and Literature Section of 1985 is affinity separation.

AFFINITY SEPARATION

PATENTS

This section identifies and gives a brief description of patents from the US patent literature from January 1984 trough June 1985. The term affinity with separation, chromatography, purification, immuno and matrix as cross-terms; the term immobilized with lectins, nucleic acids, adsorbants, and inhibitors as cross-terms; the term support with the cross-term activated; and lastly, the term group-specific were all searched. Both patent titles and abstracts were searched. Copies of US patents can be obtained for $1.50 each from the Commissioner of Patents and Trademarks, Washington, DC 20231.

Atkinson, A., Lowe, C., Mosbach, K., Small, D. A. P.
HIGH PRESSURE LIQUID AFFINITY CHROMATOGRAPHY
US 4,431,544, February 14, 1984
Assignee: The Public Health Laboratory Service Board

A process for the pressure liquid affinity chromatographic separation of at least one biological or related substance from a mixture. The contact,

washing, and eluting phases are performed on a binding material made from a ligand, containing at least one of the groups anthraquinone, phthalocyanine, or aromatic azo, coupled to a matrix through a spacer arm. The binding material is constructed so that at least one biological substance is retained on the binding material during the contact and washing phases. The ligand can be a reactive triazinyl dye with an agarose or silica matrix and with a substituted aminohexyl group spacer arm. The chromatographic procedure is performed at 100–3500 psi, at a flow rate of 0.5–2.0 mL/min. The choice of washing and eluting solutions depends on the material to be separated. However, buffer solution to wash the column and a desorbing agent to elute the material are preferred. When the biological substance is an enzyme the desorbing agent is preferably an enzyme substrate, cofactor, or inhibitor. A binding material for use in the above HPLAC procedure is also described. This is made from a ligand containing anthraquinone, phthalocyanine, or aromatic azo groups coupled to a matrix through a spacer arm.

Bishop, D. C.
LEUKOCYTE MIGRATION THROUGH ANTIGEN CONTAINING AGAROSES FOR IMMUNOCOMPETENCE TESTING
US 4,436,824, March 13, 1984
Assignee: Ortho Diagnostic Systems, Inc.

The method assesses the level of general and specific cellular immunocompetence by measuring the responses of individuals to antigens in vitro employing the phenomenon of Leukocyte Migration Inhibition (LMI). The present invention differs from the previously described LMI technique in that antigens are individually incorporated into the agarose of assay plates, requiring no preincubation of antigens with patient blood cell (leukocyte) suspensions. The LMI assay method is a practical alternative to delayed hypersensitivity skin testing to identify cellular immune deficiency and avoids the risk and inconvenience of the skin test procedure. The method also allows in vitro diagnosis of tuberculosis and monitoring of tumor therapy.

Braude, I. A.
HUMAN IMMUNE INTERFERON
US 4,440,675, April 3, 1984
Assignee: Meloy Laboratories, Inc.

A process for the purification of crude immune interferon to a near homogeneous preparation by: (a) adsorbing the crude interferon onto a column containing Controlled Pore Glass beads and eluting with ammonium sulfate, (b) adsorbing the interferon containing eluant onto a column containing either concanavalin A–Sepharose, lentil lectin–Sepharose or pea lectin–agarose and eluting with a buffer containing a sugar, (c) adsorbing the interferon containing eluant onto a column con-

taining heparin–Sepharose or Procian Red–agarose and eluting with a high salt content buffer, (d) adsorbing the interferon containing eluant onto a cationic exchange resin column and eluting with a salt buffer and (e) treating the interferon containing eluant in a gel-filtration column equilibrated in high salt to obtain a solution of immune interferon that is nearly homogeneous.

Cais, M., Shimoni, M.
METHOD FOR A NEW TYPE OF CHROMATOGRAPHY AND DEVICE THEREFOR
US 4,510,058, April 9, 1985
Assignee: Technion Research & Development Foundation, Ltd.

A new type of chromatography technique, referred to as dynamic column chromatography, for separation of one or more compounds present in a solution, which is characterized by the existence of a moving solid absorbent bed. The chromatographic system comprises a piston having at its bottom a sealing element and a longitudinal channel containing the adsorbent between two barriers and a test tube having at its bottom a multiple-way valve. By pushing the piston into the test tube, the desired eluent, which had previously been forced through the valve, is entering under intrinsic pressure of the closed system through the channel moving the adsorbed compounds to be separated between these barriers, the solution obtained going out through a nozzle located at one of the end parts of the piston. Dynamic column liquid chromatography is applicable to: silica gel chromatography, reversed-phase liquid chromatography, affinity chromatography, capillary chromatography, chromatofocusing, gel filtration, and ion exchange chromatography. In the dynamic column liquid chromatography, the equilibrium distribution of the compounds between the adsorbent and liquid is established very rapidly, resulting in sharp and narrow zones of the separated fractions.

Colman, G., Russel, R. R. B.
PROTECTION AGAINST DENTAL CARIES
US 4,442,085, April 10, 1984
and
US 4,448,768, May 15, 1984
Assignee: The Secretary of State for Social Services in Her Britannic Majesty's Government of the United Kingdom of Great Britain and Northern Ireland

An antigenic protein, termed antigen A, present on the cell walls and in cultures of *Streptococcus mutans* is separated from other antigenic proteins, notably those that cross-react with heart tissue, to give an antigenic preparation that may be used as a vaccine or to raise antibodies for use in protecting against dental caries. Antigen A is one of two major antigenic proteins remaining on cell walls of *S. mutans* genetic group I after extrac-

tion with a boiling 10 g/1 L aqueous solution of sodium dodecyl sulfate for 20 min. It has a molecular weight of about 29,000, an isoelectric point of 4.1–4.5. Its amino acid analysis is also given. The antigen also occurs in the culture filtrate and/or cell extract and may be readily purified from these sources by, for example fractional ammonium sulfate precipitation and/or affinity chromatography on immobilized antibody.

Cone, R. O., Jr., Carpenter, C. R.
PRE-PRECIPITATED DOUBLE ANTIBODY IMMUNOASSAY METHOD
US 4,481,298, November 6, 1984
Assignee: AMF Incorporated

An immunoassay process for the detection of an antigen in a sample, by (a) forming a mixture of the sample with (1) a preformed complex of a primary antibody and a secondary binding macromolecule, where the primary antibody is present at low concentrations and has substantial specificity for the antigen, the secondary binding macromolecule has substantial affinity for the Fc portion of the primary antibody, and the second binding macromolecule is affinity purified; and with (2) a detectably labeled form of the antigen; (b) incubating the mixture formed in step (a) for a time sufficient to allow the antigen and the detectably labeled antigen to competitively bind to the primary antibody of the preformed complex; (c) detecting the separated complex of the separated suspension medium.

Crane, L. J., Ramsden, H. E.
AFFINITY CHROMATOGRAPHY MATRIX WITH BUILT-IN REACTION INDICATOR
US 4,523,997, June 18, 1985
Assignee: J.T. Baker Chemical Company

p-Nitrophenylester of succinoylaminopropyl silica gel is useful as an affinity matrix for reacting with ligand for use in affinity chromatography. The affinity matrix, upon reaction with a ligand, releases one molecule of *p*-nitrophenolate ion for ever molecule of ligand that reacts turning the solution yellow as the reaction occurs.

Donahoe, P. K., Budzik, G. P., Swann, D.A.
PURIFIED MULLERIAN INHIBITING SUBSTANCE AND METHOD OF USE
US 4,510,131, April 9, 1985
Assignee: The General Hospital Corporation

A process for purifying Mullerian Inhibiting Substance (MIS) from testicular tissue by incubating testicular tissue in a culture media in the presence of a protease inhibitor in order to extract MIS from the tissue, chromatographing the extract on an anionic exchange resin followed by

chromatographing the biologically active product obtained on a cationic exchange resin; the biologically active material from the cationic exchange resin is chromatographed by lectin affinity chromatography on wheat germ lectin and then either chromatographed by lectin affinity chromatography on concanavalin A or by triazinyl dye affinity chromatography on an appropriate triazinyl dye; MIS is obtained in 8000-fold and 15,000-fold purification over original testicular tissue.

Dvorak, H. F., Senger, D.R.
VASCULAR PERMEABILITY FACTOR
US 4,456,550, June 26, 1984
Assignee: President and Fellows of Harvard College

A purified vascular permeability protein factor and method of manufacture is described where the factor has the following characteristics: (a) in an aqueous solution (0.01M, sodium phosphate, pH 7) whose concentration of NaCl is varied linearly, the factor is eluted from a heparin–Sepharose chromatography column in a peak centered at 0.4*M* sodium chloride; (b) in an aqueous solution of sodium phosphate (pH 7.0) whose concentration is varied linearly, the factor is eluted from a hydroxylapatite column in a peak centered at 0.25*M* sodium phosphate; (c) when subjected to SDS gel electrophoresis the factor is localized to a region corresponding to a molecular weight between 34,000 and 45,000 daltons.

Estis, L. F.
METHOD FOR PURIFYING GAMMA-INTERFERON
US 4,499,014, February 12, 1985
Assignee: Interferon Sciences, Inc.

A method is described for restoring some or all of the activity of gamma-interferon that has been in contact with an acidic solution comprising the steps of: (a) placing the gamma-interferon in a solution that has a pH between about 5.5 and 9.5; and (b) incubating the solution at a temperature of between about 2 and 8°C for a period of at least 24 h. The method allows antibody affinity chromatography employing acid elution to be used to purify gamma-interferon, in that, the activity of the acid-eluted gamma-interferon can be essentially completely restored using the reactivation process.

Freytag, J. W.
IMMUNOASSAY WHEREIN LABELED ANTIBODY IS DISPLACED FROM IMMOBILIZED ANALYTE-ANALOG
US 4,434,236, February 28, 1984
Assignee: E.I. Du Pont de Nemours & Co.

A method for the rapid determination of analyte in a sample is provided. The sample is contacted with a solid phase containing an immobilized analyte-analog to which there is displaceably bound a labeled anti-

analyte antibody. Because the antibody has greater affinity for the analyte than the analyte-analog, the labeled antibody is displaced from the solid phase. The complex is separated from the solid phase, and the amount of complex is measured. The measured amount is related to the amount of analyte initially present in the sample.

Gershoni, J. M.
TRANSFER OF MACROMOLECULES FROM A CHROMATOGRAPHIC SUBSTRATE TO AN IMMOBILIZING MATRIX
US 4,512,896, April 23, 1985
Assignee: Yale University

The method of transfer of macromolecules such as nucleic acid and proteins from a chromatographic substrate to an immoblizing matrix uses as the immobilizing matrix, a charge-modified microporous membrane comprising an organic microporous membrane having a charge modifying amount of cationic charge-modifying agent bonded to substantially all of the wetted surfaces of the membrane. The charge-modified microporous membrane can also be a reinforced microporous membrane, preferably a porous reinforcing web impregnated with a polymeric microporous membrane. A nucleic acid- or protein-blotting product comprising a chromatographic matrix having the charge-modified microporous membrane on a surface is also provided.

Gordon, S. G.
CHROMATOGRAPHICALLY PURIFYING PROTEOLYTIC PROCOAGULANT ENZYME FROM ANIMAL TISSUE EXTRACT
US 4,461,833, July 24, 1984
Assignee: University Patents, Inc.

A chromatographic procedure for the purification of a proteolytic procoagulant enzyme from extracts of human and animal tumors. The extracts are sequentially contacted with a first benzamide affinity chromatographic resin, an agarose filtration gel, a second benzamide affinity chromatographic resin and a phenyl-Sepharose hydrophobic chromatographic resin. The resulting enzyme is capable of producing antiprocoagulant antibody that can be used in an immunoassay diagnostic for malignancy.

Hughes, P., Lowe, C. R., Sherwood, R. F.
AFFINITY CHROMATOGRAPHY USING METAL IONS
US 4,431,546, February 14, 1984
Assignee: The Public Health Laboratory Services Board

A process for the affinity chromatographic separation of at least one biological or related substance from a mixture where at least one biological

or related substance is bound to a binding material, having a ligand containing at least one of the groups anthraquinone, phthalocyanine, or aromatic azo, in the presence of a metal ion, preferably Co(2), Ni(2), or Zn(2). The ligand may be linked directly to the matrix or via a spacer arm. The process may be performed at atmospheric pressure or under high pressure (100–3500 psi). The nature of the contact, washing, and eluting solutions depends on the substance to be separated. Generally the contact solution is made up of the substance to be separated and a metal salt dissolved in a buffer solution while the washing solution consists of the same metal salt dissolved in the same buffer. The eluting solution may be a buffer solution, either alone or containing a chelating agent, or it may be an alkali metal salt or a specific desorbing agent. Alternatively, the eluting solution may be a mixture of two or more of these solutions or two or more of these solutions used consecutively.

Jordan, R. E.
ANTITHROMBIN–HEPARIN COMPLEX AND METHOD FOR ITS PRODUCTION
US 4,446,126, May 1, 1984
Assignee: Cutter Laboratories, Inc.

A complex of antithrombin and high activity heparin is provided for use as a potent anticoagulant for humans. The complex is prepared by reversibly immobilizing it on a lectin-containing, water-insoluble gel matrix and then removing it from the matrix.

Jordan, R. E.
FRACTIONATION OF HEPARIN
US 4,446,314, May 1, 1984
Assignee: Cutter Laboratories, Inc.

A lectin-containing, water-insoluble gel matrix with a reversibly bound glycoprotein can be used for fractionating a polysaccharidic substance capable of being separated into components of differing activity by virtue of differing affinity for the glycoprotein. In this manner, heparin can be fractionated into high activity and low activity components.

Jordan, R. E.
WATER-INSOLUBLE GEL MATRIX CONTAINING LECTIN BOUND TO ANTITHROMBIN
US 4,450,104, May 22, 1984
Assignee: Cutter Laboratories, Inc.

A lectin-containing, water-insoluble gel matrix with a reversibly bound glycoprotein can be used for fractionating a polysaccharidic substance capable of being separated into components of differing activity by virtue of

differing affinity for the glycoprotein. In this manner, heparin can be fractionated into high activity and low activity components.

Lantero, O. J., Jr.
IMMOBILIZATION OF BIOCATALYSTS ON GRANULAR CARBON
US 4,438,196, March 20, 1984
Assignee: Miles Laboratories, Inc.

A process where enzymes are immobilized on activated granular carbon is described. The process involves treating the carbon with a polyamine compound having pendant amino groups to cause the polyamine to adhere to the carbon leaving pendant amine groups free to further react. The free amine groups are derivatized by treatment with a difunctional compound having amine reactive moieties, so that free amine groups of the enzyme can be covalently bound to the polyamine via the amine reactive compound. By this method, enzymes can be immobilized onto granular carbon that provides a support having excellent physical properties.

Miyashiro, Y., Ogawa, M., Yamazaki, Y., Igarasi, S.
POLYSACCHARIDE BEADS
US 4,493,894, January 15, 1985
Assignee: Takeda Chemical Industries, Ltd.

A matrix comprising a water-insoluble β-1,3-glucan gel in the shape of beads with diameters within the range of about 5–1000 μm is prepared by, for example, dispersing an alkaline aqueous solution of a water-soluble β-1,3-glucan in a water-immiscible organic solvent, and adding an organic acid to the resultant dispersion. The matrix is useful as carrier materials for immobilized enzymes, affinity chromatography, gel filtration, ion exchange, and other applications.

Olexa, S. A., Budzynski, A. Z.
INHIBITION OF FIBRIN POLYMERIZATION BY A PEPTIDE ISOLATED FROM FIBRIN FRAGMENT D(1)
US 4,455,290, June 19, 1984
Assignee: Research Corporation

A purified peptide (Thr-Arg-Trp-Tyr-Ser-Met-Lys-Lys-Thr-Thr-Met-Lys-Ile-Pro-Phe-Asn-Arg-Leu-Thr-Ile-Gly-Glu-Gly-Gln-Gln-His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val) is isolated by degrading Fragment D(1) of fibrinogen with plasmin followed by separation of the resulting peptides on the basis of molecular weight and affinity for bound fibrin monomer. The purified peptide is useful as an anticoagulant and, when suitably labeled with a gamma-emitting radioisotope, as a thrombus imaging agent.

Pancham, N., Zuffi, T.
PROCESS FOR PRODUCING A CONCENTRATE OF COAGULATION FACTORS VII AND VIIa
US 4,470,969, September 11, 1984
Assignee: Miles Laboratories, Inc.

A process is described for producing coagulation factors VII and VIIa in relatively high purity by contacting an aqueous preparation containing factors VII and VIIa with a protein precipitant and separating the coagulation factors from other proteins, contacting the resulting solution with a divalent metal salt adsorbent having selective affinity for calcium-binding coagulation factors and eluting from it the coagulaton factors and contacting the eluate with an anionic exchange resin and selectively eluting coagulation factors VII and VIIa from it by step- or gradient-elution techniques to obtain factors VII and VIIa free of other coagulation factors and other proteins.

Reynolds, R. A.
AFFINITY IMMUNOASSAY SYSTEM
US 4,504,585, March 12, 1985
Assignee: Aalto Scientific, Ltd.

An affinity immunoassay system in which a solid-phase nonimmunological, group-specific ligand is used to insolubilize the analyte of interest either simultaneously, before or after binding all of the analyte with a labeled monospecific antibody. The concentration of the analyte is then determined by measuring the label activity present in the solid phase in relation to a single-point calibrator solution containing a known amount of the analyte substance.

Russell, R. R. B.
PROTECTION AGAINST DENTAL CARIES
US 4,521,513, June 4, 1985
Assignee: The Secretary of State of Social Services in Her Britannic Majesty's Government of the United Kingdom of Great Britain and Northern Ireland

An antigenic protein, termed antigen C, present on the cell walls and in cultures of *Streptococeus mutans*, is separated from other antigenic proteins, notably those that react heart tissue, to give an antigen preparation that may be used as a vaccine or to raise antibodies for use in protecting against dental caries. Antigen C is destroyed or extracted from the cell walls by treatment with boiling aqueous sodium dodecyl sulfate (10 gm per liter) for 10 minutes. It has a molecular weight of 70,000 and an isoelectric point of 4.45. It is destroyed by proteolytic enzyme and does not cross react with heart tissue. The antigen also occurs in the culture filtrate

and/or cell extract and may readily be separated from these sources by affinity chromatography on immobilized antibody.

Sato, H., Sasaki, K., Takagi, K., Hiratani, H., Yuki, Y.
PROCESS OF TREATING INFLAMMATION WITH HUMAN URINARY THIOL PROTEASE INHIBITOR
US 4,479,937, October 30, 1984
Assignee: Zeria Shinyaka Kogyo Kabushiki Kaisha

An anti-inflammatory agent with an active component that it an inhibitor of a thiol protease is obtained from human urine by extraction and purification. This agent has an action of inhibiting a disease caused by a thiol protease. This anti-inflammatory agent is prepared by a process in which a thiol protease inhibitor is extracted and purified from human urine by adopting in combination at least two treatments selected from a treatment with a molecular filter, a treatment with an ion exchanger, a treatment with an adsorber, and an affinity chromatographic treatment.

Shamizu, F., Ohmotot, Y., Imagawa, K.
HUMAN INTERFERON-RELATED PEPTIDES, ANTIGENS, ANTIBODIES, AND PROCESS FOR PREPARING THE SAME
US 4,474,754, October 2, 1984
Assignee: Otsuka Pharmaceutical Co., Ltd.

Human interferons-related peptides and the preparation of their derivatives, antigens, antibodies, and immobilized antibodies is described. These are used for affinity chromatography, and for a novel method for assaying human interferons by using affinity chromatography.

Smith, L. H., Teplitz, R. L.
METHOD OF TESTING FOR PARTICULAR ANTIBODIES IN THE SERUM OF A PATIENT
US 4,493,899, January 15, 1985
Assignee: City of Hope

A method for testing for particular antibodies in the serum of a patient is described. The antibodies may be those of systemic lupus erythematosus and may constitute IgG and IgM immunoglobulins. The IgG and IgM immunoglobulin may be individually labeled radioactively. An antigen (such as DNA) may be attached to a support such as Sepharose. The attachment may be facilitated, as by irradiation with ultraviolet light. The DNA may be single-stranded or double-stranded. When double-stranded DNA is used, single-stranded portions in the double strands may be removed as by a suitable enzyme. The particular antibodies may be attached to the antigen, such as the supported DNA. An assay may then be provided to determine the attachment of the particular antibodies to the supported DNA. When the particular antibodies constitute immunoglobins of systemic lupus erythematosus, the assay may actually

provide a determination of the amounts of the IgG and IgM immunoglobulins individually attached to the single-stranded and double-stranded DNA.

Tan, Y. H., Smith J. H.
ISOLATION OF HUMAN INTERFERON BY IMMUNOSORBENT AND HIGH PERFORMANCE LIQUID CHROMATOGRAPHY
US 4,485,017, November 27, 1984
Assignee: Cetus Corporation

An improved process for the isolation and purification of HIFNs is disclosed in which a partially purified preparation of the HIFN is sequentially passed through an antibody affinity column and a reversed-phase high-performance liquid chromatographic column. Organic solvents used during the elution are extracted and the protein concentrated for subsequent use.

Zuffi, T. R., Pancham, N.
PROCESS FOR PRODUCING A LIPOPROTEIN-POOR CONCENTRATE OF COAGULATION FACTORS VII AND VIIa
US 4,473,553, September 25, 1984
Assignee: Miles Laboratories, Inc.

A process is described for producing coagulation factors VII and VIIa in relatively high purity by contacting an aqueous preparation containing factors VII and VIIa with a lipoprotein binding adsorbent and separating out the coagulation factors and other proteins, contacting the resulting lipoprotein-poor solution with a divalent metal salt adsorbent having selective affinity for calcium-binding coagulation factors, and eluting the coagulation factors from it and contacting the eluate with an anionic exchange resin and selectively eluting coagulation factors VII and VIIa therefrom by step- or gradient-elution techniques to obtain factors VII and VIIa free of other coagulation factors and other proteins.

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