UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BOEHRINGER INGELHEIM PHARMACEUTICALS, INC.
Petitioner,

v.

GENENTECH, INC.
Patent Owner

U.S. Patent No. 6,870,034

Title: PROTEIN PURIFICATION

Inter Partes Review No. IPR2017-02029

PETITION FOR INTER PARTES REVIEW OF
U.S. PATENT NO. 6,870,034 B2

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1002	Declaration of Daniel G. Bracewell, Ph.D. ("Bracewell Dec.")
1003	Curriculum Vitae of Daniel G. Bracewell, Ph.D.
1004	van Sommeren et al., *Effects of Temperature, Flow Rate and Composition of Binding Buffer on Adsorption of Mouse Monoclonal lgG1 Antibodies to Protein A Sepharose 4 Fast Flow*, in PREPARATIVE BIOCHEMISTRY, 22(2), 135–49, 1992 ("van Sommeren")
1005	Godfrey et al., *A Sensitive Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Staphylococcal Protein A (SpA) Present as a Trace Contaminant of Murine Immunoglobulins Purified on Immobilized Protein A*, JOURNAL OF IMMUNOLOGICAL METHODS, 149, 21–27, 1992 ("Godfrey")
1006	U.S. Patent No. 4,801,687 ("the '687 patent")
1007	U.S. Patent No. 5,098,829 ("the '829 patent")
1008	U.S. Patent No. 6,127,526 ("the '526 patent")
1009	Fang et al., *Real-Time Isoform Analysis by Two-Dimensional Chromatography of a Monoclonal Antibody During Bioreactor Fermentations*, JOURNAL OF CHROMATOGRAPHY A, 816: 39–47 ("Fang")
1010	Seetharam & Sharma, PURIFICATION AND ANALYSIS OF RECOMBINANT PROTEINS, Marcel Dekker, Inc., 1991 ("Seetharam")
1011	Elgert, *Antibody Structure and Function* in IMMUNOLOGY: UNDERSTANDING THE IMMUNE SYSTEM, 1996 ("Elgert")
1012	Levin, PHARMACEUTICAL PROCESS SCALE-UP, Marcel Dekker, Inc., 2002 ("Levin")
1013	U.S. Patent No. 6,265,542 ("the '542 patent")
1014	Lehninger et al., PRINCIPLES OF BIOCHEMISTRY, Second Edition: http://www.bioinfo.org.cn/book/biochemistry/start.htm
1015	Gagnon, *Protein A Affinity Chromatography*, in PURIFICATION TOOLS FOR MONOCLONAL ANTIBODIES, in Validated Biosystems, Inc., 1996 ("Gagnon")
1016	Chadha & Sulkowski, *Chromatography of Human Leukocyte Interferon on Controlled Pore Glass*, PREPARATIVE BIOCHEMISTRY, 11:4, 467–82, 1981 ("Chadha")
1017	Flynn, *Buffers – pH Control within Pharmaceutical Systems*, PDA

	J. PHARM. SCI AND TECH., 34: 139–62, 1980 ("Flynn")
1018	Reifsnyder et al., *Purification of insulin-like growth factor-I and related proteins using underivatized silica*, JOURNAL OF CHROMATOGRAPHY A, 753:73–80, 1996 ("Refisnyder")
1019	Prosecution History, U.S. Patent Application No. 10/356,974
1020	Robyt, *Chromatographic Techniques* in BIOCHEMICAL TECHNIQUES THEORY AND PRACTICE, 1987 ("Robyt")

Petitioner Boehringer Ingelheim Pharmaceuticals, Inc. ("BI" or "Petitioner") seeks *inter partes* review and cancellation of claims 13 and 16 of U.S. Patent 6,870,034 ("the '034 patent").

I. INTRODUCTION

The '034 patent concerns one discrete step in the process of preparing proteins, specifically antibodies, for therapeutic use. Antibody preparation may be divided into two general stages, the "upstream" stage and the "downstream" stage. During the upstream stage, the antibodies are produced as one of many products of living cells, all present in the "cell culture fluid." During the downstream stage, the antibodies, *i.e.*, "the target protein," are separated from the other products made by the cells and present in the cell culture fluid, purified, and otherwise prepared for therapeutic use in humans. One well-known step in the downstream stage, protein A chromatography, can be designed to separate the target protein from the other components of the cell culture fluid with a purity as high as 95%. Protein A chromatography is described in the prior art as "simple," requiring just three steps (1) load, (2) wash, and (3) elute.

In the "load" step of protein A chromatography, a sample of cell culture fluid is dissolved in a "binding buffer" and poured through the protein A column. The target protein then specifically and strongly binds to the protein A in the column, while other proteins, *i.e.*, the "contaminant" proteins, either flow through

the column, or non-specifically and weakly bind to the glass or silica column support material. In the "wash" step, compositions are poured through the column to break the non-specific bonds between the contaminant proteins and the column support material. The contaminant proteins then flow through the column. In the "elute" step, the chemical bonds between the protein A and the target protein are broken by the application of another buffer, and the target protein then elutes from the column, purified from the contaminant proteins.

The '034 patent describes this general protein A chromatography method, and admits that this well-known method is part of the prior art. As its alleged inventive concept, the '034 patent concentrates on the "wash" step of the method, and claims various wash solutions that are to be applied to the protein A chromatography column to wash the other components of the cell culture fluid out of the column while the target protein is bound to the protein A. However, the claimed wash solutions are all fully described in the prior art, and had been used for years to effect successful protein separations prior to the earliest filing date of the '034 patent. The challenged claims of the '034 patent are therefore anticipated by and obvious over the prior art, and should never have issued.

This petition is supported by the expert declaration of Dr. Daniel G. Bracewell, Ph.D. (Ex. 1002.) Dr. Bracewell is a Professor in the Bioprocess

Analysis Department of Biochemical Engineering at University College London in the United Kingdom.

II. MANDATORY NOTICES

A. Real Parties-in-Interest (37 C.F.R. § 42.8(b)(1))

The real partes in interest are Boehringer Ingelheim, GmbH, Boehringer Ingelheim Corporate Center GmbH, Boehringer Ingelheim Pharma GmbH & Co. KG, Boehringer Ingelheim International GmbH, Boehringer Ingelheim USA Corporation, and Boehringer Ingelheim Pharmaceuticals, Inc.

B. Related Matters (37 C.F.R. § 42.8(b)(2))

Petitioner is not aware of any matters involving the ’034 patent.

C. Lead And Back-Up Counsel (37 C.F.R. § 42.8(b)(3))

Lead counsel is Ira J. Levy, Reg. No. 35,587. Backup counsel are Elaine Herrmann Blais (to seek *pro hac vice* admission), Brian A. Fairchild, Reg. No. 48,645, and Sarah Fink, Reg. No. 64,886. All counsel are with Goodwin Procter, LLP. Mr. Levy and Ms. Fink are at 620 Eighth Avenue, New York, NY 10018, tel. 212-813-8800, fax 212-355-3333. Ms. Blais and Dr. Fairchild are at 100 Northern Avenue Boston, MA 02210, tel: (617) 570-1000, fax: (617) 523-1231. Email contact for counsel is ilevy@goodwinlaw.com, eblais@goodwinlaw.com, bfairchild@goodwinlaw.com, and sfink@goodwinlaw.com.

D. Service Information (37 C.F.R. § 42.8(b)(4))

Please direct all correspondence to counsel at the contact information above. Petitioner consents to service by electronic mail at ilevy@goodwinlaw.com, eblais@goodwinlaw.com, bfairchild@goodwinlaw.com, sfink@goodwinlaw.com and DG-BI034@goodwinlaw.com.

II. CERTIFICATION OF GROUNDS FOR STANDING

Petitioner certifies pursuant to 37 C.F.R. § 42.104(a) that the patent for which review is sought is available for *inter partes* review and that Petitioner is not barred or estopped from requesting an *inter partes* review challenging the patent claims on the grounds identified in this Petition.

III. FEES

The Commissioner is hereby authorized to charge all fees due in connection with this matter to Attorney Deposit Account 506989.

IV. SUMMARY OF THE ’034 PATENT

The ’034 patent issued on March 22, 2005, from Application No. 10/356,974 (“the ’974 application”), which claims a provisional application filed on February 5, 2002 as its priority application. The prosecution history of the ’974 application is included as Exhibit 1019. For purposes of this petition only, Petitioner assumes that the ’034 patent is entitled to this claim of priority.

A. The Challenged Claims

Petitioner challenges claims 13 and 16. These claims are reproduced below.

13. A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:

(a) adsorbing the protein to Protein A immobilized on a solid phase;

(b) removing contaminants by washing the solid phase with a composition comprising a buffer at a concentration of greater than about 0.8M; and

(c) recovering the protein from the solid phase.

16. A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:

(a) adsorbing the protein to Protein A immobilized on a solid phase;

(b) removing contaminants by washing the solid phase with a composition comprising salt and a solvent selected from the group consisting of ethanol, methanol, isopropanol, acetonitrile, hexylene glycol, propylene glycol, and 2,2-thiodiglycol; and

(c) recovering the protein from the solid phase.

B. The Specification

The abstract of the ’034 patent states that the patent is directed to a “method for purifying proteins by Protein A chromatography […] which comprises

removing contaminants by washing the solid phase with various intermediate wash buffers." ('034 patent, Ex. 1001 at abstract.)

The '034 patent discusses the use of protein A chromatography as one of the purification steps used to purify proteins for therapeutic use. (*Id*. at 1:64–2:4.) For protein A chromatography, "preferably, the solid phase is a controlled pore glass column or a silicic acid column." (*Id*. at 16:52–53.) The patent describes the protein A chromatography process as follows. First, the preparation containing the protein of interest is loaded onto the solid phase. (*Id*. at 16:66–67.) "As the contaminated preparation flows through the solid phase, the protein is adsorbed to the immobilized Protein A and other contaminants (such as Chinese Hamster Ovary Proteins, CHOP, where the protein is produced in a CHO cell) may bind nonspecifically to the solid phase." (*Id*. at 17:2–6.) Then, the contaminants must be removed "by washing the solid phase in an intermediate wash step." (*Id*. at 17:7–10.) Last, "following the intermediate wash step… the protein of interest is recovered from the column." (*Id*. at 17:57–59.)

Regarding the composition used for the intermediate wash step, called the "intermediate wash buffer" in the '034 patent (*id*. at 4:29–37), the patent discloses that the composition "may comprise salt and a further compound" where the further compound is either detergent, solvent or polymer. (*Id*. at 17:13–17.) The specification states that the preferred solvent is an organic, non-polar solvent and

lists the following: ethanol, methanol, isopropanol, acetonitrile, hexylene glycol, propylene glycol, and 2,2-thiodiglycol. (*Id*. at 4:42–47.) The intermediate wash step may also involve "the use of a highly concentrated buffer solution, e.g., a buffer at a concentration of greater than about 0.8M, *e.g.*, up to about 2M, and preferably in the range from about 0.8M to about 1.5M, most preferably about 1M. In this embodiment, the buffer is preferably a Tris buffer, such as Tris acetate." (*Id*. at 45–51.)

III. OVERVIEW OF CHALLENGE AND PRECISE RELIEF REQUESTED

Petitioner challenges claims 13 and 16 of the '034 Patent based on seven Grounds.

In **Ground 1**, Petitioner challenges claim 13 as anticipated by Van Sommeren, *Effects of Temperature, Flow Rate and Composition of Binding Buffer on Adsorption of Mouse Monoclonal IgG1 Antibodies to Protein A Sepharose 4 Fast Flow*, PREPARATIVE BIOCHEMISTRY, 22:2, 135–49 (1992) ("van Sommeren," Ex. 1004). In **Ground 2**, Petitioner challenges claim 13 as anticipated by Godfrey, *A Sensitive Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Staphylococcal Protein A (SpA) Present as a Trace Contaminant of Murine Immunoglobulins Purified on Immobilized Protein A*, JOURNAL OF IMMUNOLOGICAL METHODS, 149, 21–27 (1992) ("Godfrey," Ex. 1005). In **Ground 3**, Petitioner challenges claim 13 as anticipated by United States Patent

No. 4,801,687 (the "'687 patent," Ex. 1006.) In **Ground 4**, Petitioner challenges claim 13 as anticipated by United States Patent No. 5,098,829 (the "'829 patent," Ex. 1007). In **Ground 5**, Petitioner challenges claim 13 as anticipated by U.S. Patent No. 6,127,526 (the "'526 patent," Ex. 1008).

In **Ground 6**, Petitioner challenges claim 16 as anticipated by Fang, *Real-Time Isoform Analysis by Two-Dimensional Chromatography of a Monoclonal Antibody During Bioreactor Fermentations*, JOURNAL OF CHROMATOGRAPHY A, 816, 39–47 (2998) ("Fang," Ex. 1009). In **Ground 7** Petitioner challenges claim 16 as obvious over the '526 patent and Refisnyder, *Purification of Insulin-Like Growth Factor-I and Related Proteins Using Underivatized Silica*, JOURNAL OF CHROMATOGRAPHY A, 753, 73–80 (1996) ("Reifsnyder," Ex. 1018).

IV. LEVEL OF ORDINARY SKILL IN THE ART

As Dr. Bracewell explains, a person of ordinary skill in the art ("POSA") would have experience with protein manufacturing and purification. (Ex. 1002 (Bracewell Dec.) at ¶ 28.) This person would understand the mechanics of and the science behind protein A chromatography because that is one of the common steps performed during protein purification. (*Id*.) This person would also have hands on experience with other protein purification methods, including other types of chromatography. (*Id.*) The relevant experience could be gained by formal education, such as by a Ph.D., in chemical engineering, physical chemistry,

analytical chemistry, biotechnology, biochemistry, or a related field, along with at least three years' post-education experience. (*Id*.)

V. BACKGROUND ON ANTIBODY MANUFACTURING AND PURIFICATION

An antibody is a molecule produced naturally as part of a body's immune response to foreign substances, called "antigens." (Ex. 1011 (Elgert) at 59.) Antibodies are proteins, and are also known as immunoglobulins. (*Id*. at 59.) There are five types of immunoglobulins, differing from each other based on their structures: "IgG," "IgA," "IgM," "IgD," and "IgE." (*Id*. at 67–70.) This Petition concerns the IgG class of immunoglobulins. All IgG antibodies have a structural region known as the "C_H2/C_H3 region." (*Id*. at 63.)

As of the priority date for the '034 patent, many antibodies were already known and in use for therapeutic purposes. (*See.*, *e.g*., the "Molecular inhibitors/antagonists" portion of the list of Biotechnology-Derived Products in Ex. 1012 (Levin) at 97. The products that are listed as "Mab" are antibodies. (Ex. 1002 (Bracewell Dec.) at p. 16, n. 1).)

A. Antibody Manufacturing and Purification is Accomplished in an "Upstream" Stage and a "Downstream" Stage.

Antibody manufacturing and purification on a large scale for commercial therapeutic use is accomplished in two stages. In the first stage of the process, called the "upstream" stage, many copies of the target antibody, *i.e.*, the antibody

that is to be produced, are produced in large bioreactors, as a product of living cells. (Ex. 1010 (Seetharam) at 214-16.) Following production of the antibodies, the "cell culture fluid," or "CCF," is harvested from the bioreactor; the CCF includes the antibodies, other proteins and other cellular materials dissolved in water. (*Id.*) The CCF is then centrifuged and passed through various filtration media to separate the proteins from the other material. (*Id.*) At this point, the CCF is comprised mostly of proteins dissolved in an aqueous (water-based) solution.

In the next stage, the target antibody must be separated from the other proteins present in the CCF to a high degree of purity. This separation is accomplished during the "downstream" phase by a series of chromatography steps. (*Id.* at 38, 222-31.)

B. Chromatography is a Method by Which Different Materials are Separated Based on their Physical and Chemical Properties.

Many types of chromatography are used to separate different types of proteins. (Ex. 1002 (Bracewell Dec.) at ¶ 35.) Gel filtration chromatography, for example, uses a column packed with sieve-like material that separates proteins based on their molecular sizes. (Ex. 1010 (Seetharam) at 11.)

Adsorption/elution chromatography separates proteins based on the strength with which the proteins adsorb to or bind materials in the chromatography column. (*Id*. at 10; Ex. 1002 (Bracewell Dec.) at ¶¶ 31-34.) Adsorption/affinity chromatography can be used to purify proteins to a purity level of over 90%. (Ex.

1010 (Seetharam) at 10.) Some types of adsorption/elution chromatography relevant to this Petition are ion exchange chromatography, reverse phase chromatography and affinity chromatography.

In ion exchange chromatography, a sample with various types of proteins is flowed through a column packed with a material that binds to the proteins via "electrostatic interactions," i.e., attractive interactions between materials that have the opposite electric charged and repulsive interactions between materials that have the same charge. (*Id*. at 12; Ex. 1002 (Bracewell Dec.) at ¶ 36.) Most often, the packing material is glass, silica or agarose. (Ex. 1010 (Seetharam) at 39; Ex. 1002 (Bracewell Dec.) at ¶ 35.) The strength of the electrostatic interactions is affected by the charge and other characteristics of the proteins, such as their three-dimensional shape. (Ex. 1010 (Seetharam) at 32.) Thus, different proteins bind with different strengths to the packing material. Once the proteins are bound to the packing material, wash solutions with decreasing concentrations of salt are flowed through the column. (*Id*. at 12–13; Ex. 1002 (Bracewell Dec.) at 12-13.) The electrostatic interactions between the different proteins and the column material are interrupted by different concentrations of salt. Therefore, by using washes with different salt concentrations, different proteins are un-bound from the packing material at different times and may be separately collected at the bottom of the column. (Ex. 1010 (Seetharam) at 12-13; Ex. 1002 (Bracewell Dec.) at ¶¶ 34, 37.)

Reverse phase chromatography is similar to ion exchange chromatography but the interactions between the proteins and the packing material are "hydrophobic interactions," a term used to describe the tendency of non-polar materials to aggregate in the presence of water. (Ex. 1010 (Seetharam) at 12–13, 34; Ex. 1002 (Bracewell Dec.) at ¶¶ 34, 37.) Breaking the bonds between the proteins and the packing material cannot be accomplished by using high salt concentration solutions, and is therefore is done by washing the column with solutions of different concentrations of organic solvents, such as acetonitrile, along with water and salt. (Ex. 1010 (Seetharam) at 13; Ex. 1002 (Bracewell Dec.) at ¶¶ 34, 37.)

The organic solvents used in reverse phase chromatography can cause denaturing of some types of proteins when used at high concentrations. (Ex. 1010 (Seetharam) at 34; Ex. 1002 (Bracewell Dec.) at ¶ 39.) Despite the possibility of denaturing proteins, because of its ability to effect high levels of protein purification, reverse phase chromatography was recognized as of 2002 as one method of purification that could be used to purify antibodies intended for therapeutic use. (Ex. 1002 (Bracewell Dec.) at ¶ 72.) For example, U.S. Patent 6,127,526 ("the '526 patent") cited in the '034 patent and assigned to Patent Owner, states that, in addition to purification by protein A chromatography, the fluid with the target antibody "may be subjected to additional purification steps

either prior to, or after, the Protein A chromatography step. Exemplary further purification steps include…reverse phase HPLC…" (Ex. 1008 ('526 patent) at 14:64–15:5; Ex. 1002 (Bracewell Dec.) at ¶ 72.)

As another example, U.S. Patent 6,265,542 ("the '542 patent"), also assigned to Patent Owner, describes and claims the use of reverse phase chromatography for antibodies using hexylene glycol as a wash and eluting solution. (Ex. 1013 ('542 patent) at claim 2, claiming antibodies as one polypeptide that may be purified using the reverse phase chromatography method claimed in claim 1, which uses hexylene glycol as the elution buffer.) According to the '542 patent, the commonly used organic solvents for reverse phase chromatography (acetonitrile, ethanol, methanol, and isopropanol) are flammable, but may be used with "expensive nonflammable-capable equipment and facilities." (*Id*. at 6–11.) Of these solvents, only acetonitrile "has a denaturing effect." (*Id*. at 2:4–5; 3:11–12; *see also* Ex. 1002 (Bracewell Dec.) at ¶ 72.)

In yet another example, Reifsnyder teaches the use of an ethanol-salt solution to wash the protein insulin growth factor from a silica column. (Ex. 1018 (Reifsnyder).) The ethanol-salt solution was successful in washing some protein off of the column that had not been washed out with a high salt (TMAC) concentration solution. Specifically, some of the protein bound with strong

hydrophobic interactions were washed out of the column with the ethanol solution and not with the TMAC solution. (*Id*. at 78; Ex. 1002 (Bracewell Dec.) at ¶ 70.)

As Dr. Bracewell explains, all solutions that are used to wash chromatography columns must be tested for compatibility with the materials to be separated. (*See, e.g.*, Ex. 1016 (Chadha) at 471-72, testing different concentrations of TMAC salt for compatibility for use in a wash solution of a chromatography column used to separate types of proteins; Ex. 1002 (Bracewell Dec.) at ¶ 39.) A POSA would have known how to test different solutions for compatibility, and would know to adjust the solution, by for example, changing the concentrations of some components or adding protective materials, if needed. (Ex. 1002 (Bracewell Dec.) at ¶ 39.)

Another type of adsorption chromatography relevant to this petition is Protein A affinity chromatography which separates materials based on their ability to bind to a protein called "protein A." (Ex. 1010 (Seetharam) at 223–24.)

C. Protein A Chromatography is Effective at Purifying Antibodies From CCF.

Protein A is bacterial cell wall protein that binds specifically to antibodies. It binds selectively and strongly to the C_H2/C_H3 region found on all IgG proteins,

and is therefore particularly suited as a ligand for purification of IgG antibodies.[1] (Ex. 1010 (Seetharam) at 223–24; Ex. 1002 (Bracewell Dec.) at ¶ 40.) Antibody purification by protein A chromatography is fully described in the prior art, including for antibodies intended for therapeutic use. (*See, e.g.*, Ex. 1012 (Levin) Ex. 1012 at 97; Ex. 1002 (Bracewell Dec.) at p. 16, n. 1.)

Protein A chromatography of CCF is performed as follows. First, the CCF consisting of mostly proteins, including the target antibody and other proteins, is loaded onto a protein A column. A protein A column has protein A immobilized on a support material, called the "solid phase," often a glass or silica based material. (Ex. 1002 (Bracewell Dec.) at ¶¶ 32, 41.) Once the CCF is loaded onto the column, the target antibody binds strongly to the protein A. This is a "specific" bond, occurring between protein A and the C_H2/C_H3 region of an antibody. (*Id*. at ¶ 41; Ex. 1015 (Gagnon) at 157.) Other proteins, the "contaminant" proteins that lack the C_H2/C_H3 portion, do not bind as easily to protein A. Upon loading of the sample, some of these immediately flow through the column, while others may be retained on the column support by "non-specific" interactions between the proteins and the glass or silica support material. (Ex. 1002 (Bracewell Dec.) at ¶ 41.) These non-specific interactions include, for example, electrostatic interactions and

[1] Protein A binds well to IgG_1, IgG_2, and IgG_4 type antibodies, but does not bind as strongly to IgG_3 type antibodies.

hydrophobic interactions. A wash step, discussed in the next section, is then used to interrupt these interactions and wash the non-specifically bound proteins out of the column. (*Id*.; Ex. 1008 ('526 patent) at 2:8-28; Ex. 1015 (Gagnon) at 163-65.)

The last step of protein A chromatography is to elute the IgG, strongly bound to the protein A, from the column. This is most often done by washing the column with an acidic "elution buffer," with a low pH value. (Ex. 1010 (Seetharam) at 224; Ex. 1002 (Bracewell Dec.) at ¶ 41.) The low pH breaks the bonds between the protein A and the IgG, allowing the IgG to flow through and elute from the column.

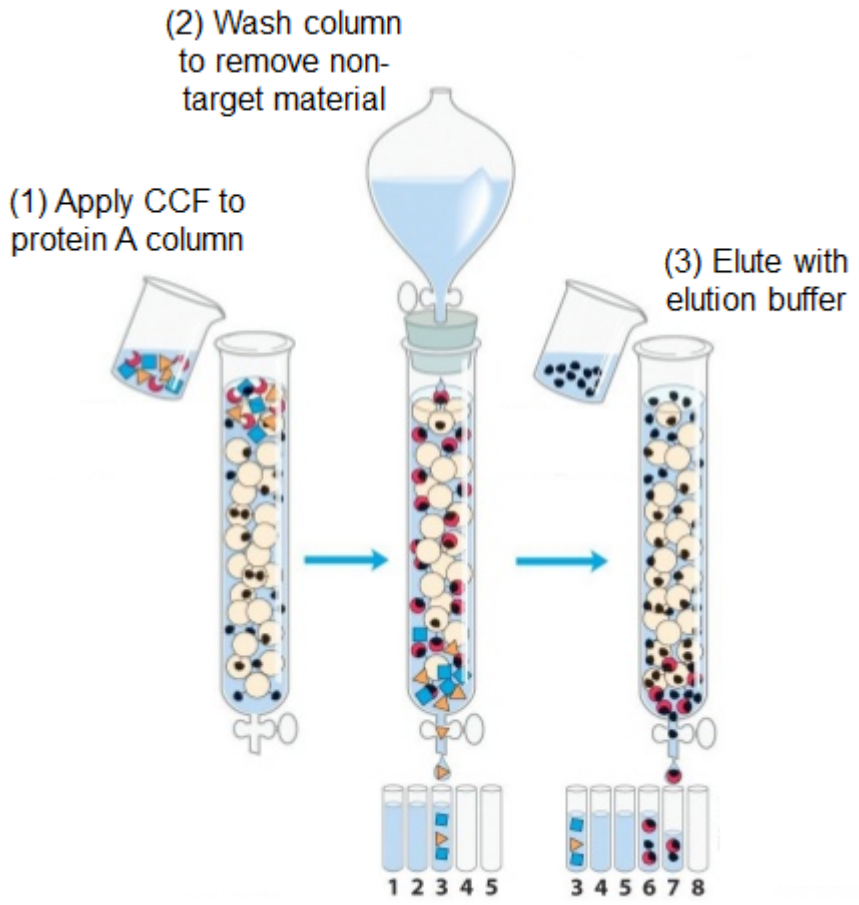

Figure 1: Affinity chromatography separates proteins by their binding specificities. The proteins retained on the column are those that bind specifically and strongly to protein A. After proteins that are non-specifically bound to the column are washed through and eluted from the column, the bound protein of particular interest is eluted by a buffer solution. (Modified from Lehninger et al., *Principles of Biochemistry, Second Edition*, available at http://www.bioinfo.org.cn/book/biochemistry/start.htm, Ex. 1014)

Protein A chromatography may be optimized to purify antibodies to as high as 95% purity. (Ex. 1015 (Gagnon) at 163.) It is a simple process requiring only three steps: load, wash, and elute. (*Id.*) For these reasons, protein A chromatography is often incorporated into antibody manufacturing and purification processes. (Ex. 1010 (Seetharam) at 224.)

D. In Protein A Chromatography, Wash Buffers Are Used to Wash Proteins Bound to the Column with Non-Specific Interactions from the Column.

A sample loaded onto a protein A column is normally dissolved in a buffer, sometimes called a "binding buffer." A buffer is "a buffered solution that resists changes in pH by the action of its acid-base conjugate pairs." (Ex. 1001 ('034 patent) at 4:1–2; Ex. 1002 (Bracewell Dec.) at ¶¶ 43-44.) Studies have shown that the binding between protein A and IgGs is optimized at high pH values, for example at between neutral pH (7) and basic pH values of about pH = 9. (Ex. 1004 (van Sommeren) at 136; 146; Ex. 1002 (Bracewell Dec.) at ¶ 43.) Therefore, buffers that are able to maintain a solution at these pH values are often used as the binding buffer for dissolving a protein sample to be purified by protein A chromatography. (Ex. 1002 (Bracewell Dec.) at ¶¶ 43-44.)

In addition to maintaining pH values, the binding buffer may also have some additives that are known to aid various functions of the chromatography process. (Ex. 1015 (Gagnon) at 158–60.) For example, salts are often added to the buffer to

help some IgG proteins bind to the column.[2] High concentrations of salts are also added to binding buffers because this helps the dissociation between DNA residues that may be present in the CCF and the IgG antibodies so that the DNA may be washed from the column separately from the target antibody. (*Id*. at 165.)

Other than salts, glycine, a building block of proteins, is often added to binding buffer to increase binding between protein A and IgG antibodies. (*Id*. at 159.) Glycine is also added to enhance antibody stability. (*Id*.)

Once the sample is loaded onto the protein A column and the IgG binds to the protein A, the remaining proteins in the sample are washed out of the column with a solution. (Ex. 1002 (Bracewell Dec.) at ¶ 41.) This solution is called the "intermediate wash buffer" or the "intermediate wash solution" in the '034 patent. (Ex. 1001 ('034 patent) at 4:29-36.) Often the solution used to wash the other proteins out of the column is the same as the binding buffer. (*See*, *e.g.*, Ex. 1004 (van Sommeren); Ex. 1002 (Bracewell Dec.) at ¶ 43.) Thus, the wash solution also promotes the continued binding between protein A and the IgG antibody. (Ex. 1002 (Bracewell Dec.) at ¶ 43.)

[2] Most IgGs bind to the column without any particular salt concentration. Some IgGs, for example, those derived from human IgG3, require high salt concentrations in order to bind strongly to protein A. (Ex. 1015 (Gagnon) at 162; Ex. 1002 (Bracewell Dec.) at n. 1.)

As discussed above, oftentimes, when the sample is loaded onto a column and the target protein binds strongly to the protein A on the column, contaminant proteins will non-specifically bind to the column: "[i]t has been observed that in Protein A chromatography using a glass or silica surface for adsorbing the Protein A…contaminants in the protein preparation (such as Chinese Hamster Ovary Proteins (CHOP), where the protein preparation is derived from a CHO cell) adhere to the glass or silica surface of the solid phase." ('526 patent at 2:8–17; Ex. 1002 (Bracewell Dec.) at ¶ 41.) Unless these contaminant proteins are removed before the target protein is eluted from the column, they will be present in the product with the target protein, decreasing the level of purity of the concentrated antibody fluid. (Ex. 1002 (Bracewell Dec.) at ¶ 41.) The '034 patent is directed to the wash solution that is used to wash these non-specifically bound proteins from the column.

Before the filing date of the '034 patent, POSAs knew that these non-specifically bound proteins may be washed out of the column before eluting the protein A by washing a wash buffer through the column. (*Id*. at ¶¶ 41-44.) For the composition of the wash solution, POSAs looked to other forms of adsorption chromatography for guidance regarding what solutions successfully wash proteins that are bound to glass or silica columns. (*Id*. at ¶ 42.)

For example, the ’526 patent cited in the ’034 patent at 2:10–16, teaches the use of the salt tetramethylammonium chloride (TMAC) dissolved in a wash buffer to remove these proteins. (Ex. 1008 (’526 patent) at 2:25–27.) Prior to the filing of the ’526 patent in 1996, POSAs knew that TMAC was useful for washing proteins from glass columns during various types of chromatography. (*See, e.g.*, Ex. 1016 (Chadha), published in 1981 and cited in the ’034 patent at 2:5–9. Chadha identified the interactions between the proteins and the glass column as electrostatic interactions.) Thus, the use of TMAC to elute the non-specifically bound contaminant proteins from protein A columns was developed based on its known use to elute proteins from glass columns during adsorption chromatography. (Ex. 1002 (Bracewell Dec.) at ¶¶ 67-68.)

High concentrations of salts other than TMAC were also used to wash bound proteins out of protein A columns. (*See, e.g.*, Ex. 1004 (van Sommeren).) As discussed above, the use of salts to elute proteins from columns has its source in older forms of chromatography, specifically ion exchange chromatography. (Ex. 1002 (Bracewell Dec.) at ¶ 68.)

Fang, *Real-time Isoform Analysis by Two-Dimensional Chromatography of a Monoclonal Antibody During Bioreactor Fermentation*, JOURNAL OF CHROMATOGRAPHY A, 816, 39–47 (1998) teaches the use of a salt and isopropanol solution as a wash buffer during protein A chromatography. (Ex. 1009 (Fang) at

40–41.) The use of organic solvents with salt such as isopropanol to elute proteins from glass or silica chromatography columns also has its source in another form of chromatography, specifically reverse-phase chromatography. (Ex. 1010 (Seetharam) at 12; Ex. 1018 (Reifsnyder); Ex. 1002 (Bracewell Dec.) at ¶ 70.)

V. CLAIM CONSTRUCTION

Because the ’034 patent will not expire during the pendency of this proceeding, the challenged claims should be given their broadest reasonable construction in light of the patent specification. 37 C.F.R. § 42.100(b); *see also Cuozzo Speed Techs. LLC v. Lee*, 136 S. Ct. 2131, 2142 (2016).

A. “buffer”

The ’034 patent defines the term “buffer” at col 4, lines 1–2: “A “buffer” is a buffered solution that resists changes to pH by the action of its acid-base conjugate components.” (*See also*, Ex. 1002 (Bracewell Dec.) at ¶ 46.)

For purposes of this Petition only, Petitioner construes the term “buffer” to include solutions with dissolved substances that are commonly used as buffers in pharmaceutical applications. Petitioner does not here attempt to define and list all substances that may fit into this definition, but asserts that POSAs are familiar with these substances and would readily be able to determine whether a given substance was commonly used as a buffer in pharmaceutical applications. As a starting guide, Petitioner refers to “Buffers—pH Control within Pharmaceutical Systems,”

("Flynn," published in PDA Journal of Pharmaceutical Sciences and Technology in 1980, Ex. 1017) and particularly its Table V, which lists substances that may be used as buffers for pharmaceutical compositions. Certainly, all of the substances listed in Table V, when dissolved in water at the specified pH values, are "buffers" within the meaning of claim 13 of the '034 patent, as a POSA would readily recognize that each of these substances was commonly used as a buffer.[3] (Ex. 1002 (Bracewell Dec.) at ¶ 47.)

B. "composition comprising a buffer at a concentration of greater than about 0.8M"

Petitioner provides two alternate claim constructions for the term "composition comprising a buffer at a concentration of greater than about 0.8M." The term, on its face, is unclear with respect to what substance must be "at a concentration of greater than about 0.8M." (Ex. 1002 (Bracewell Dec.) at ¶ 48.) Under one reading, it is the total concentration of the solutes (i.e., the materials dissolved) in the buffered solution that must be greater than about 0.8M. Under another reading, it is the "buffer" as that term has been construed above that, alone

[3] Different buffers have the ability to control the pH of a solution at different pH values. Flynn lists in Table V the approximate pH range(s) at which each listed substance has buffering capacity. This Petition relies on these values throughout.

within the overall composition, must have a concentration of greater than about 0.8M. (*Id*.)

The "first construction," that the total concentration of the solutes in the buffered solution must be greater than about 0.8M, is the broadest reasonable construction of the term in light of the specification. Indeed, the '034 patent defines "buffer" as "a buffered solution," and does not limit the term "buffer" to just the substance that lends the buffering property to the solution. In other words, under the broadest reasonable construction in light of the specification, the solution as a whole is a "buffer," should have a concentration of dissolved substances that is "greater than about 0.8M," and should, as a whole, have buffering capacity. Any amount of a dissolved buffer as that term is defined above will lend buffering capacity to a solution, with the strength of the buffer increasing with the concentration of the dissolved buffering material itself.

Under the "second construction," it is the substance that provides the buffering capacity to the composition that must be present at a concentration of greater than about 0.8M. This construction, while not as broad as the "first construction," may nonetheless be compelled by the '034 patent specification's reference to the '526 patent.

According to the '034 patent's specification, the '526 patent discloses an intermediate wash step using toxic substances. (Ex. 1001 ('034 patent) at 18:42–

54.) Also according to the ’034 patent’s specification, the instantly claimed intermediate wash buffers are superior to substances used in the ’526 patent because they are not toxic. (*Id*.) The ’526 patent, however, describes wash solutions that include TMAC or TEAC, the substances described as “toxic” in the ’034 patent, dissolved at concentrations “in the range from about 0.1 to about 1.0 M” in “a buffered solution.” (’526 patent at 14:52–55.) The ’526 patent lists “suitable buffers for this purpose” including Tris and phosphate—both of which are included in the Flynn Table V list of buffers used in the pharmaceutical sciences. In other words, the wash substance disclosed in the ’526 patent includes electrolytes at concentrations up to at least 1M, which is “greater than about 0.8M” dissolved in a buffered solution. Thus, in order to follow the ’034 patent’s logic that differentiates the ’526 patent’s wash substances from the wash substances claimed in the ’034 patent, the construction of “concentration greater than about 0.8M” must refer to only the buffering substance, alone, within the entire wash composition.

C. “C_H2/C_H3 Region”

The patent defines “C_H2/C_H3 region” as “those amino acid residues in the Fc region of an immunoglobulin molecule which interact with Protein A.” (Ex. 1001 (’034 patent) at 3:47–50.) Ex. 1011 confirms that all antibodies of the IgG type have a C_H2/C_H3 region. (Ex. 1011 (Elgert) at 64.)

VI. GROUND 1 – CLAIM 13 IS ANTICIPATED BY VAN SOMMEREN

Van Sommeren is an article titled "*Effects of Temperature, Flow Rate and Composition of Binding Buffer on Adsorption of Mouse Monoclonal IgG_1 Antibodies to Protein A Sepharose 4 Fast Flow*." Van Sommeren published in 1992 in the Journal Preparative Biochemistry, which is well known and well respected by POSAs. (Ex. 1002 (Bracewell Dec.) at ¶ 54.) Van Sommeren is therefore a printed publication and prior art to the '034 patent under 35 U.S.C. § 102(b). Van Sommeren was not before the examiner during prosecution of the '974 application. (*See* Ex. 1019 (Prosecution History of '974 application).)

As its title suggests, van Sommeren explores, among other things, the effect of the composition of the binding buffer on the binding strength between protein A and an IgG antibody. Van Sommeren describes the procedure used to test the various buffers; in that procedure, the "washing buffer," used to wash the non-bound proteins out of the column after loading the sample and before elution of the antibody was the same buffer used as the binding buffer. (Ex. 1004 (Van Sommeren) at 138.) Van Sommeren tested 15 different binding buffers and determined the strength of the binding when each of those buffers was used. Of the 15 binding buffers tested, 12 of them comprised "a buffer at a concentration of greater than about 0.8M" as claimed in claim 13. (Ex. 1002 (Bracewell Dec.) at ¶ 56.) The 12 binding buffers are listed below:

Binding Buffer Used in the Wash Step	Buffer is Within Scope of Claim 13
0.5 M glycine, 0.5 M NaCl, pH 8.9	This buffer has a total concentration of approximately 1 M, which is "greater than about 0.8 M" under Petitioner's first construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.
0.5 M glycine, 1.0 M NaCl, pH 8.9	This buffer has a total concentration of approximately 1.5 M, which is "greater than about 0.8 M" under Petitioner's first construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.
0.5 M glycine, 2.0 M NaCl, pH 8.9	This buffer has a total concentration of approximately 2.5 M, which is "greater than about 0.8 M" according to Petitioner's first construction. Glycine is listed in Flynn Table V as

	having a pH buffering range of approximately from 8.8–10.8.
0.5 M glycine, 3.0 M NaCl, pH 8.9	This buffer has a total concentration of approximately 3.5 M, which is "greater than about 0.8 M" under Petitioner's first construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.
1.5 M glycine, 0.5 M NaCl pH 8.9	This buffer has a total concentration of approximately 2 M, which is "greater than about 0.8 M" according to Petitioner's first construction. Glycine alone is present at a concentration of 1.5 M, which is "greater than about 0.8M" according to Petitioner's second construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.

1.5 M glycine, 1 M NaCl pH 8.9	This buffer has a total concentration of approximately 2.5 M, which is "greater than about 0.8 M" according to Petitioner's first construction. Glycine alone is present at a concentration of 1.5 M, which is "greater than about 0.8M" under Petitioner's second construction. Glycine's pH buffering range is approximately from 8.8–10.8.
1.5 M glycine, 2 M NaCl pH 8.9	This buffer has a total concentration of approximately 3.5 M, which is "greater than about 0.8 M" under Petitioner's first construction. Glycine alone is present at a concentration of 1.5 M, which is "greater than about 0.8M" according to Petitioner's second construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.

1.5 M glycine, 3.0 M NaCl, pH 8.9	This buffer has a total concentration of approximately 4.5 M, which is "greater than about 0.8 M" according to Petitioner's first construction. Glycine alone is present at a concentration of 1.5 M, which is "greater than about 0.8M" according to Petitioner's second construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.
0.025 M glycine, 1.2 M K_2HPO_4, pH 9.0	This buffer has a total concentration of approximately 1.225 M, which is "greater than about 0.8 M" according to Petitioner's first construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.
0.025 M glycine, 1.2 M K_2HPO_4, pH 10.0	This buffer has a total concentration of approximately 1.225 M, which is

	"greater than about 0.8 M" according to Petitioner's first construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8.
0.1 M Tris, 1.0 M Na_2SO_4, pH 7.5	This buffer has a total concentration of approximately 1.1 M, which is "greater than about 0.8 M" according to Petitioner's first construction. Tris is listed in Flynn Table V as having a pH buffering range of approximately from 7.1–9.1.
0.1 M Tris, 1.5 M $(NH_4)_2SO_4$, pH 7.5	This buffer has a total concentration of approximately 1.6 M, which is "greater than about 0.8 M" according to Petitioner's first construction. Tris is listed in Flynn Table V as having a pH buffering range of approximately from 7.1–9.1.

Dr. Bracewell confirms that these twelve buffered solutions are within the scope of claim 13. (Ex. 1002 (Bracewell Dec.) at ¶ 56.)

Because van Sommeren discloses successfully carried out purifications, its disclosure is enabled. (*Id*. at ¶ 54.) The disclosure in van Sommeren meets all of the limitations of claim 13, as set forth below, and therefore anticipates claim 13, under either of Petitioner's alternative claim constructions of "composition comprising a buffer at a concentration of greater than about 0.8M." (*Id*. at ¶ 55.)

Claim 13 Limitation	**Disclosure in van Sommeren**
A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:	"In this paper it is described in which way the composition of binding buffer (concentration and ion type) affects purification of these mouse IgG_1 mabs with respect to the dynamic binding capacity of the protein A Sepharose 4 Fast Flow gel, and the purity of the final IgG solution." (Ex. 1004 (Van Sommeren) at 326.) Because the protein that was purified was IgG, it had a C_H2/C_H3 region. (Ex. 1002 (Bracewell Dec.) at ¶ 55; Ex. 1011

	(Elgert) at 63.)
(a) adsorbing the protein to Protein A immobilized on a solid phase;	"The cell culture supernatant was diluted with an equal volume of binding buffer and filtered through a 0.2 μm pore size membrane filter. Subsequently a volume containing a fixed amount of mab was loaded onto the column." (Ex. 1002 (Van Sommeren) at 138.)
(b) removing contaminants by washing the solid phase with a composition comprising a buffer at a concentration of greater than about 0.8M; and	"The non-bound fraction was washed from the column with binding buffer." (*Id*.) The identities of 12 buffers that are within the scope of this limitation under either of Petitioner's alternative claim constructions are described in the table above. (Ex. 1002 (Bracewell Dec.) at ¶ 56.)
(c) recovering the protein from the solid phase.	"The fraction bound to the column was desorbed with 0.1 M citric acid (pH

	5.0.)" (Ex. 1004 (Van Sommeren) at 138.)

VII. GROUND 2 – CLAIM 13 IS ANTICIPATED BY GODFREY

Godfrey is an article titled "*A Sensitive Enzyme-Linked Immunosorbent Assay (ELISA) for the Detection of Staphylococcal Protein A (SpA) Present as a Trace Contaminant of Murine Immunoglobulins Purified on Immobilized Protein A.*" Godfrey published in 1992 in the Journal of Immunological Methods, which was read by POSAs as part of their work on purifying antibodies for immunological therapies. (Ex. 1002 (Bracewell Dec.) at ¶ 57.) Godfrey is therefore a printed publication and prior art to the '034 patent under 35 U.S.C. § 102(b). Godfrey was not before the examiner during prosecution of the '974 application. (*See* Ex. 1019 (Prosecution History of '974 application.)

Godfrey describes an assay developed to measure the amount of protein A that elutes with the target antibody during the elution step of protein A chromatography. Because Godfrey discloses successfully carried out purification, its disclosure is enabled. (Ex. 1002 (Bracewell Dec.) at ¶ 57.) Godfrey describes a protein A chromatography method that anticipates claim 13 under either of Petitioner's alternative claim constructions, as demonstrated below. (*Id*. at ¶ 58.)

Claim 13 Limitation	Disclosure in Godfrey
A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:	"The utility of immobilized protein A (SpA) preparations for the affinity purification of antibodies for therapeutic applications is widely acknowledged." (Ex. 1005 (Godfrey) at 21.) "isoloated murine IgG1 monoclonal antibody…" (*Id*. at 22.) "Prosep A, high capacity, and Protein A-Sepharose CL-4B (preswollen) were suspended in washing buffer (glycine, 1 M; sodium chloride, 0.15M; pH 8.6)…they were then poured into disposable columns…" (*Id*. at 23.) Because the protein that was purified was an IgG, it had a C_H2/C_H3 region. (Ex. 1002 (Bracewell Dec.) at ¶ 58; Ex. 1011 (Elgert) at 63.)
(a) adsorbing the protein to Protein A immobilized on a solid phase;	"The columns were then loaded with murine IgG1 containing bioreactor

	supernatant…" (Ex. 1005 (Godfrey) at 23.)
(b) removing contaminants by washing the solid phase with a composition comprising a buffer at a concentration of greater than about 0.8M; and	"…and washed with 10 vols of washing buffer." (*Id*.) "washing buffer (glycine, 1 M; sodium chloride, 0.15 M; pH 8.6)." (*Id*.) The total concentration of the washing buffer is approximately 1.15 M, which is "greater than about 0.8 M" according to Petitioner's first construction. The concentration of glycine alone is 1 M, which is "greater than about 0.8M" according to Petitioner's second construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8-10.8. Glycine is expected to have some buffering capacity at pH = 8.6. (Ex. 1002 (Bracewell Dec.) at ¶ 58.)

(c) recovering the protein from the solid phase.	"Purified antibodies were eluted in 5.5 column vols. of elution buffer…" (Ex. 1005 (Godfrey) at 23.)

VIII. GROUND 3 –CLAIM 13 IS ANTICIPATED BY THE '687 PATENT

United State Patent 4,801,687 (the "'687 patent"), titled "*Monoclonal Antibody Purification Process*," issued on January 31, 1989, and is a patent and prior art to the '034 patent under 35 U.S.C. § 102(b). The '687 patent was not before the examiner during prosecution of the '974 application. (*See* Ex. 1019 (Prosecution History of '974 application).)

The abstract of the '687 patent states: "Immunoglobulins are purified by adsorption upon an immobilized protein A adsorbent using a buffer having a pH of 7.5 to 10 and containing a number of monovalent cations and polybasic anions in a concentration of about 0.6M to 1.75M." (Ex. 1006 ('687 patent) at 1.) With respect to the binding buffer that is also used as the wash buffer, the '687 patent says: "[t]he first step in the process of the present invention requires a buffer having a pH in the range of about pH 7.5 to pH 10 and a combination of monovalent cations and polybasic anions in a concentration of about 0.6 M to 1.75 M. Any buffer may be used to provide the desired pH. For example, glycine

buffer, borate buffer or tris buffer can be used. The concentration of the buffer should be in the range of about 0.01 M to 0.25 M." (*Id.* at 3:32–35.)

A number of examples in the '687 patent anticipate claim 13; for purposes of this Petition, Petitioner relies on Example 5. As the '687 patent is an issued U.S. patent, its disclosure is presumed to be enabled. *See, e.g.*, *Amgen Inc., v. Hoechst Marion Roussel, Inc.* 314 F.3d 1313, 1354 (Fed Cir. 2003). Example 5 anticipates claim 13 under either of Petitioner's alternative claim constructions, as demonstrated below. (Ex. 1002 (Bracewell Dec.) at ¶ 59.)

Claim 13 Limitation	**Disclosure in the '687 Patent Example 5**
A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:	"The process of the present invention is useful in purifying immunoglobulins of various types including both monoclonal and polyclonal antibodies. It is applicable to many IgG subclasses…" (Ex. 1006 ('687 patent) at 2:36–39.) "Immunoglobulins are purified by adsorption upon an immobilized protein A adsorbent using a buffer having a pH

	of 7.5 to 10 and containing a number of monovalent cations and polybasic anions in a concentration of about 0.6M to 1.75M." (*Id*. at abstract.) "To a 3 ml column was added 1 ml of immobilized protein A…A quantity of 1 ml of mouse monoclonal antibody from ascites fluid was diluted with 1 ml of buffer and applied to the column." (*Id*. at 4:52–59.) Because the protein that was purified was an IgG, it had a C_H2/C_H3 region. (Ex. 1002 (Bracewell Dec.) at ¶ 59; Ex. 1011 (Elgert) at 63.)
(a) adsorbing the protein to Protein A immobilized on a solid phase;	"A quantity of 1 ml of mouse monoclonal antibody from ascites fluid was diluted with 1 ml of buffer and applied to the column." (Ex. 1006 ('687 patent) at 6:30–32.)
(b) removing contaminants by washing	"The column was equilibrated with 10

the solid phase with a composition comprising a buffer at a concentration of greater than about 0.8M; and	ml of a 0.05 M Tris (hydroxymethyl) aminomethane (Tris) buffer, pH 8.5, containing 1.0 M K_2HPO_4. A quantity of 1 ml of mouse monoclonal antibody from ascites fluid was diluted with 1 ml of buffer and applied to the column. Then the column was washed with 5–10 ml of buffer." (*Id*.) The total concentration of the wash buffer was 1.05, which is "greater than about 0.8M" according to Petitioner's first construction. The concentration of the K_2HPO_4 alone was 1.0 M, which is "greater than about 0.8M" under Petitioner's second construction. Tris is listed in Flynn Table V as having a pH buffering range of approximately from 7.1–9.1. Phosphoric buffers such as K_2HPO_4 are listed in Flynn Table V as having a pH

	buffering range of approximately 6.2–8.2. Thus, K_2HPO_4 has some buffering capacity at pH = 8.5. (Ex. 1002 (Bracewell Dec.) at ¶ 59.)
(c) recovering the protein from the solid phase.	"The immunoglobulins which were adsorbed on the column were eluted with 5 ml of 0.1 M acetic acid-sodium acetate buffer, pH 3.5." (Ex. 1006 ('687 patent) at 5:29–31.)

IX. GROUND 4 – CLAIM 13 IS ANTICIPATED BY THE '829 PATENT

United States Patent 5,098,829 (the "'829 patent"), titled "*Anti-thrombin-binding substance monoclonal antibodies, hybridomas producing same, as well as purification process and assay of thrombin-binding substance making use of said monoclonal antibodies*," issued on March 23, 1992, and is a patent and prior art to the '034 patent under 35 U.S.C. § 102(b). The '829 patent was not before the examiner during prosecution of the '974 application. (*See* Ex. 1019 (Prosecution History of '974 application).)

The abstract of the '829 patent states: "The present invention provides a monoclonal antibody specific to a thrombin-binding substance (TM), hybridomas

producing the monoclonal antibody, a purification process of TM featuring the use of the monoclonal antibody as an immunoadsorbent, as well as an immunoassay of TM featuring the use of the monoclonal antibody." (Ex. 1007 ('829 patent) at 1.) The '829 patent gives six exemplary antibodies; all are of the IgG_1 class. All therefore have the C_H2/C_H3 region. (*Id*. at 2:45–52.) As an issued U.S. Patent, the '829 patent is presumed to be enabled. *See, e.g.*, *Amgen Inc., 314 F.3d at* 1354. Example 2 anticipates claim 13 according to either of Petitioner's alternative claim constructions as illustrated below. (Ex. 1002 (Bracewell Dec.) at ¶ 60.)

Claim 13 Limitation	**Disclosure in The '829 Patent, Example 2**
A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:	"… About 10 days later, ascetic fluid was collected from the mice. The fluid was centrifuged at 3,000 RPM for 10 minutes to collect a supernatant. To 4.8 ml of the supernatant, an equal amount of 1.5 M glycine buffer (pH 8.9) containing 3 M of sodium chloride was added. The resultant mixture was subjected to chromatography on a column packed with 5 ml of "Protein A

	Sepharose CL-4B" (trade name) with had been equilibrated with the same buffer. After washing the column thoroughly with the same buffer the column was eluted with 0.1 M citrate buffer (pH 4.0). (Ex. 1007 ('829 patent) at 11:37–40.) The '829 patent gives six exemplary antibodies; all are of the IgG1 class, and therefore, all have the C_H2/C_H3 region. (*Id*. at 2:45–52; *see* Ex. 1002 (Bracewell Dec.) at ¶ 60; Ex. 1011 (Elgert) at 63.)
(a) adsorbing the protein to Protein A immobilized on a solid phase;	"The resultant mixture was subjected to chromatography on a column packed with 5 ml of 'Protein A Sepharose CL-4B' (trade name) with had been equilibrated with the same buffer." (*Id*.)
(b) removing contaminants by washing the solid phase with a composition comprising a buffer at a concentration of	"To 4.8 ml of the supernatant, an equal amount of 1.5 M glycine buffer (pH 8.9) containing 3 M of sodium chloride was

greater than about 0.8M; and	added. The resultant mixture was subjected to chromatography on a column packed with 5 ml of "Protein A Sepharose CL-4B" (trade name) with had been equilibrated with the same buffer. After washing the column thoroughly with the same buffer… " (*Id*.) The total concentration of the wash buffer was 4.5M, which is "greater than about 0.8M" according to Petitioner's first construction. The concentration of the glycine alone was 1.5 M, which is "greater than about 0.8M" according to Petitioner's second construction. Glycine is listed in Flynn Table V as having a pH buffering range of approximately from 8.8–10.8. (Ex. 1002 (Bracewell Dec.) at ¶ 60.)
(c) recovering the protein from the solid	"After washing the column thoroughly

phase.	with the same buffer the column was eluted with 0.1 M citrate buffer (pH 4.0)." (*Id*.)

X. GROUND 5 – CLAIM 13 IS ANTICIPATED BY THE '526 PATENT

United States Patent 6,127,526 (the "'526 patent"), titled "*Protein Purification by Protein A Chromatography*," issued on October 3, 2000, and is a patent and is prior art to the '034 patent under 35 U.S.C. § 102(b). Patent Owner admitted that the '526 patent is prior art to the '034 patent in the specification at col. 2, ll. 10–17. As it is a United States patent, the '526 patent is presumed to be enabled. *See, e.g., Amgen Inc.*, 314 F.3d at 1354.

The abstract of the '526 patent states: "A method for purifying proteins by Protein A chromatography is described which comprises the steps of: (a) adsorbing the protein to Protein A immobilized on a solid phase comprising silica or glass; (b) removing contaminants bound to the solid phase by washing the solid phase with a hydrophobic electrolyte solvent, and (c) recovering the protein from the solid phase." (Ex. 1008 ('526 patent) at abstract.)

The disclosure of the '526 patent anticipates claim 13 under Petitioner's first claim construction, as illustrated below.

Claim 13 Limitation	**Disclosure in ’526 Patent**
A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:	“Protein A immobilized on a solid phase is used to purify the C_H2/C_H3 region-containing protein.” (Ex. 1008 (’526 patent) at 14:16–19.)
(a) adsorbing the protein to Protein A immobilized on a solid phase;	“The contaminated preparation derived from the recombinant host cells is loaded on the equilibrated solid phase using a loading buffer which may be the same as the equilibration buffer. As the contaminated preparation flows through the solid phase, the protein is adsorbed to the immobilized Protein A.” (*Id*. at 14:31–34.)
(b) removing contaminants by washing the solid phase with a composition comprising a buffer at a concentration of greater than about 0.8M; and	“The next step performed sequentially entails removing the contaminants bound to the solid phase by washing the solid phase with a hydrophobic electrolyte solvent in an intermediate wash step. The hydrophobic electrolyte

	is preferably added to a pH buffered solution having a pH in the range from about 4 to about 8, and preferably in the range from about 5 to about 7. Suitable buffers for this purpose include Tris, phosphate, MES, and MOPSO buffers. The preferred final concentration for the hydrophobic electrolyte in the wash solvent is in the range from about 0.1 to about 1.0 M, and preferably in the range from about 0.25 to about 0.5M." (*Id*. at 14:40–55.) The total concentration of the wash buffer may be up to about 1.0M, which is "greater than about 0.8M" according to Petitioner' first construction. Tris is listed in Flynn Table V as having a pH buffering range of approximately from 7.1–9.1. (Ex. 1002 (Bracewell Dec.) at ¶ 61.)

(c) recovering the protein from the solid phase.	"Following the intermediate wash step of the preceding paragraph, the protein of interest is recovered from the column." (*Id.* at 14:56–58.)

XI. GROUND 6 – CLAIM 16 IS ANTICIPATED BY FANG

Fang is an article titled "*Real-time Isoform Analysis by Two-Dimensional Chromatography of a Monoclonal Antibody During Bioreactor Fermentations*," published in 1998 in the Journal of Chromatography A. As Dr. Bracewell states, the Journal of Chromatography A is well respected and commonly read by POSAs. (Ex. 1002 (Bracewell Dec.) at ¶ 63.) Fang is therefore a printed publication and is prior art to the '034 patent under 35 U.S.C. § 102(b). Fang was not before the examiner during prosecution of the '974 application. (*See* Ex. 1019 (Prosecution History of '974 application).)

Fang discloses the purification of HuDREG-55, an IgG_4 antibody, by protein A chromatography. (Ex. 1009 (Fang) at abstract.) Because HuDREG-55 is an IgG antibody, it, by definition, contains a C_H2/C_H3 region. (Ex. 1011 (Elbert) at 63; Ex. 1002 (Bracewell Dec.) at ¶ 64.) The antibody in Fang is purified in a series of two steps. The first step is protein A chromatography, done in the conventional manner, by loading an impure sample onto the column where the target antibody

binds to protein A, washing the column to remove loosely bound proteins, and eluting the target antibody. Fang discloses that the protein A conditions were "optimized," indicating that all of the steps in the protein A worked as intended. (Ex. 1008 (Fang) at 41; Ex. 1002 (Bracewell Dec.) at ¶ 64.) The second step is anion-exchange chromatography, a type of chromatography that separates materials based on their charges.

For the wash composition during the protein A chromatography, Fang teaches the use of a potassium phosphate/potassium chloride/isopropanol solution. (Ex. 1008 (Fang) at Table 1, Table 2.) This solution is within the scope of claim 16's wash composition "comprising salt and a solvent selected from the group consisting of ethanol, methanol, isopropanol, acetonitrile, hexylene glycol, propylene glycol, and 2,2-thiodiglycol." (Ex. 1002 (Bracewell Dec.) at ¶ 64.) Claim 16 includes a Markush group of solvents, including isopropanol. The disclosure of one member of a Markush group in the prior art discloses the entire Markush group element. *See, e.g., Schering Corp. v. Geneva Pharms, Inc*., 339 F.3d 1373, 1380 (Fed. Cir. 2003); *see also Fresenius USA, Inc. v. Baxter Intern., Inc*., 582 F.3d 1288 (Fed. Cir. 2009) ("Element (a) is written in Markush form, such that the entire element is disclosed by the prior art if one alternative in the Markush group is in the prior art."). Thus, Fang's use of a composition comprising salt and isopropanol as the wash buffer during the protein A purification of an IgG antibody anticipates claim 16.

Because Fang discloses a successfully carried out purification, its disclosure is enabled. (Ex. 1002 (Bracewell Dec. at ¶ 64.) Fang anticipates claim 16 as illustrated below. (*Id*.)

Claim 16 Limitation	**Disclosure in Fang**
A method for purifying a protein, which comprises a C_H2/C_H3 region, from a contaminated solution thereof by Protein A chromatography comprising:	"The column used in the number 1 position on the Integral was a Poros PA ImmunoDetection cartridge (3 cm x 2.1 mm) packed with protein A-immobilized 20 μm particles from Perseptive Biosystems." (Ex. 1008 (Fang) at 40.) "During purification of HuDREG-55, it was observed that affinity purified antibody…" (*Id*. at 39.) "HuDREG-55 is a humanized IgG4 monoclonal antibody…" (*Id*.) The sample that was injected into the protein A column "was spiked into culture medium," to add impurities. (*Id*. at 41.) Therefore, the HuDREG-55 was

	"purified" by Protein A chromatography which removed these impurities. (Ex. 1002 (Bracewell Dec.) at ¶ 64.)
(a) adsorbing the protein to Protein A immobilized on a solid phase;	*See* Table 2, listing step 2 as "sample loading." (*Id.*) Because the protein A chromatography step was successful in Fang, the target protein must have adsorbed onto the protein A. (*Id.*)
(b) removing contaminants by washing the solid phase with a composition comprising salt and a solvent selected from the group consisting of ethanol, methanol, isopropanol, acetonitrile, hexylene glycol, propylene glycol, and 2,2-thiodiglycol; and	*See* Table 3, listing step 3 is "Column 1 wash" with "30 CV [column volumes] of 60% 1A-40% 2A." (Ex. 1008 (Fang) at 41.) *See* Table 2, listing buffer 1A as "25 mM potassium phosphate, 75 mM potassium chloride, 2.5% isopropanol, pH 7.3" and listing buffer 2A as "25 mM potassium phosphate, 75 mM potassium chloride, 2.5% isopropanol, pH 1.7." (Ex. 1008 (Fang) at 40.)

	Buffers 1A and 2A, both contain salts (potassium phosphate and potassium chloride) and one of the solvents listed in the ’034 patent (isopropanol). They are therefore within the scope of claim 16, as is a 60/40 mixture of the two. (Ex. 1002 (Bracewell) at ¶ 64.)
(c) recovering the protein from the solid phase.	*See* Table 2, listing step 4 as “column 1 elution to column 2.” (Ex. 1008 (Fang) at 41.)

XII. GROUND 7 – CLAIM 16 IS OBVIOUS OVER THE ’526 PATENT AND REIFSNYDER

U.S. Patent 6,127,526, (the “’526 patent”), titled *Protein Purification by Protein A Chromatography*, issued on October 3, 2000. It is therefore prior art to the ’034 patent under 35 U.S.C. § 102(b). The ’526 patent is discussed in the ’034 patent at 2:10–16.

Reifsnyder published in the Journal of Chromatography A in 1996. As Dr. Bracewell explains, the Journal of Chromatography A is commonly read by POSAs. (Ex. 1002 (Bracewell Dec.) at ¶ 66.) Reifsnyder is a printed publication and prior art to the ’034 patent under 35 U.S.C. § 102(b). Reifsnyder was not

before the examiner during prosecution of the ’794 application. (*See* Ex. 1019 (Prosecution History of ’974 application).)

A. Level of Ordinary Skill in the Art

The level of ordinary skill in the art is described above in section IV.

B. Scope and Content of the Prior Art

The scope and content of the prior art includes the discussion above in section V, and the disclosures of all of the references included in Grounds 1–6. The scope and content of the prior art also includes the following discussion.

As explained above, the wash solution used during the intermediate wash step of protein A chromatography often contains high concentrations of salts to wash the non-specifically bound proteins off of the column. (*See, e.g.*, Ex. 1004 (van Sommeren); Ex. 1002 (Bracewell Dec.) at ¶ 42.) In some cases, however, the use of certain types of salts are not desirable for various reasons. For example, some antibodies form chemical interactions with some salts used in buffers. (Ex. 1015 (Gagnon) at 185–86.)

Fang illustrates another situation in which a wash solution with a high salt concentration was undesirable. In Fang, a salt concentration of 200 mM interfered with a later chromatography step while a salt concentration of 100 mM did not. (Ex. 1009 (Fang) at 42–43; Ex. 1002 (Bracewell Dec.) at ¶ 71.) The Fang study included two chromatographic purifications, protein A chromatography and ion

exchange chromatography, done sequentially. Fang described the optimization of each step. With respect to the protein A chromatography, the first experimental condition used a wash buffer comprised 150 mM potassium chloride and 50 mM potassium phosphate, for a total of 200 mM salt. (Fang at 42, Fig. 1.) However, with that concentration of salt, the eluted antibody protein did not bind as was required during the second chromatographic step. (*Id.* at 42–43 and Fig. 5.) The researchers then optimized the method, and the resulting optimized method included a wash buffer with only 25 mM potassium phosphate and 75 mM potassium chloride, for a total of 100 mM salt. (*Id*. at Table 1, Table 2, and page 41.) Both the initial experimental conditions and the optimized conditions also had isopropanol in the wash buffer, with the experimental buffer having a higher concentration of isopropanol. Fang thus illustrates that wash compositions, including the amount of salt and organic solvent used to wash the loosely bound proteins from the column, sometimes must be adjusted for different circumstances. (Ex. 1002 (Bracewell Dec.) at ¶ 71.)

In other cases, high concentrations of salt, used to disrupt electrostatic interactions, are not successful in washing proteins that are bound to glass or silica columns with hydrophobic interactions. Reifsnyder teaches that an ethanol-salt solution successfully washed proteins that are bound to silica column with strong

hydrophobic interactions when even high concentrations of the salt TMAC could not. (*Id*.; Ex. 1018 (Reifsnyder) at 76-77.)

The ’526 patent, which teaches the purification of antibodies via protein A chromatography, illustrates that methods from one type of chromatography may be successfully used in other types of chromatography. In that patent, the wash buffer is a high-concentration salt solution, specifically, high concentration TMAC. Prior to this use in protein A chromatography to wash out the contaminant proteins from the column, TMAC had been used for decades to wash proteins that were bound to silica and glass chromatography columns with electrostatic interactions. (*See, e.g.*, Chadha.) The use of TMAC in protein A chromatography therefore developed as an extension of its old use of breaking electrostatic interactions between proteins and silica or glass columns in other types of adsorption chromatography. (Ex. 1002 (Bracewell Dec.) at ¶ 71.)

C. Differences Between the Claims and the Prior Art and Conclusion of Obviousness

A POSA, faced with a protein A chromatography situation in which, for a reason such as the reasons explained above, high concentrations of the salts normally used for wash solutions do not perform well, would have known about other options for use as the wash buffer, based on other types of adsorption chromatography. (Ex. 1002 (Bracewell Dec.) at ¶ 69.) A POSA would have expected that other solutions that were used to elute proteins bound to silica or

glass chromatography columns during other types of chromatography would also work to elute the proteins that were bound to the silica or glass column in protein A chromatography. (*Id*.) The ’526 patent, disclosing the use of TMAC to elute proteins that are bound as contaminants on a glass or silica protein A column, when TMAC had been known for years to elute proteins from these types of columns during other chromatography, illustrates this point. (Ex. 1002 (Bracewell Dec.) at ¶ 68.)

A POSA who needed a wash solution for protein A chromatography when a high-salt concentration solution did not work well would have been motivated to use the ethanol-salt solution taught in Reifsnyder to wash proteins from silica or glass chromatography columns during protein A chromatography. (Ex. 1002 (Bracewell Dec.) at ¶ 71.) Reifsnyder teaches that the ethanol-salt solution was able to wash proteins that were strongly bound with hydrophobic interactions to a silica column even when high concentration TMAC could not. (Ex. 1018 (Reifsnyder) at 76–77.) In addition to Reifsnyder, Fang confirms that using alcohols, in that case isopropanol, along with salt successfully works as a wash buffer for protein A chromatography. (Ex. 1008 (Fang) at 40.) Therefore, a POSA would have had a reasonable expectation that using a solution of a salt and solvent including at least ethanol (disclosed in Reifsnyder) or isopropanol (disclosed in Fang) would successfully perform as a wash solution in protein A chromatography.

(Ex. 1002 (Bracewell Dec.) at ¶ 70.) The disclosure of (1) ethanol and salt and (2) isopropanol and salt, two members of the claimed Markush group of solvents, renders the claim obvious. *See, e.g., Fresenius USA, Inc.* 582 F.3d at 1380.

In a similar situation, the Federal Circuit affirmed a district court's obviousness determination, finding "there were a finite number of identified, predictable solutions to the problem…and that the [claimed] combination was the product not of innovation but of ordinary skill and common sense." *Wm. Wrigley Jr. Co. v. Cadbury Adams USA LLC*, 683 F.3d 1356, 1365 (Fed. Cir. 2012). In *Wrigley*, a claim to a chewing gum composition recited a combination of elements that were all known in the prior art, "and all that was required to obtain that combination was to substitute one well-known cooling agent for another." (*Id*. at 1364.) Because the claimed cooling agent was "obvious to try" within the chewing gum composition, and the combination resulted in nothing unexpected, the court found that the claim was obvious over the prior art.

Here, there are a finite number of materials that were known and used to wash and elute proteins that are bound to silica or glass chromatography columns during adsorption chromatography. (Ex. 1002 (Bracewell) at ¶ 69.) The claimed composition of "salt and a solvent" selected from the Markush group was an "identified and predictable" composition for this purpose. As evidenced by Reifsnyder, Fang and the '034 patent itself, there is nothing unexpected about the

resulting protein fluid produced by using the claimed wash solution as the intermediate wash buffer. (*Id*.) Claim 16 is therefore obvious over the '526 patent and Reifsnyder. (*Id*. at ¶ 65.)

D. No Secondary Considerations Support the Patentability of Claim 16.

Petitioner is unaware of any secondary considerations that may support the patentability of claim 16. (*Id*. at ¶ 73.)

Petitioner reserves the right to respond to any allegations of secondary considerations brought by Patent Owner in this proceeding.

XIII. CONCLUSION

For the reasons set forth above, Petitioner respectfully submits that it has established a reasonable likelihood of success of showing that the challenged claims are unpatentable, requests that this petition be granted, and requests that the claims be finally found unpatentable as both obvious over the prior art and anticipated by the prior art and cancelled.

Respectfully submitted,

Dated: August 31, 2017

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CERTIFICATE OF WORD COUNT

The undersigned certifies that the attached Petition for *Inter Partes* Review of U.S. Patent 6,870,034 B2 contains 10,973 words (as calculated by the word processing system used to prepare this Petition), excluding the parts of the Petition exempted by 37 C.F.R. §42.24(a)(1).

Dated: August 31, 2017

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CERTIFICATE OF SERVICE

Pursuant to 37 C.F.R. §§ 42.6(e) and 42.105, I certify that on this 31st day of August, 2017, I caused to have served a copy of this PETITION FOR INTER PARTES REVIEW and copies of all supporting materials and exhibits by Federal Express Next Business Day Delivery on the following addresses for Patent Owner:

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