Appeal No. 2018-1993

United States Court of Appeals for the Federal Circuit

AMGEN INC., AMGEN MANUFACTURING LIMITED,

Plaintiffs-Appellants,

– v. –

COHERUS BIOSCIENCES INC.,

Defendant-Appellee.

Appeal from the United States District Court for the District of Delaware in Case No. 1:17-cv-00546-LPS, Chief Judge Leonard P. Stark

NON-CONFIDENTIAL BRIEF FOR PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING, LIMITED

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August 20, 2018

U.S. Patent No. 8,273,707, Claim 1

1. A process for purifying a protein on a hydrophobic interaction chromatography column such that the dynamic capacity of the column is increased for the protein comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, wherein the first and second salts are selected from the group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, respectively, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.

CERTIFICATE OF INTEREST

1. The full name of every party represented by me is:

   AMGEN INC. and AMGEN MANUFACTURING, LIMITED

2. The name of the real party in interest (if the party named in the caption is not the real party in interest) represented by me is:

   AMGEN INC. and AMGEN MANUFACTURING, LIMITED

3. All parent corporations and any publicly held companies that own 10 percent or more of the stock of the party represented by me are:

   AMGEN INC.

4. The names of all law firms and the partners and associates that appeared for the party now represented by me in the trial court or are expected to appear in this Court (and who have not or will not enter an appearance in this case) are

   MORRIS, NICHOLS, ARSHT & TUNNELL LLP: Jack B. Blumenfeld, Maryellen Noreika

5. The title and number of any case known to counsel to be pending in this or any other court or agency that will directly affect or be directly affected by this court's decision in the pending appeal. *See* Fed. Cir. R. 47.4(a)(5) and 47.5(b).

   None

Date: August 20, 2018

/s/ *Nicholas Groombridge*
Nicholas Groombridge

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CONFIDENTIAL MATERIAL OMITTED

Pursuant to Federal Circuit Rule 28(d)(2)(B), Plaintiffs-Appellants prepared this public version of their brief which redacts certain information designated confidential pursuant to the district court's Protective Order entered on December 7, 2017. Specifically, the material omitted on pages ii, 3, 4, 5, 20, 23, 25, 36, 50, 51, and 52 contains references to Defendant-Appellee's accused process, and was designated confidential by Defendant-Appellee during discovery under the terms of the Protective Order.

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STATEMENT OF RELATED CASES

Amgen is aware of one pending district court case that may be directly affected by this Court's decision in this appeal: *Amgen Inc. v. Mylan Inc.*, Case No. 2:17-cv-01235-MRH, which is currently pending before the United States District Court for the Western District of Pennsylvania. Amgen has asserted the patent-at-issue here, U.S. Patent No. 8,273,707, against defendants in the *Mylan* Action. No other related cases are known to counsel for Amgen to be pending in this or any other court that will directly affect or be affected by this Court's decision on appeal.

STATEMENT OF JURISDICTION

The district court has subject-matter jurisdiction over this case under 28 U.S.C. §§ 1331 and 1338(a). This Court has jurisdiction over this appeal under 28 U.S.C § 1295(a). Amgen timely appealed under 28 U.S.C. § 2107 and Fed. R. App. P. 4(a) on May 17, 2018. (Appx46-48.) The appeal is from a final judgment that disposes of all parties' claims.

STATEMENT OF THE ISSUES

1. Whether the district court erred in dismissing Amgen's Complaint, with prejudice, based on prosecution history estoppel where the single file-wrapper statement relied upon by the district court—that the pending claims recite a "particular combination of salts"—is at best ambiguous as to whether it was meant to limit the claims to three salt-pairs or whether it referred to the discovery that the salt combinations useful to obtain the benefits of the invention (*i.e.*, increasing the dynamic capacity of the HIC column) are particularly selected for any specific protein to be purified, and in view of this ambiguity the statement is not a clear and unmistakable disclaimer of claim scope.

2. Whether the district court erred in dismissing Amgen's Complaint, with prejudice, based on the dedication-disclosure doctrine where the specification does not explicitly disclose the subject matter—use of the [redacted] practiced by Coherus—that was found to be dedicated to the public.

STATEMENT OF THE CASE

This appeal arises from a patent infringement lawsuit filed under the Biologics Price Competition and Innovation Act of 2009 ("BPCIA"). The accused product ("Coherus's pegfilgrastim biosimilar") and process are described in Coherus's abbreviated biologics license application ("aBLA") to FDA referencing Amgen's Neulasta® (pegfilgrastim) product. (Appx4-11 at Appx5-6; Appx100-115 at Appx101-102, Appx105-106.) As of this filing, Coherus has not announced publicly, nor has it informed Amgen, that FDA has approved its aBLA.

Amgen filed the Complaint in the U.S. District Court for the District of Delaware on May 10, 2017, asserting that Coherus uses a process for the manufacture of its pegfilgrastim biosimilar, as described in its aBLA, that infringes Amgen's U.S. Patent No. 8,273,707 ("the '707 Patent"). (Appx100; Appx107-113.) Amgen's '707 Patent relates to a process for purifying proteins. Specifically, the Complaint alleges that the [redacted] of [redacted] used in Coherus's purification process is equivalent to the salt pair combinations listed in the claims of the '707 Patent, and Coherus thus infringes the '707 Patent under the doctrine of equivalents. (Appx107-113.) On June 1, 2017, Coherus moved to dismiss the Complaint for failure to state a claim on which relief may be granted. (Appx132.)

On December 7, 2017, the magistrate judge issued a Report and Recommendation that Coherus's motion to dismiss under Rule 12(b)(6) of the Federal Rules of Civil Procedure be granted with prejudice. (Appx12-30.) The district court then adopted that Report and Recommendation and granted Coherus's motion to dismiss finding that (1) Amgen "clearly and unmistakably surrendered claim scope beyond the salt combinations listed in the claims of the '707 patent – i.e., citrate and sulfate, citrate and acetate, and sulfate and acetate" (Appx6); and (2) Amgen "dedicated to the public" the use of the [redacted] being practiced by Coherus. (Appx9-10.) The district court did not address Coherus's other non-infringement arguments regarding salt concentrations. (Appx10.) The district court then entered final judgment against Amgen and dismissed the Complaint. (Appx1-2.) Amgen timely appealed. (Appx46-47.)

STATEMENT OF THE FACTS

A. Amgen's Neulasta® and Coherus's aBLA Referencing That Product

Amgen Inc. discovers, develops, manufactures, and sells innovative therapeutic products based on advances in molecular biology and chemistry. (Appx100.) Amgen Manufacturing, Limited manufactures and sells biologic medicines for treating human diseases. (Appx100-101.) Biologic medicines "constitute therapeutic proteins that are manufactured inside living cells" using recombinant technology. (Appx13.) Pegfilgrastim is an example of a recombinant protein, *i.e.*, a recombinantly expressed 175-amino acid form of a protein ("filgrastim") known as human granulocyte-colony stimulating factor ("G-CSF") that is then conjugated to a 20kD monomethoxypolyethlene glycol (m-PEG) at the N-terminus of this G-CSF protein. (Appx104-105.) Amgen's Neulasta® (pegfilgrastim) stimulates the production of neutrophils, a type of white blood cell. (Appx105.) Neulasta® counteracts neutropenia, a neutrophil deficiency that makes a person highly susceptible to life-threatening infections and is a common side effect of certain chemotherapeutic drugs. (*Id.*)

Amgen's Neulasta® was approved by FDA under the traditional biologics regulatory pathway, 42 U.S.C. § 262(a), which requires that the applicant independently demonstrate that the biologic is "safe, pure, and potent." 42 U.S.C. § 262(a)(2)(C)(i)(I). (Appx101-102, Appx105-106.) In contrast, Coherus, which

develops and seeks to manufacture and sell "biosimilar" products (Appx101)—filed an aBLA under the BPCIA's abbreviated pathway, 42 U.S.C. § 262(k), requesting FDA approval to market a pegfilgrastim product based on biosimilarity, using Amgen's Neulasta® as the reference product. (*Id.*)

B. The '707 Patent

1. The Invention

Before a recombinant protein such as filgrastim can be therapeutically useful, it must first be purified from contaminants. (*See* Appx13.) One method used in the purification process of these proteins is chromatography, which is a method of separating molecules in solution based on their chemical or physical interactions with a solid matrix. (*Id.*; '707 Patent, 1:19-35.) Amgen's '707 Patent—which issued on September 25, 2012 to inventors Anna Senczuk and Ralph Klinke—is directed to a process for protein purification using a particular separation process called hydrophobic interaction chromatography ("HIC"). (Appx13; '707 Patent, 1:36-39.) HIC "is used to separate proteins on the basis of hydrophobic interactions between the hydrophobic moieties of the protein and insoluble, immobilized hydrophobic groups on the matrix." '707 Patent, 1:36-39. For example, a protein solution together with associated impurities is loaded onto a HIC column filled with solid particles called the matrix. *See id.* at 1:40-45; *id.* at 3:53-64. "Usually, a decreasing salt gradient is used to elute proteins from a

column. As the ionic strength decreases, the exposure of the hydrophilic regions of the protein increases and proteins elute from the column in order of increasing hydrophobicity." *Id.* at 1:45-49.

The invention of the '707 Patent addresses a problem with HIC known as "breakthrough" in which significant amounts of protein are washed away with the impurities before elution. (Appx13-14; '707 Patent, 3:37-52.) The '707 Patent provides a process that increases the "dynamic capacity" of the column for the protein being purified. The dynamic capacity of the column is "the maximum amount of protein in solution which can be loaded onto a column without significant breakthrough or leakage of the protein into the solution phase of a column before elution." (*See* Appx14; '707 Patent, 3:67–4:3.)

Before the invention of the '707 Patent, HIC purification relied on high salt concentrations to increase dynamic capacity. (*See* Appx14; '707 Patent, 3:16-30, 3:37-40.) But "high salt can be detrimental to protein stability" because it "increases the viscosity of a solution, results in increased formation of aggregates, results in protein loss due to dilution and filtration of the protein after elution from the column, and can lead to reduced purity." '707 Patent, 3:41-45. The '707 Patent increases dynamic capacity for "a particular protein while reducing the concentration of the salts used, without reducing the quality of the protein separation or raising manufacturing issues," *id.* at 3:47-52:

> The present invention provides combinations of salts useful for increasing the dynamic capacity of an HIC column compared with the dynamic capacity of the column using separate salts alone. These combinations of salts allow for a decreased concentration of at least one of the salts to achieve a greater dynamic capacity, without compromising the quality of the protein separation.

Id. at 2:9-16.

2. The Prosecution of the Parent Application, Which Issued as the '395 Patent

The original application in the chain that resulted in the'707 Patent was filed on July 21, 2004. (Appx611-642.) This application issued as U.S. Patent No. 7,781,395 ("the '395 Patent"). (*See id.*; Appx215.) The '707 Patent issued from a divisional application and shares the same specification as the '395 Patent. As discussed above, the specification describes an invention in which the use of combinations of salt-pairs increased the dynamic capacity of a HIC column compared to the dynamic capacity of the column using single salts alone.

This original application included claims to a process for purifying a protein which did not include a limitation to the use of specific salt pairs. (Appx635-636.) For example, originally-filed claim 1 recited: "A process for purifying a protein comprising mixing a preparation containing the protein with a solution containing a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the column, wherein the first and second salts have different lyotropic values, and wherein at least one salt has a buffering

capacity at a pH at which the protein is stable." (Appx635.) This original application also included dependent claims with limitations to a selection of specified salts. (Appx635-636.)

On December 14, 2006 and in response to the originally-filed claims, the Patent Office issued an Office Action that required restriction, *i.e.*, "an election of species under 35 U.S.C. § 121 and 37 C.F.R. § 1.146 to one first and one second salt selected from the following combinations of salts: citrate and sulfate, citrate and acetate, citrate and phosphate, acetate and sulfate, or sulfate and phosphate." (*See* Appx266-271 at Appx267.)

On April 13, 2007, applicants traversed the Patent Office's restriction requirement but elected "the combination of citrate and phosphate salts to be fully compliant." (*Id.*) Applicants pointed out that its amended claims 1 and 20 are generic claims, and stated that, "Upon the allowance of a generic claim, Applicants will be entitled to consideration of claims to additional species that are written in dependent form or otherwise include all the limitations of an allowed generic claim, as provided by 37 C.F.R.§ 1.146. and MPEP § 809.02(a)." (*Id.*)

Then, following applicants' election of citrate and phosphate, applicants amended the then-pending claims on November 16, 2007 to recite the use of a citrate and phosphate salt pair. (*See* Appx190-195 at Appx192-193.) Applicants expressly stated that this amendment was made "in response to the previously

issued restriction requirement." (Appx194.) However, the then-pending claims did not require that there be an increase in dynamic capacity. (Appx192-193.) For example, then-pending claim 1 recited:

> 1. (currently amended) A process for purifying a protein on a hydrophobic interactive chromatography column comprising mixing a preparation containing the protein with a ~~solution containing~~ combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein ~~column~~, wherein the first and second salts are citrate and phosphate salts, ~~have different lyotropic values, and wherein at least one salt has a buffering capacity at a pH at which the protein is stable,~~ and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0 M.

(Appx192.)

In response to applicants' November 16, 2007 amendment, the Patent Office issued an Office Action on February 14, 2008 rejecting the then-pending claims as anticipated and rendered obvious by a prior art reference, Holtz.[1] (Appx197-203.) The Office Action reasoned that Holtz disclosed particular salts including citrate salts (sodium citrate) and phosphate salts (potassium phosphate) useful in the purification of insulin-like growth factor (IGF-1). (Appx200.) The Patent Office's statements were, of course, directed to the then-pending claims that did not require an increase in dynamic capacity of the HIC column and instead required simply

[1] The Holtz reference is U.S. Patent No. 5,231,178, *available at* http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=1&f=G&l=50&co1=AND&d=PTXT&s1=5,231,178.PN.&OS=PN/5,231,178&RS=PN/5,231,178.

that the claimed process comprise mixing a preparation containing the protein with a "combination of a first salt and a second salt" where "the first and second salts are citrate and phosphate salts." (*See* Appx192-193.)

Applicants responded on July 14, 2008 with amendments and argument addressing the then-pending claims, which still did not require an increase in dynamic capacity. (*See* Appx205-213.) For example, then-pending claim 1 recited:

> 1. (currently amended) A process for purifying a protein on a hydrophobic ~~interactive~~ interaction chromatography column comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, wherein the first and second salts are citrate and phosphate salts, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.

(Appx207.) Specifically, in response to the obviousness rejection, applicants argued that Holtz does not describe the "use of two salts, let alone the particular combination of salts of the claimed method since, as described above, more than two salts are used in the protein solutions for every HIC column described in Holtz."

> Applicants submit first that there are significant differences between what is disclosed in Holtz et al. and the claimed process, as pointed out in detail above. The methods employed in Holtz et al. represents the typical methods used prior to the instant invention, that is, adding a high concentration of ammonium sulfate to a low concentration of a buffer solution to prepare a protein for a HIC column (see the instant application, page 4). Holtz et al. merely describes in detail methods for purifying a single protein IGF-1 so that the protein is intact and correctly folded. Holtz et al. does not describe optimizing the purification process for commercial production of any protein by increasing the dynamic capacity of the HIC column(s) through the novel use of particular combinations of only two salts. Further, there is no suggestion in Holtz et al. to use two salts, let alone the particular combination of salts of the claimed method, since, as described above, more than two salts are used in the protein solutions for every HIC column described in Holtz et al.

(Appx212.)

The Patent Office maintained the rejection of the then-pending claims under 35 U.S.C. §103(a). (Appx969-974.) Applicants continued to press its contention that:

> it is the use of a particular *combination* of salts that confers the advantageous properties described in the instant application. The unexpected and advantageous properties of the particular combination of salts described in the instant claims are clearly shown in the application, . . . which shows that the dynamic capacity of the combined citrate and phosphate salts is much greater than either salt alone. While Holtz, et. al. lists potential salts for use in a HIC column for IGF-1, there is no guidance or suggestion for increasing the dynamic capacity of a HIC column for proteins in general by combining salts, nor by using the specific combination of salts recited in the instant claims.

(Appx972.) It was only later, after this response addressing Holtz, that applicants amended the claims to require that the dynamic capacity of the column be

increased. The amended claims progressed to issuance. Specifically, the '395 Patent claims require:

> 1. A process for purifying a protein on a hydrophobic interaction chromatography column such that the dynamic capacity of the column is increased for that protein comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, wherein the first and second salts are citrate and phosphate salts, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.

('395 Patent, 15:17-26 (highlighting added).)

3. The Prosecution of the '707 Patent Application

The '707 Patent application was filed on June 23, 2010 as a divisional. (*See* Appx32.) The originally-filed claims of the '707 Patent application—like the claims that issued—require that dynamic capacity be increased for the column. (*See* Appx178-184, '707 Patent, 15:8-18.) For example, original claim 1 recited:

> 1. (Original) A process for purifying a protein on a hydrophobic interaction chromatography column such that the dynamic capacity of the column is increased for the protein comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, wherein the first and second salts are selected from the group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.

(Appx179 (highlighting added).)

On October 13, 2010, the Patent Office issued a first Office Action rejecting those pending claims, among other things, for obviousness in view of Holtz. (Appx170-176.) Applicants responded to that Office Action on January 26, 2011. (Appx178-184; Appx186-188.) With respect to Holtz, applicants argued that the pending claims were not obvious in view of Holtz because there is no suggestion in Holtz that "any particular combination of salts would have the result demonstrated in the instant application of increasing dynamic capacity of a HIC." (Appx183.) That is, Holtz does not disclose "any connection at all between dynamic capacity and combination of salts." (*Id.*)

> The court further explained that the analysis of obviousness should focus on whether the combination [of elements] giving rise to the improvement is "more than the *predictable* use of prior art elements according to their established functions." *Id* at 13. Thus, "[a] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *Id* at 14. There must, in addition, be some technical or logical basis for asserting that the *advantages of the combination would have been predictable*. Again, there is no suggestion in Holtz et al. that any particular combinations of salts would have the result demonstrated in the instant application of increasing dynamic capacity of a HIC. There is no mention in Holtz et al. of any connection at all between dynamic capacity and combinations of salts.

(*Id.* (highlighting added).) Applicants also submitted a Declaration of co-inventor Anna Senczuk as evidence of non-obviousness. (*See* Appx183-184, Appx186-188.)

The Senczuk Declaration explained that the invention made by Dr. Senczuk and her co-inventor was that "using certain combinations of salts will greatly improve the dynamic capacity" of a HIC column, and that this "result was not expected in light of any information on HIC available from the scientific literature or other sources at the time of our invention." (Appx186.) Indeed, "[p]reviously, it was not known that salt combinations had anything to do with improving dynamic capacity of a HIC." (*Id.*) Dr. Senczuk further explained that "[i]ncreasing the dynamic capacity of the HIC is very significant in a commercial manufacturing setting" because "this allows more protein to be purified per purification cycle. This greatly improves the efficiency and reduces the cost of manufacturing a therapeutic protein." (Appx186-187.)

Specifically, Dr. Senczuk described her testing and experiments, in which the use of the dual salt combination of sodium sulfate plus sodium citrate was calculated to have a dynamic capacity of 33 g/L-r for the particular IgG2 monoclonal antibody, whereas the single sulfate salt of sodium sulfate was calculated to have a dynamic capacity of only 24 g/L-r for the same IgG2 monoclonal antibody. (Appx187.) The dual salt combination in that instance thus resulted "an increase of 38%" for the dynamic capacity of the column. (*Id.*) The dual salt combination of sodium acetate plus sodium sulfate for the particular IgG2 monoclonal antibody resulted in a 550% increase in dynamic capacity compared to

the single acetate salt of sodium acetate for the same IgG2 monoclonal antibody. (*Id.*)

As the Senczuk Declaration explains, the "increase in dynamic capacity for the HIC resulting from the use of the dual salt combination in the HIC" has the benefit of allowing "for 2 instead of 3 cycles of purification for each bioreactor harvest" in the case of the citrate and acetate dual salt combination versus the single sulfate salt for the particular IgG2 monoclonal antibody. (*Id.*) With respect to the acetate and sulfate dual salt combination versus the single acetate salt for the particular IgG2 monoclonal antibody, the increase in dynamic capacity allowed for "2 instead of 12 cycles for each bioreactor harvest." (*Id.*) Further, the increase in dynamic capacity reduced the processing time from 10 hours to 7 hours, and 32 hours to 10 hours, respectively. (*Id.*)

The Senczuk Declaration further explains that using salt combinations to increase dynamic capacity reduced the estimated cost/kg of the produced product from an estimated $3,961/kg with the single sulfate salt to $2,664/kg with the sulfate/citrate or sulfate/acetate salt combinations; and from an estimated $15,636/kg with the single acetate salt to $3,961/kg with the acetate/citrate salt combination and to $2664/kg with the sulfate/acetate salt combination. (Appx187-188.) Thus, "[u]se of this particular combination of salts greatly improves the cost-effectiveness of commercial manufacturing by reducing the number of cycles

required for each harvest and reducing the processing time for each harvest." (Appx188.)

Following submission of the Senczuk Declaration, the Patent Office maintained its rejection of the pending claims based on Holtz, after which applicants re-submitted the Senczuk Declaration, together with Exhibit A which was inadvertently omitted from the earlier submission. (Appx158-168.) In this response, applicants argued that the Patent Office's rejection of the pending claims as obvious in view of Holtz was incorrect because the claimed method uses "a *combination* of salts in a HIC operation, and the *enhancement of the dynamic capacity of a HIC column* imparted by applicants' method." (Appx160 (emphases in original).) Applicants explained that "Holtz et al. Does Not Disclose a Combination of Salts" and "Holtz et al. Does Not Disclose Enhancing the Dynamic Capacity of a HIC Column," and that the Senczuk Declaration supports the non-obviousness of the claimed invention. The claims were then allowed, and issued. (*See generally* '707 Patent.)

C. Amgen's Complaint

Amgen's Complaint asserts that the "process by which Coherus manufactures the Coherus Pegfilgrastim Product satisfies each limitation of at least claim 1 and dependent claims 2, 3, 4, and 7" of the '707 Patent. (Appx109.) Claim 1 recites:

> A process for purifying a protein on a hydrophobic interaction chromatography column such that the dynamic capacity of the column is increased for the protein comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, wherein the first and second salts are selected from the group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, respectively, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.

'707 Patent, 15:8-18. Amgen's Complaint alleges that, "With respect to the use of dual salts, in the Coherus process, a preparation containing protein is mixed with a combination of a first salt and a second salt, which combination is the equivalent of one or more of the recited salt pairs." (Appx110.) Amgen's Complaint also refers to its detailed statement describing infringement exchanged with Coherus, which stated that the salts used in Coherus's process "will be shown to be insubstantially different from one or more of the claimed salt pairs, as to its ability to increase the dynamic capacity" of the column for G-CSF. (Appx109-110; Appx279-280.)

SUMMARY OF THE ARGUMENT

The district court erred in dismissing Amgen's Complaint on the grounds that argument-based prosecution history estoppel bars Amgen from succeeding on its infringement claim under the doctrine of equivalents; and Amgen dedicated to the public the particular [redacted] used in Coherus's accused process.

First, the district court erred in limiting Amgen's '707 Patent claims at issue to only the recited salt pairs based on a single statement in the '707 Patent prosecution history—that the claims at issue recite a "particular combination of salts"—that is at best ambiguous as to whether the particular recited salt pairs are a point of distinction over the prior art. The district court correctly recognized that the discovery of the '707 Patent is a way to increase the "dynamic capacity" of a hydrophobic interaction chromatography ("HIC") column by using a combination of two salts, which is an advance over the prior art's disclosures of the use of a high concentration of one salt. (Appx7-9.) But, the district court then ignored that discovery in reading Amgen's statements from the prosecution. Each of the two passages from the '707 Patent prosecution history relied on by the district court that have this statement show that the point of novelty over the Holtz prior art is Holtz's failure to disclose a purification process that uses a combination of salts to increase dynamic capacity. Amgen did not limit its claims to only the recited salt pair combinations by distinguishing Holtz during prosecution. Indeed, the '707

Patent claims were allowed after applicants submitted a declaration from one of the co-inventors, Dr. Senczuk, and not because applicants disclaimed all salts other than the "particular" salts recited in the claims. As discussed below, none of the arguments that applicants made in their last submission prior to allowance—which presumably must be the focus of any argument-based estoppel analysis—addressed let alone limited the invention to the "particular" salts recited in the claims.

The district court found that Amgen had limited its claims to the particular recited salt pair combinations based on a passage that says first that "the pending claims recite a particular *combination* of salts." (Appx182 (emphasis in original); Appx8.) The emphasis in Amgen's statement there is on the combination of salts, which is important because Holtz uses the prior art approach to HIC chromatography—the use of a high concentration of a ***single*** salt—and thus does not disclose any combination of salts to achieve an increase in dynamic capacity of the column. Amgen's next statement is that "No combinations of salts is taught nor suggested in the Holtz et al. patent, nor is the *particular* combinations of salts recited in the pending claims taught nor suggested in this reference." (Appx182.) The district court seized on the latter clause beginning with "nor" to hold that Amgen had made a clear and unmistakable surrender of all salts except for the "particular" combinations recited in the '707 Patent claims. (*Id.*) This is error. That clause simply observes (correctly) as a factual matter that Holtz does not

disclose the particular combinations recited in the claims. But it is clear from the first part of that sentence, the remainder of the paragraph, and indeed the prosecution history as a whole, that the reason Holtz is different is because it does not disclose nor suggest any combination of salts in order to increase the dynamic capacity of the HIC column.

Further, the district court erred in relying on Amgen's statements in the parent application prosecution because the pending claims in the parent application are materially different from the '707 Patent claims. (Appx7.) Specifically, the parent application claims—that were pending when Amgen made the statements relied on by the district court to find estoppel—did not require that the claimed salt pair combination increase dynamic capacity. Nor did the parent application's pending claims require the particular salt pairs recited in the '707 Patent. Rather, the patent application claims are directed to a specific salt combination that is not claimed in the '707 Patent claims. This is because the Patent Office required restriction to just one salt pair, applicants selected citrate and phosphate as that salt pair, and applicants expressly reserved their right to seek "consideration of claims to additional species." (Appx266-271 at Appx267.) Therefore, any statements by applicants limiting the claims of the parent application to citrate and phosphate cannot reasonably be read as disclaiming coverage of the other salt pairs in the

'707 Patent application (citrate and sulfate, citrate and acetate, and sulfate and acetate). The district court erred in holding otherwise.

Second, the district court erred in determining that the dedication-disclosure doctrine prevents Amgen from asserting infringement against Coherus's process under the doctrine of equivalents. (Appx9-10.) The district court relied on a single passage of the '707 Patent specification that lists a series of anions, but does not identify particular salts, let alone the [redacted] of [redacted] used in Coherus's process. Thus, there is no disclosure in the '707 Patent of the particular [redacted] used in Coherus's process, and the district court erred in finding that such subject matter is dedicated to the public.

Accordingly, Amgen respectfully requests that that this Court reverse, vacate, and/or remand the district court judgment dismissing Amgen's Complaint.

ARGUMENT

This Court reviews a district court decision to dismiss a complaint for failure to state a claim under Fed. R. Civ. P. 12(b)(6) under the law of the regional circuit. *McZeal v. Sprint Nextel Corp.*, 501 F.3d 1354, 1355–56 (Fed. Cir. 2007). The Third Circuit reviews challenges to a dismissal for failure to state a claim *de novo*. *Sands v. McCormick*, 502 F.3d 263, 267 (3d Cir. 2007). When evaluating a dismissal for failure to state a claim, the Third Circuit "accept[s] all factual allegations as true, construe[s] the complaint in the light most favorable to the plaintiff, and determine[s] whether, under any reasonable reading of the complaint, the plaintiff may be entitled to relief." *Id.* at 267–68. (quoting *Pinker v. Roche Holdings Ltd.*, 292 F.3d 361, 374 n.7 (3d Cir. 2002)). The Court "need not credit a plaintiff's 'bald assertions' or 'legal conclusions' when deciding a motion to dismiss." *Id.* at 268. (quoting *Morse v. Lower Merion Sch. Dist.*, 132 F.3d 902, 906 (3d Cir. 1997)).

Here, Amgen's Complaint asserts infringement of the '707 Patent because Coherus's manufacturing process "satisfies each limitation of at least claim 1 and also dependent claims 2, 3, 4, and 7." (Appx109-110.) With respect to the claim limitation that "the first and second salts are selected from a group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, respectively," Amgen's Complaint alleges: "With respect to the use of dual salts, in the Coherus

process, a preparation containing protein is mixed with a combination of a first salt and a second salt, which combination is the equivalent of one or more of the recited salt pairs." (Appx109.) The district court rejected these allegations, and dismissed Amgen's Complaint because: (1) "argument-based prosecution history estoppel prevented Amgen from being able to prevail on this theory of infringement under the doctrine of equivalents" (Appx6); and (2) Amgen "dedicated to the public the [redacted] being practiced by Coherus" (Appx9-10). Each holding is incorrect as a matter of law.

I. The District Court Erred in Determining That Argument-Based Prosecution History Estoppel Bars Amgen From Succeeding on its Claim of Infringement Under the Doctrine of Equivalents

"Prosecution history estoppel applies as part of an infringement analysis to prevent a patentee from using the doctrine of equivalents to recapture subject matter surrendered from the literal scope of a claim during prosecution." *Trading Techs. Int'l, Inc. v. Open E Cry, LLC*, 728 F.3d 1309, 1322 (Fed. Cir. 2013). The Court reviews the application of prosecution history estoppel *de novo*. *Id.* at 1318. While prosecution history estoppel is a question of law, it "has traditionally been viewed as equitable in nature, its application being 'guided by equitable and public policy principles.'" *Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 344 F.3d 1359, 1367 (Fed. Cir. 2003). The form of prosecution history estoppel at issue here is argument-based prosecution history estoppel. (Appx9.) Argument-

based prosecution history estoppel applies only where the prosecution history "evince[s] a clear and unmistakable surrender of subject matter." *Intendis GMBH v. Glenmark Pharm. Inc., USA*, 822 F.3d 1355, 1365 (Fed. Cir. 2016). In determining whether there has been a clear and unmistakable surrender of subject matter, the prosecution history must be examined as a whole. *See Pharmacia & Upjohn Co. v. Mylan Pharms., Inc.*, 170 F.3d 1373, 1376 (Fed. Cir. 1999). An objective standard is applied when looking at the prosecution history, the proper inquiry being "whether a competitor would reasonably believe that the applicant had surrendered the relevant subject matter." *Cybor Corp. v. FAS Techs., Inc.*, 138 F.3d 1448, 1457 (Fed. Cir. 1998).

As discussed below, Amgen's statements in the '707 Patent prosecution history do not evince a clear and unmistakable surrender of subject matter. Further, Amgen's statements made during the prosecution of the parent application—on which the district court relied—do not apply to the '707 Patent because the pending claims of the parent application that are the subject of Amgen's statements are materially different from the '707 Patent claims. The district court erred in ignoring these differences and finding estoppel based on Amgen's statements in the parent application.

A. Amgen's Statements in the '707 Patent Prosecution Are Not a Clear and Unmistakable Surrender

1. The Invention of the '707 Patent is Increasing the Dynamic Capacity of the HIC Column for a Particular Protein

Arguments made by a patentee during prosecution are not analyzed in isolation and "must be viewed in context." *Read Corp. v. Portec, Inc.*, 970 F.2d 816, 824 (Fed. Cir. 1992); *see also Ecolab, Inc. v. FMC Corp.*, 569 F.3d 1335, 1342 (Fed. Cir. 2009), *amended on reh'g on other grounds,* 366 F. App'x 154 (Fed. Cir. 2009). Relevant to the context here is the discovery of the '707 Patent that certain salt pair combinations together "increase the dynamic capacity of the HIC column for a particular protein" more than using a single salt alone at the high concentrations reported in the prior art. (*See*'707 Patent, 4:52-60; *see also* '707 Patent, 5:25-28; *id.* at 2:9-15; *id.* at 4:33-42; *id.* at 15:8–16:26.) By increasing the dynamic capacity of a HIC column—the maximum protein "load at which no significant [protein] breakthrough occurs" in the column ('707 Patent, 4:14-16)—and using a lower salt concentration than in the prior art, the invention improves the efficiency of the HIC purification process. *See id.* at 1:54-62. This then decreases the cost and time required to purify a batch of protein, which is particularly useful in commercial production and purification of proteins, especially therapeutic proteins. *See id.* at 10:4-24; *id.* at 11:36-46.

The specification repeatedly discloses that the selection of the salt pair combinations that increase dynamic capacity is specific to each individual protein, and the salt pair combinations are selected for each such protein.

- "The first and second salt combinations are ***selected for each particular protein*** through a process of establishing precipitation curves for each salt individually, and precipitation curves for the combination of salts holding one salt constant and varying the second." *Id*. at 2:16-20 (emphasis added).

- "The two salt buffers of the present invention result in an increase in dynamic capacity of an HIC column ***for a particular protein*** compared with the dynamic capacity achieved by single salts." *Id.* at 2:39-42 (emphasis added).

- "It is an objective of the present invention to produce conditions for ***particular proteins*** which maximize the amount of protein which can be loaded and retained by an HIC column with little or no reduction in the quality of separation of the protein." *Id.* at 4:52-56 (emphasis added).

- "It is now understood that several factors influence the hydrophobic interactions which control the retention of a native protein to the hydrophobic groups attached to the matrix. These include van der Waals forces, or electrostatic interactions between induced or permanent dipoles; hydrogen bonding, or electrostatic interactions between acidic donor and basic acceptor groups; ***the hydrophobicity of the protein itself; and the influence of various salts on hydrophobic interactions.***" *Id.* at 4:61–5:2 (emphasis added).

- "According to the present invention a first salt and a second salt are selected which have differing lyotropic values. This combination of salts acts together to increase the dynamic capacity of the HIC column ***for a particular protein***." *Id.* at 5:25-28 (emphasis added).

The specification examples confirm this. For instance, Example 1 describes various combinations of salt solutions "for their ability to increase the dynamic capacity of an HIC column used for purifying" a particular protein: an antibody against epidermal growth factor receptor (EGFR). *Id.* at 11:53-56. For this particular protein (antibody), Table 1 identifies the increase in dynamic capacity of the salt pair combinations recited in the claims: "citrate and sulfate, citrate and acetate, and sulfate and acetate."

Table 1 shows that the combinations of citrate/sulfate, acetate/citrate, phosphate/citrate, acetate/sulfate, citrate/acetate, sulfate/acetate, sulfate/citrate, and citrate/phosphate increased the dynamic capacity of the HIC column for the antibody by factors varying from approximately 1.5 to 2 times or more that of each salt alone. The phosphate/sulfate

TABLE 1

Dynamic capacities of antibody against EGFR with four salts and their combinations. Only anions are listed; the cations were sodium for every salt

Experimental Conditions	Dynamic Capacity (mg/ml-r)
0.55M Citrate	24
0.5M Phosphate	12
0.8M Sulfate	24
1.2 M Acetate	5
0.55M Citrate/0.3M Sulfate	30
0.6M Acetate/0.5M Citrate	29
0.35M Phosphate/0.6M Citrate	39
0.6M Acetate/0.7M Sulfate	27
0.5M Citrate/1M Acetate	34
0.5M Sulfate/1M Acetate	33
0.4M Phosphate/0.3M Sulfate	15
0.5M Sulfate/0.3M Citrate	33
0.5M Sulfate/0.3M Phosphate	17
0.3M Citrate/0.6M Phosphate	35

Id. at 13:40-64 (highlighting added).

2. Amgen's Statements in the '707 Patent Prosecution Distinguish Holtz as Failing to Disclose the Use of Salt Combinations to Increase the Dynamic Capacity

The relevant Amgen statements from the prosecution of the '707 Patent application were made in response to Office Actions rejecting the pending claims as obvious over Holtz. Holtz discloses a traditional prior-art method of using a high concentration single salt to purify a particular protein (IGF-1). (Appx160-161.) Amgen responded to the Patent Office's obviousness rejections over Holtz by arguing that the point of novelty for the '707 Patent claims was the use of a combination of salts that "increase the dynamic capacity" of the HIC columns for the protein. (*See, e.g.*, Appx182-183.) That combination of salts is specific to the protein because, as the specification describes, not all combinations of salt pairs for the particular protein being tested (an EGFR antibody) increase dynamic capacity. '707 Patent, 13:64–14:5. For example, the specification discloses that the "phosphate/sulfate combination did not increase the dynamic capacity" relative to that of sulfate alone. *Id.* The specification explains that "sulfate in combination with phosphate resulted in a precipitate, so that lower concentrations of sulfate were required to prevent precipitation. These low concentrations proved too low to improve dynamic capacity." *Id.*

Amgen's statements regarding Holtz in the '707 Patent prosecution thus properly distinguish the single salt used in the Holtz process as not increasing

dynamic capacity, as opposed to the salt pair combinations recited in the claims each of which increases dynamic capacity for the EGFR antibody. The district court erred in ignoring the requirement to increase dynamic capacity, and reading Amgen's prosecution history statements as limiting the claimed inventions to the specific salt pairs recited in the claims.

Neither of the two '707 Patent prosecution passages relied on by the district court evinces a clear and unmistakable surrender of combinations of salts other than the ones recited in the '707 Patent claims. (*See* Appx6-9.) The district court relied on two sentences from Amgen's January 26, 2011 response to the Patent Office's October 13, 2010 non-final rejection of the pending claims as obvious over Holtz prior art. (Appx8.)[2] According to the district court, Amgen in this response "distinguished its application by (1) the use of dual salts and (2) 'the particular combination of salts' recited in the application that became the '707 patent." (*Id.*) This ignores the invention of the '707 Patent and the context for those statements.

In contrast to Holtz which uses a traditional prior-art method of using a high concentration single salt to purify a particular protein (IGF-1) (Appx160-161), the

[2] In the October 13, 2010 Office Action, the examiner mistakenly identified "citrate and phosphate salts" as claimed in the '707 Patent (the same salts claimed in the parent patent), but the claims the '707 Patent as filed recited the same salts as the claims that ultimately issued in the '707 Patent (citrate and sulfate, citrate and acetate, and sulfate and acetate). (Appx174; *see* Appx179-180.)

point of novelty for the '707 Patent claims was to use a combination of salts that "increase the dynamic capacity" of the HIC columns for the protein being purified. (*See* '707 Patent, 4:52-60; *see also* '707 Patent, 5:25-28; *id.* at 2:9-15; *id.* at 4:33-42; *id.* at 15:8–16:26.) Amgen thus distinguished its pending claims from Holtz under *KSR Int'l. Co. v. Teleflex Inc.*, 550 U.S. 398 (2007) on the ground that there is no "suggestion in Holtz et al. that any particular combinations of salts would have the result demonstrated in the instant application of increasing dynamic capacity of a HIC;" indeed, there is no mention in Holtz of "any connection at all between dynamic capacity and combinations of salts." (Appx183.)

> The court further explained that the analysis of obviousness should focus on whether the combination [of elements] giving rise to the improvement is "more than the *predictable* use of prior art elements according to their established functions." *Id* at 13. Thus, "[a] patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art." *Id* at 14. There must, in addition, be some technical or logical basis for asserting that the *advantages of the combination would have been predictable*. Again, there is no suggestion in Holtz et al. that any particular combinations of salts would have the result demonstrated in the instant application of increasing dynamic capacity of a HIC. There is no mention in Holtz et al. of any connection at all between dynamic capacity and combinations of salts.

(*Id.* (highlighting added).) In other words, the critical difference between Holtz and the '707 Patent claims is Holtz's failure to describe a process that increases the

dynamic capacity of the HIC column for the protein. And, Amgen was not clearly and unmistakably distinguishing Holtz based on the recited salt pairs in the claims.

The district court incorrectly read two of Amgen's statements in the preceding page of the response as limiting the invention to only those salt pairs recited in the claims. (Appx8.) The first sentence below says that the pending claims "recite a particular ***combination*** of salts," with the emphasis on "combination." The next sentence then makes clear that Holtz is different because it does not teach or suggest the particular combinations of salts recited in the pending claims, with the emphasis on "particular:"

> Applicants point out that the pending claims recite a particular *combination* of salts. No combinations of salts is taught nor suggested in the Holtz et al. patent, nor is the *particular* combinations of salts recited in the pending claims taught nor suggested in this reference. Applicants point out that the patent to Holtz et al. is directed to "a

(Appx182.) The district court based its decision on prosecution history estoppel on this sentence, holding that applicants had distinguished Holtz not only on the basis that it failed to disclose the use of a combination of salts, "but also for the independent reason that the invention recited the use of ***particular*** combinations of salts." (Appx9, emphasis in original.) In so holding the district misunderstood the sentence in question and disregarded the rest of the paragraph in which that sentence appears, which makes clear that Holtz does not disclose any combinations of salts at all. As the paragraph explains later in the blue-highlighted text, this is

important because the claimed subject matter is "directed to the use of combinations of salts that ***increase the dynamic capacity*** of the hydrophobic interaction chromatography columns."

> Applicants submit that a *prima facie* case of obviousness has not been made. Applicants point out that the pending claims recite a particular *combination* of salts. No combinations of salts is taught nor suggested in the Holtz et al. patent, nor is the *particular* combinations of salts recited in the pending claims taught nor suggested in this reference. Applicants point out that the patent to Holtz et al. is directed to "a method for recovery and purification of intact, correctly-folded, monomeric insulin-like growth factor-1 peptide" (Abstract of the patent), that is, this patent is directed to optimizing a purification scheme for a particular protein. The claimed subject matter is directed to use of combinations of salts that *increase the dynamic capacity* of the hydrophobic interaction chromatography columns. There is no description or suggestion in Holtz et al. for the use of any combination of salts to increase the dynamic capacity of a HIC. Applicants point out that optimizing a purification scheme for a particular protein is not the same as increasing dynamic capacity of HIC.

(Appx182 (highlighting added).)

As described above, the specification is clear that a particular combination of salts that will increase dynamic capacity must be determined for each individual protein. (*See*'707 Patent, 4:52-60; *see also* '707 Patent, 5:25-28; *id.* at 2:9-15; *id.* at 4:33-42; *id.* at 15:8–16:26.) Nowhere did the applicants say that combinations other than citrate/sulfate, citrate/acetate, and sulfate/acetate will not work, and no reasonable competitor could read the prosecution history in that way. On the contrary, the reference to "the *particular* combinations of salts recited in the

pending claims" means particular combinations that will work to increase dynamic capacity, a property that was recited in the claims at the time of this exchange. By seizing on this language in isolation to hold that Amgen had made a clear and unmistakable surrender of all salt pairs except for the "particular" combinations recited in the '707 Patent claims, the district court committed error. That clause simply observes (correctly) as a factual matter that Holtz does not disclose the particular combinations recited in the claims—because Holtz does not disclose using combinations of salts in the first instance. But it is clear from the first part of that sentence and the rest of the paragraph that Holtz is different than the pending '707 Patent claims because Holtz does not disclose the use of any combination of salts that increase the dynamic capacity of the HIC column whatsoever.

Thus, the clause "nor is the *particular* combinations of salts recited in the pending claims taught nor suggested in this reference" (Appx182) is not a clear and unmistakable surrender of all salt pair combinations that increase dynamic capacity of the column for the protein being purified, other than the recited salt pair combinations. Indeed, in the very next Office Action, the Patent Office read Amgen's words "particular combination of salts" to refer to Holtz's failure to disclose "salt***s***" (which is consistent with Amgen's position in this case) rather than reading those words to refer to Holtz's failure to disclose the ***particular*** salt combinations recited in the claims (as the district court held):

> Applicant's arguments were fully considered but were not found persuasive. Applicant contends that the instant claims recite a particular combination of salts. However, the examiner contends that the cited reference does disclose salts used in a method of purification and that the adjustment of particular conventional working conditions (if not expressly taught) is deemed merely a matter of judicious selection and routine optimization which is well within the purview of the skilled artisan.

(Appx949 (highlighting added).)

In addition, the district court appears to have assumed that Amgen intended to argue that the accused process's use of [redacted] is an equivalent to a claimed limitation. This is incorrect. Amgen alleged in its Complaint—which is taken as true here—that the [redacted] used in Coherus's accused process [redacted] is equivalent to the claimed salt combinations. (Appx109-110.) Specifically, the district court stated in its decision that "in an October 2010 Office Action, the Patent Office once more rejected the '707 patent claims as obvious over Holtz, again listing the various salts disclosed by Holtz, including [redacted]," and that applicants responded by distinguishing Holtz based on "(1) the use of dual salts and (2) 'the particular combinations of salts' recited in the application that became the '707 patent." (Appx8.) To be clear, Amgen is not asserting that the use of [redacted] in the accused process meets a claimed limitation; it is the use of the [redacted] of [redacted] and [redacted] to increase dynamic capacity that meets the claims under the doctrine of equivalents.

For the same reasons, the district court erred in determining that Amgen's statements in its August 22, 2011 response to the Patent Office's April 7, 2011 Final Rejection of the pending claims as obvious over Holtz are a clear and unmistakable surrender of all salt pairs other than the particular recited combinations. (*See id.*) As an initial matter, applicants' August 22, 2011 response again makes clear that the claimed invention involved a determination of "what combinations of salts would increase the dynamic capacity for the proteins on the HIC column." (Appx162.) This is consistent with the specification's disclosure that the particular salt combinations are specific to each protein. And it supports an interpretation of the patent claims that extends to processes that increase dynamic capacity for the HIC column as to each protein even if the salt pair combinations used in the process are not the ones recited in the claims.

No reasonable competitor could conclude from the prosecution of the '707 Patent that the '707 Patent is limited to the recited salt pairs in those issued claims (which are different than the citrate and phosphate salt pair recited in the parent application claims that issued as the '395 Patent). As discussed above in the Statement of Facts, the '707 Patent claims were allowed after applicants submitted a response distinguishing Holtz as failing to disclose "combinations" of salts and enhancement of dynamic capacity. (Appx159-163.) The January 26, 2011 response also resubmitted the Senczuk Declaration, and together that response and

the Senczuk Declaration make clear that the claimed invention is the connection between the combination of salt pairs and the enhancement of dynamic capacity. (Appx183-184; Appx186-188.) As the Senczuk Declaration avers, the benefit of using dual salts in HIC is to increase dynamic capacity which then greatly improves the cost-effectiveness of commercial manufacturing by reducing the number of cycles required for each harvest and reducing the processing time for each harvest. (Appx186-188.) Simply using dual salt combinations without a resulting increase in dynamic capacity does not achieve these benefits, which is why the '707 Patent claims so require an increase. (*See id.*)

Specifically, in the August 22, 2011 response, applicants made clear that Holtz fails to disclose "two elements of the claimed method—the use of a *combination* of salts in a HIC operation, and the *enhancement of the dynamic capacity of a HIC column* imparted by applicant's method." (Appx160-161.) With respect to the second element, applicants' statements distinguishing Holtz for failure to disclose increasing the dynamic capacity of HIC column do not surrender claim scope for salts other than the particular recited salt combinations in the claim for the reasons discussed above.

With respect to the first element, applicants explained that Holtz "simply does not disclose, suggest, or contemplate any steps involving a combination of two salts for any purpose whatsoever" and that Holtz describes "a single salt

system." (Appx160-161.) This statement simply means that applicants' claims must have a combination of salts, which of course the claims require as one of its limitations. It is not a clear and unmistakable surrender of claim scope such that the claims are limited to the particular combinations of salts recited in the claims.

Further, the district court incorrectly interpreted applicants' statement that "merely adding a second salt to the traditional HIC process, as the Patent Office appears to suggest, will not produce applicants' claimed method." (Appx162; Appx8). According to the district court, this statement meant that applicants were "again telling the Patent Office that merely using two salts, while different from Holtz, was not within the scope of its claims." (Appx8.) It is correct that merely adding a salt to the traditional HIC process will not necessarily produce applicants' claimed method—and is therefore not necessarily within the scope of the claims—because the claims require an increase in dynamic capacity (as Amgen's Complaint alleges), and not just the use of a combination of salts. The district court erred in ignoring the dynamic capacity requirement to interpret this statement as a clear and unmistakable disclaimer of claim scope.

The Senczuk Declaration is also not a clear and unmistakable disavowal of salts not recited in the claims. As the district court correctly determined, the Senczuk Declaration merely discussed specific "experiments testing single salts and combinations of salts (dual salts) described in the ['707 Patent]" and showing

the benefits of increased dynamic capacity. (Appx186; *see* Appx9.) The Senczuk Declaration thus only demonstrates "[t]he benefits that result from the use of dual salts in the HIC column." (Appx187.) Therefore, the Senczuk Declaration does not clearly and unmistakably surrender of claim scope as the district court correctly found. (Appx9.)

If anything, applicants' August 22, 2011 response resubmitting the Senczuk Declaration confirms that Amgen did not surrender other salt pairs than the ones recited in the claims. The claims were allowed following that response resubmitting the Senczuk Declaration. (*See* Appx158-168; Appx940-942.) In that response, applicants argued that Holtz does not render the pending claims obvious because Holtz's method does not disclose (1) a "combination" of salts and (2) "the idea of enhancing the dynamic capacity of the HIC column." (Appx158-168.) Thus, none of the arguments that applicants made in their last submission prior to allowance—which presumably must be the focus of any argument-based estoppel analysis—addressed let alone limited the invention to the "particular" salts recited in the claims.

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In sum, only one of the portions relied upon by the district court from the '707 Patent prosecution says that Holtz does not teach or suggest the "particular" combinations of salts recited in the pending claims. Read in context, however, that

statement was made to overcome the obviousness rejection by distinguishing Holtz's use of a single salt system from the pending claims' use of salt pairs that increase dynamic capacity of the HIC column. Indeed, the Examiner understood Amgen's statements in that passage to refer to Holtz's failure to disclose a process using a combination of "salts" rather than to refer to Holtz's failure to disclose "particular" salts. The district court erred in holding otherwise. An "equivocal" assertion by the applicant during prosecution will not give rise to estoppel. *Athletic Alts., Inc. v. Prince Mfg., Inc.*, 73 F.3d 1573, 1582 (Fed. Cir. 1996). Further, where, as here, Amgen did not amend its claims and was simply making arguments to the Examiner, equity is not served by barring Amgen from asserting infringement under the doctrine of equivalents. Arguments to the examiner "do not always evidence the same clear disavowal of scope that a formal amendment to the claim would have." *Conoco, Inc. v. Energy Envtl., L.C.*, 460 F.3d 1349, 1364 (Fed. Cir. 2006).

The consequence of the district court's decision is that Amgen's claims are limited to the particular proteins known to have increased dynamic capacity in a HIC column when mixed with the recited salt pairs. *See, e.g.*, Example 1 (showing that not all combinations of salt pairs increase dynamic capacity for the particular protein being tested (an EGFR antibody)). But, the claims are not so limited. The claims on their face cover a process of purification for proteins beyond the ones

described in Example 1. For example, Claim 1 simply recites "a protein." Indeed, the specification expressly discloses that the claimed method "is directed to all types of proteins" and is "particularly suitable for purifying protein-based drugs, also known as biologics." '707 Patent, 7:55-58, 15:27-28. Consistent with this breadth, the claims also encompass the full range of equivalents to the salt combinations recited in the claims that are mixed with the claimed proteins to increase dynamic capacity of the HIC column. So long as the accused salt combinations perform substantially same function in substantially the same way to achieve substantially the same result in the accused process as in the claimed process, there is infringement under the doctrine of equivalents as Amgen's Complaint alleges. *See Warner-Jenkinson Co., Inc. v. Hilton Davis Chem. Co.*, 520 U.S. 17, 40 (1997); *Mylan Institutional LLC v. Aurobindo Pharma Ltd.*, 857 F.3d 858, 866–67 (Fed. Cir. 2017).

Finally, to the extent that the prosecution history requires interpretation, or the claims require construction in resolving this issue, it was error for the district court to dismiss Amgen's Complaint without considering evidence from one of ordinary skill in the art. *See, e.g., Mass. Inst. of Tech. v. Shire Pharms., Inc.*, 839 F.3d 1111, 1119–22 (Fed. Cir. 2016) (looking to how a skilled artisan would read statements made during prosecution). Indeed, this Court has cautioned that the question of infringement under the doctrine of equivalents "rarely come[s] clear on

a premature record." *Mylan Institutional LLC*, 857 F.3d at 866 (quoting *Jeneric/Pentron, Inc. v. Dillon Co.*, 205 F.3d 1377, 1384 (Fed. Cir. 2000)).

B. Amgen's Statements in the Parent Application About Different Pending Claims Do Not Create Estoppel That Limits the Scope of Equivalents for the '707 Patent Claims

The functional requirement in the '707 Patent claims that the dynamic capacity of the column is increased for a protein by mixing the protein with a salt pair combination is a point of novelty for the invention, as discussed above. Amgen thus alleges that the claims can be met equivalently by showing an increase in dynamic capacity of the column for the protein, even if the salt pairs used in the accused process are not literally the ones recited in the '707 Patent as Amgen alleged in its Complaint.

Unlike the '707 Patent claims and as illustrated in the chart below, the pending claims of the parent application as of July 14, 2008 did not require an increase in dynamic capacity of the column for the protein; Amgen did not amend the claims to include such a limitation until November 11, 2009, after which the Examiner allowed the claims and the claims then issued. (Appx207; '395 Patent, 15:17-26.)

'707 Patent, Claim 1 (emphases added)	**Parent Application, Pending Claim 1 as of July 14, 2008 (emphasis added)**
A process for purifying a protein on a hydrophobic interaction chromatography column ***such that the dynamic capacity of the column is increased for the protein comprising***	A process for purifying a protein on a hydrophobic interaction chromatography column comprising
mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein,	mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein,
wherein the first and second salts are selected from the group consisting of ***citrate and sulfate, citrate and acetate, and sulfate and acetate***, and	wherein the first and second salts are ***citrate and phosphate salts***, and
wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.	wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.

The fact that Amgen emphasized a particular combination of salts in the parent application is not surprising given that the pending claims had been limited to one combination only—citrate plus phosphate—because of the Patent Office's restriction requirement. And the claims as then pending lacked any functional limitation that this combination increases the dynamic capacity of the HIC column for the protein. Thus, Amgen's statements in the parent application cannot be used

to prove that Amgen made a clear and unmistakable surrender of equivalents in the materially different '707 Patent claims.

The district court erred as a matter of law in ignoring the differences in the claims of the pending parent application on July 14, 2008 and the '707 Patent claims in finding that argument-based estoppel applied to bar doctrine of equivalents for the '707 Patent claims. This legal error was made clear by the district court's statement in the footnote:

> The parent patent's claim 1 recited a combination "wherein the first and second salts are citrate and phosphate salts," while the larger group of three combinations of salts – "citrate and sulfate, citrate and acetate, and sulfate and acetate" – is claimed in the '707 Patent as issued.

(Appx7 (citing Appx207).) While "[p]rosecution history estoppel can extend from a parent application to subsequent patents in the same lineage," "arguments made in a related application do not automatically apply to different claims in a separate application." *Trading Techs.*, 728 F.3d at 1323 (citations omitted). Further, even if the "prosecution history regarding a particular limitation in one patent is presumed to inform the later use of that same limitation in related patents," the intrinsic record may compel a different result. *Id.*

That is the case here, where differences in the claims render any parent prosecution statements inapplicable to the '707 Patent. *See id.*; *Invitrogen Corp. v. Clontech Labs., Inc.*, 429 F.3d 1052, 1078 (Fed. Cir. 2005) ("[T]he prosecution of

one claim term in a parent application will generally not limit different claim language in a continuation application."); *Biogen, Inc. v. Berlex Labs, Inc.*, 318 F.3d 1132, 1141 (Fed. Cir. 2003) ("When the applicant is seeking different claims in a divisional application, estoppel generally does not arise from the prosecution of the parent."). As the district court itself recognized, the parent application claims recite different salt pairs than the '707 Patent claims. (Appx7.)

Even if Amgen's statements during the prosecution of the parent patent to distinguish Holtz surrendered the use of salt pairs other than the recited ones in the pending claims, the surrender would extend only to salts other than citrate and phosphate claimed in the parent. There is no clear and unmistakable surrender of the use of salt combinations other than the different salt combinations recited in the '707 Patent claims. Indeed, Amgen could not have limited the pending parent application claims in July 2008 to the salts recited in the '707 Patent claims (citrate and sulfate, citrate and acetate, and sulfate and acetate) because the claims pending in the parent application in July 2008 did not recite those salts. (Appx207.) Rather, the parent application claims are limited to one salt pair: citrate and phosphate.

The reason that the parent application claims are limited to only the citrate and phosphate salt pair is because of the Patent Office's restriction requirement in

the parent application prosecution that one salt pair be selected for the claims. (*See* Appx266-271.)

Response to Restriction Requirement

The Examiner has required an election of species under 35 U.S.C. § 121 and 37 C.F.R. § 1.146 to one first and one second salt selected from the following combinations of salts: citrate and sulfate, citrate and acetate, citrate and phosphate, acetate and sulfate, or sulfate and phosphate. Applicants traverse this restriction and point out that it would not create an undue burden on the Examiner to search several combinations of salts, such as citrate in combination with sulfate, acetate, and phosphate. However Applicants provisionally elect the combination of citrate and phosphate salts to be fully compliant. Upon the allowance of a generic claim, Applicants will be entitled to consideration of claims to additional species that are written in dependent form or otherwise include all the limitations of an allowed generic claim, as provided by 37 C.F.R.§ 1.146, and MPEP § 809.02(a).

(Appx267.) To comply with the Patent Office's restriction requirement, applicants elected "the combination of citrate and phosphate." (*Id.*) However, applicants expressly pointed out that "Upon allowance of a generic claim, Applicants will be entitled to consideration of claims to additional species . . . ." (*Id.*) Further, such generic claims were included in the original application for the parent application and eventually issued in the '395 Patent. (*See* Appx611-642.) Thus, no reasonable competitor could conclude that Amgen was abandoning coverage to other salt pairs than just citrate and phosphate based on the prosecution of the parent application to the '707 Patent.

As applicants preserved in their response to the restriction requirement in the parent application, Amgen later filed the '707 Patent independent claims reciting additional salt pairs (citrate and sulfate, citrate and acetate, and sulfate and acetate) on June 23, 2010. '707 Patent, Cover. The issuance of the '707 Patent claims reciting those salt pair combinations confirms that there was no surrender of salt pairs other than phosphate and citrate in the parent application prosecution. Had Amgen surrendered the use of all salt pair combinations except for phosphate and citrate, the Examiner would not have been able to issue claims directed to the use of other combinations. But the Examiner allowed the '707 Patent claims reciting combinations other than phosphate and citrate (citrate and sulfate, citrate and acetate, and sulfate and acetate). Thus, for these reasons and as discussed above, no reasonable competitor could conclude from the prosecution of the parent application or the '707 Patent that the '707 Patent claims are limited to the recited salt pairs.

C. Amgen's Statements in the Parent Application Are Not a Clear and Unmistakable Surrender

Were the Court to consider Amgen's parent application statements relevant to evaluating estoppel as to the '707 Patent claims, there is no clear and unmistakable surrender of claim scope as to the '707 Patent claims. Specifically, the district court erred in finding surrender based on Amgen's statements in a July 14, 2008 response to a February 14, 2008 Patent Office Non-Final Rejection of the

claims pending in the parent application as obvious over Holtz. (Appx7.) At the time, those pending claims did not require a resultant increase in dynamic capacity of the column from use of the claimed combination of salts. (Appx21; *see* Appx207.)

As a result, Amgen asserted in its response that the "advantages of the particular combinations of salts of the claimed process" would not have been "*predictable* based on Holtz" because Holtz "neither describes nor suggests the particular combination of two salts of the claimed process, nor were the advantages of the claimed two salt processes predictable based on Holtz." (Appx213.) As Amgen noted, Holtz "represents the typical methods [use of a single salt system] used prior to the instant invention": "that is, adding a high concentration of ammonium sulfate to a low concentration of a buffer solution to prepare a protein for a HIC column." (Appx212.) Thus, Amgen stated that the pending claims had "significant differences" from the process disclosed in Holtz. (*Id.*) The fact that Amgen described the then-pending claims of the parent application by identifying particular salt combinations simply reflects that the pending claims had not yet been amended to require the novel functionality or effect of increasing the dynamic capacity of the HIC column for the protein.

Similarly, Amgen's statement that Holtz does not describe "the novel use of particular combinations" addresses the distinction between Holtz's one-salt

purification process and the claimed invention's purification process using a combination of two salts to increase dynamic capacity. (*Id.*) It is not a clear and unmistakable disclaimer of all salt combinations except the ones recited in the pending parent application claims. The district court erred in finding otherwise.

II. The District Court Erred in Determining that Amgen Dedicated to the Public the Particular [redacted] Used in Coherus's Process

The district court erred in determining that the dedication-disclosure doctrine prevents Amgen from asserting infringement against Coherus's process, which uses a [redacted] of [redacted] and [redacted], under the doctrine of equivalents. (Appx9.) The dedication-disclosure doctrine prevents the application of the doctrine of equivalents "to recapture subject matter deliberately left unclaimed." *Johnson & Johnson Assocs. Inc. v. R.E. Serv. Co.*, 285 F.3d 1046, 1054 (Fed. Cir. 2002). When a patentee discloses but declines to claim subject matter, that unclaimed subject matter is dedicated to the public. *Id.* However, the dedication-disclosure doctrine "does not mean that any generic reference in a written specification necessarily dedicates all members of that particular genus to the public." *Sandisk Corp. v. Kingston Tech. Co.*, 695 F.3d 1348, 1363 (Fed. Cir. 2012) (quoting *PSC Comp. Prods. v. Foxconn Int'l, Inc.*, 355 F.3d 1353, 1360 (Fed. Cir. 2004)). "[B]efore unclaimed subject matter is deemed to have been dedicated to the public, that unclaimed subject matter must have been identified by

the patentee as an alternative to a claim limitation." *Id.* at 1363–64 (quoting *Pfizer, Inc. v. Teva Pharms., USA, Inc.*, 429 F.3d 1364, 1379 (Fed. Cir. 2005)).

Here, the district court found that the dedication-disclosure doctrine applies based on the following passage of the '707 Patent specification that lists a series of anions—including [redacted] and [redacted]—by order of relative lyotropic effect. (Appx9-10.)

> As used herein, the term "lyotropic" refers to the influence of different salts on hydrophobic interactions, more specifically the degree to which an anion increases the salting out effect on proteins, or for cations, increases the salting-in effect on proteins according to the Hofmeister series for precipitation of proteins from aqueous solutions (Queiroz et al. *J. Biotechnology* 87: 143-159 (2001), Palman et al. *J. Chromatography* 131, 99-108 (1977), Roe et al. *Protein Purification Methods: A Practical Approach*. IRL Press Oxford, pp. 221-232 (1989)). The series for anions in order of decreasing salting-out effect is: PO_4^{3-}->SO_4^{2-}->CH_3COO–>Cl–>Br–>NO_3–>ClO_4–>I–>SCN–, while the series for cations in order of increasing salting-in effect: NH_4+<Rb+<K+<Na+<Li+<Mg^{2+}+<Ca^{2+}+<Ba^{2+}+ (Queiroz et al., supra). According to the present invention, combining two different salts having different lyotrophic values with a protein preparation allows more protein to be loaded onto a column with no or negligible breakthrough compared with higher salt concentrations of each single salt.

'707 Patent, 4:33-51.

The district court's rationale is incorrect because the list above does not disclose any salt pairs and therefore cannot be a dedication of any such salt pairs to

the public. Rather, the list is simply a generic reference to the well-known lyotropic series for anions and cations without providing for particular salt pairs. Indeed, the list does not even identify a single salt (*i.e.*, a cation with an anion, like [redacted]), let alone a combination of salt pairs such as the [redacted] used by Coherus. As Amgen has laid out in detail above, the teaching of the '707 Patent is how to identify and use particular salt pairs that will increase the dynamic capacity of a HIC column when purifying a specific protein. This subject matter is described elsewhere in the specification, but not in the part quoted by the district court. Thus, passage relied on by the district court does not identify to one of ordinary skill in the art that the [redacted] specifically is an alternative to the claimed invention. *See PSC Comp. Prods.*, 355 F.3d at 1360.

To hold otherwise would mean that subject matter can be dedicated to public even if it is not specifically disclosed in the specification. The law is otherwise. "The disclosure must be of such specificity that one of ordinary skill in the art could identify the subject matter that had been disclosed and not claimed." *Id.* "Whether a person of ordinary skill ultimately could employ the disclosures of the patent to implement a purported equivalent does not amount to actually disclosing to one of ordinary skill that equivalent 'as an alternative to a claim limitation.'" *Sandisk Corp.*, 695 F.3d at 1364 (quoting *Pfizer*, 429 F.3d at 1379).

Finally, the district court erred in applying the disclosure-dedication doctrine on the pleadings, without the benefit of a fully-developed record. The inquiry requires a showing that "one of ordinary skill in the art can understand the unclaimed disclosed teaching upon reading the written description." *PSC Computer Prods.*, 355 F.3d at 1360; *see Pfizer, Inc.*, 429 F.3d at 1378. Thus, expert testimony on how one of skill in the art would understand the specification's disclosure of a lyotropic series may be relevant to resolution of the dispute.

CONCLUSION

For the foregoing reasons, Amgen respectfully requests that this Court reverse, vacate, and/or remand the district court judgment dismissing Amgen's Complaint.

Dated: August 20, 2018

Respectfully submitted,

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ADDENDUM

INDEX TO ADDENDUM

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AMGEN INC. and AMGEN MANUFACTURING, LIMITED,	)	
Plaintiffs,	)	Case No. 17-546 (LPS) (CJB)
v.	)	
COHERUS BIOSCIENCES, INC.	)	
Defendant.	)	

FINAL JUDGMENT AND [PROPOSED] ORDER

WHEREAS the Court's Memorandum Order (D.I. 72) dated March 26, 2018, among other things, adopted Magistrate Judge Burke's Report & Recommendation (D.I. 50) to grant Coherus BioSciences, Inc.'s ("Coherus") Motion to Dismiss pursuant to Federal Rule 12(b)(6) and dismissed with prejudice the patent infringement complaint filed by Amgen Inc. and Amgen Manufacturing Limited ("Amgen") alleging infringement of U.S. Patent No. 8,273,707;

WHEREAS the Court's Memorandum Order (D.I. 72) denied Amgen's request for leave to amend;

IT IS HEREBY ORDERED by the Court as follows:

1. For the reasons stated in the Court's Memorandum Order (D.I. 72) and Magistrate Judge Burke's Report & Recommendation (D.I. 50), final judgment is entered against Amgen on all claims in the Complaint and this case is dismissed with prejudice;
2. Amgen's request for leave to amend is denied;
3. Amgen takes nothing by its claim for infringement of the '707 patent; and

4. Any request for attorneys' fees and/or costs shall be deferred until after the final resolution of the appeal of this action.

Dated: April 16, 2018

APPROVED AS TO FORM;

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IT IS SO ORDERED, this 18th day of April, 2018.

United States District Court Chief Judge

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AMGEN INC. and AMGEN MANUFACTURING LIMITED,	:	
Plaintiffs,	:	
v.	:	C.A. No. 17-546-LPS-CJB
	:	**SEALED**
COHERUS BIOSCIENCES INC.,	:	
Defendant.	:	

MEMORANDUM ORDER

WHEREAS, Magistrate Judge Burke issued a 19-page Report and Recommendation (the "Report") (D.I. 50), dated December 7, 2017, recommending that the Court grant Defendant Coherus Biosciences Inc.'s ("Defendant" or "Coherus") motion to dismiss (D.I. 9) Plaintiffs Amgen, Inc. and Amgen Manufacturing, Limited's ("Plaintiffs" or "Amgen") Complaint for failure to state a claim upon which relief may be granted;

WHEREAS, on December 21, 2017, Amgen objected to the Report ("Objections") (D.I. 53), specifically objecting to the Report's interpretation of the prosecution history in finding that Amgen "clearly and unmistakably" surrendered claim scope regarding particular combinations of salts that were not specifically listed in the claims of U.S. Patent No. 8,273,707;

WHEREAS, on January 9, 2018, Coherus responded to Amgen's Objections ("Response") (D.I. 57), asserting that the Report properly interpreted the prosecution history, and further, that two additional grounds support granting its motion to dismiss: (1) Amgen dedicated the surrendered claim scope to the public by disclosing alternative salt combinations in the '707

patent specification but not claiming them; and (2) Amgen surrendered salt combinations in concentrations greater than the amount Coherus uses;

WHEREAS, the Court has considered the parties' objections and responses *de novo*, *see St. Clair Intellectual Prop. Consultants, Inc. v. Matsushita Elec. Indus. Co., Ltd.*, 691 F. Supp. 2d 538, 541-42 (D. Del. 2010); 28 U.S.C. § 636(b)(l); Fed. R. Civ. P. 72(b)(3);

NOW THEREFORE, IT IS HEREBY ORDERED that:

1. Amgen's Objections (D.I. 53) are OVERRULED, Judge Burke's Report (D.I. 50) is ADOPTED, and Coherus's Motion to Dismiss (D.I. 9) is GRANTED.

2. The '707 patent is directed to a process for purifying proteins during which the dynamic capacity – "the amount of protein that can be loaded onto a column without 'breakthrough' or loss of protein to the solution phase before elution" – of a hydrophobic interaction chromatography ("HIC") column is increased by using a combination of two salts in the loading solution. '707 patent at 2:39-42, 3:38-41. Independent claim 1 of the patent reads:

> A process for purifying a protein on a hydrophobic interaction chromatography column such that the dynamic capacity of the column is increased for the protein comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, ***wherein the first and second salts are selected from the group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, respectively, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.***

'707 patent at claim 1 (emphasis added).

3. Amgen alleges that Coherus's abbreviated Biologic License Application ("aBLA") infringes the '707 patent [redacted] (D.I. 1 at

¶¶ 48-50; D.I. 17 at 13-15) In describing the equilibrium buffer used in this step, Coherus's aBLA explains: [redacted] (D.I.10 Ex. 9 at 1701-CHS-00343268) Thus, Coherus contends there cannot be infringement because [redacted] (*See* D.I.10 at 8-9) Amgen counters that the aBLA infringes the '707 patent under the doctrine of equivalents because "in the Coherus process, a preparation containing protein is mixed with a combination of a first salt and a second salt, which combination is the equivalent of one or more of the recited salt pairs." (D.I. 1 at ¶ 50) The Report found argument-based prosecution history estoppel prevented Amgen from being able to prevail on this theory of infringement under the doctrine of equivalents. (Report at 17)

4. The Report found that the patent applicants clearly and unmistakably surrendered claim scope beyond the salt combinations listed in the claims of the '707 patent – i.e., citrate and sulfate, citrate and acetate, and sulfate and acetate. (Report at 12-13) In doing so, according to Amgen, the Report "misinterpret[s] the arguments Amgen made to the Patent Office during the prosecution of the '707 patent" and "ignores context." (Objections at 5-6) The Court agrees with the Report and disagrees with the Objections.[1]

5. The prosecution history, namely, the patentee's correspondence in response to two

[1]The Court also disagrees with Coherus' contention that Amgen waived the arguments it is making in its Objections because they were not presented to the Magistrate Judge. (*See* Response at 6) Amgen has always contested the application of prosecution history estoppel, and in its motion briefing contended that "'highlighting' the use of specific salt pairs is not a clear and unmistakable surrender of other salt pairs that are not discussed in the prosecution history." (D.I. 17 at 16) While this statement is not as detailed as Amgen's Objections, the Court finds Amgen has not waived it.

office actions and a final rejection, shows a clear and unmistakable surrender of claim scope by the patentee. *See Intendis GmbH v. Glenmark Pharms. Inc., USA*, 822 F3d 1355, 1365 (Fed. Cir. 2016) (stating that argument-based prosecution history estoppel applies when prosecution history "evinces a clear and unmistakable surrender of subject matter").

6. First, in a February 2008 Office Action, the Patent Office rejected the parent patent of the '707 patent as obvious over Holtz, a United States patent that disclosed "a method for purification of insulin-like growth hormone," in which salts are used to "improve the hydrophobic interaction" between the protein and the HIC matrix. (D.I. 10 Ex. 6 at 4) Holtz specifically listed salts such as "sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate and the like." (*Id.*) In July 2008, the patentee responded that "Holtz et al. does not describe optimizing the purification process for commercial production of any protein by increasing the dynamic capacity of the HIC column(s) through the novel use of ***particular combinations*** of only two salts." (*Id.* Ex. 7 at 8) (emphasis added) The patentee continued, "there is no suggestion in Holtz et al. to use two salts, ***let alone the particular combination of salts of the claimed method*** . . . ." (*Id.*) (emphasis added) The patentee made this statement – referencing the "particular combinations of salts of the claimed method" – even though the Patent Office had specifically pointed out that Holtz disclosed [redacted] which Amgen now alleges is equivalent to salts claimed in its patent.[2]

[2]The parent patent's claim 1 recited a combination "wherein the first and second salts are citrate and phosphate salts," while the larger group of three combinations of salts – "citrate and sulfate, citrate and acetate, and sulfate and acetate" – is claimed in the '707 patent as issued. (D.I. 10 Ex. 7 at 3)

7. Later, in an October 2010 Office Action, the Patent Office once more rejected the '707 patent claims as obvious over Holtz, again listing the various salts disclosed by Holtz, [redacted] (D.I. 10 Ex. 2 at 4) On January 26, 2011, the patentee argued in its response that the pending claims were different from Holtz because they "recite a particular ***combination*** of salts [and n]o combination of salts is taught nor suggested in the Holtz et al. patent, nor is the ***particular*** combinations of salts recited in the pending claims taught nor suggested in this reference." (D.I. 10 Ex. 3 at 5) Thus, the patentee once more distinguished its application by (1) the use of dual salts and (2) "the particular combinations of salts" recited in the application that became the '707 patent.

8. In April 2011, the Examiner issued a Final Rejection, maintaining its rejection of the claims as obvious over Holtz. (D.I. 10 Ex. 1 at 1, 4) The patentee responded in August 2011, arguing that "the Patent Office's argument again overlooks two elements of the claimed method – the use of a ***combination*** of salts in a HIC operation, and the ***enhancement of the dynamic capacity of a HIC column*** imparted by the applicants' method." (*Id.* at 5) The patentee further stated that "merely adding a second salt to the traditional HIC process, as the Patent Office appears to suggest, will not produce applicants' claimed method." (*Id.* at 7) The patentee, therefore, was again telling the Patent Office that merely using two salts, while different from Holtz, was not within the scope of its claims.

9. Amgen's correspondence with the Patent Office shows "that the patentee clearly and unmistakably – and indeed, repeatedly – indicated to competitors that it surrendered processes using combinations of salts different from the '***particular*** combinations of salts recited in the claims.'" (Report at 12; *see also PODS, Inc. v. Porta Stor, Inc.*, 484 F.3d 1359, 1368 (Fed.

Cir. 2007) (describing prosecution history estoppel inquiry depends on "whether a competitor would reasonably believe that the applicant had surrendered the relevant subject matter")) As the Report correctly stated, "if all that was in the prosecution history was the patentee's general focus on the fact that its invention disclosed the use of a ***combination*** of salts (in contrast to Holtz's disclosure of the use of a single salt) then its conclusion [recommending granting Coherus's motion to dismiss] would not be warranted. But in order to overcome the Examiner's rejection of the claims over Holtz, the patentee distinguished its invention not only on that ground, but also for the independent reason that the invention recited the use of ***particular*** combinations of salts." (*Id.* at 12-13) Accordingly, argument-based prosecution history estoppel bars Amgen from succeeding on its claim of infringement under the doctrine of equivalents.[3]

10. While the Report understandably and appropriately did not reach Coherus' other arguments, the Court agrees with Coherus that another reason Amgen's claim for infringement of the '707 patent must be dismissed is that the patentee dedicated to the public [redacted] (*See* '707 patent at 4:42-51) [redacted] "combining two different salts having different lyotrophic values with a protein preparation allows more protein to be loaded onto a column with no or negligible breakthrough compared with higher salt concentrations of each single salt") But [redacted] was not claimed – and,

[3]While the Court agrees with the Report's ultimate conclusion about prosecution history estoppel, the Court does not find inventor Senczuk's Declaration (D.I. 10 Ex. 4 at 1) to provide the strong support for this conclusion that the Report found (*see* Report at 13) (noting, correctly, that "patentee supported its position with an inventor declaration providing test results for those ***particular claimed combinations***"). The Court understands the Declaration to be principally concerned with comparing combinations of salts to use of a single salt and improving dynamic capacity of a HIC column.

thus, has been dedicated to the public. *See Johnson & Johnston Assocs., Inc. v. R.E. Serv. Co.*, 285 F.3d 1046, 1054 (Fed. Cir. 2002) ("[W]hen a patent drafter discloses but declines to claim subject matter, as in this case, this action dedicates that unclaimed subject matter to the public. Application of the doctrine of equivalents to recapture subject matter deliberately left unclaimed would conflict with the primacy of the claims in defining the scope of the patentee's exclusive right.") (internal quotation marks omitted).[4]

11. Amgen argues that dismissal is premature because the Court has not had the benefit of a developed record and because there are factual disputes. (Objections at 8-9; D.I. 68 at 1) The Court disagrees. Unlike in *Amgen Inc. v. Alkem Labs. Ltd.*, 2017 WL 6493150, at *2 n.2 (D. Del. Dec. 19, 2017), the facts in the prosecution history here are undisputed. Amgen acknowledges each of the statements to which Coherus points and does not identify any conflicting evidence. (*See generally* Report at 15) ("Amgen makes no attempt to explain how claim construction or discovery would shed light on the objective inquiry regarding whether argument-based prosecution history estoppel applies here. Nor is that clear to the Court.") Amgen merely disputes the interpretation of those facts, contending that, in context, they do not support a finding of estoppel. But the Court has sufficient context in this case to make a decision of law that prosecution history estoppel applies. *See EMD Millipore Corp. v. AllPure Techs., Inc.*, 768 F.3d 1196, 1201 (Fed. Cir. 2014).[5]

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[4]The Court has chosen not to address Coherus' third argument for dismissal, based on the amount of [redacted] Coherus uses. (*See* D.I. 10 at 13-15)

[5]Amgen's citation to the Federal Circuit's very recent decision in *Nalco Co. v. Chem-Mod, LLC*, 883 F.3d 1337 (Fed. Cir. 2018), is equally unavailing. In *Nalco* the parties had disputes over the proper construction of claim terms that were inappropriate to resolve on a motion to dismiss. Here no such disputes have been identified – only a legal dispute that, in the

12. Given the Court's conclusions about the clear and unmistakable disclaimer in the prosecution history, necessitating dismissal of Amgen's claim for infringement under the doctrine of equivalents, and given Amgen's acknowledgment that Coherus does not literally infringe, amendment of the complaint would be futile. Therefore, the Court denies Amgen's request for leave to amend.

As the Court has issued this Memorandum Order under seal, IT IS FURTHER ORDERED that the parties shall meet and confer and, no later than March 28, submit a proposed redacted version of it.

IT IS FURTHER ORDERED that, no later than March 28, the parties shall submit a joint status report advising the Court as to their position(s) as to how this case should now proceed.

March 26, 2018
Wilmington, Delaware

HONORABLE LEONARD P. STARK
UNITED STATES DISTRICT JUDGE

Court's view, turns on the clear and unambiguous prosecution history.

IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AMGEN INC. and AMGEN MANUFACTURING LIMITED,	)	
	)	
Plaintiffs,	)	
	)	
v.	)	Civil Action No. 17-546-LPS-CJB
	)	
COHERUS BIOSCIENCES INC.,	)	
	)	
Defendant.	)	

REPORT AND RECOMMENDATION

In this patent infringement action filed by Plaintiffs Amgen Inc. and Amgen Manufacturing Limited ("Plaintiffs" or "Amgen") against Defendant Coherus Biosciences Inc. ("Defendant" or "Coherus"), pending is Coherus's motion to dismiss the Complaint, filed pursuant to Federal Rule of Civil Procedure 12(b)(6) (the "Motion"). (D.I. 9) For the reasons that follow, the Court recommends that Coherus's Motion be GRANTED with prejudice.[1]

I. BACKGROUND

The Biologics Price Competition and Innovation Act ("BPCIA") was enacted by Congress to establish a regulatory process for an applicant to obtain approval by the United States Food and Drug Administration ("FDA") to market biological products that are "biosimilar" to biological reference products that have been FDA-approved. 42 U.S.C. § 262; (*see also* D.I. 1 at ¶ 8). In August 2016, Coherus filed an abbreviated Biologic License Application ("aBLA"), seeking FDA approval to market a biosimilar version of Amgen's pegfilgrastim product, Neulasta®. (D.I. 1 at ¶¶ 9-10) The parties then engaged in the exchange

[1] The Motion has been referred to the Court for resolution, (D.I. 6), and was fully briefed on June 22, 2017, (D.I. 22). Coherus's pending motion to seal its reply brief, (D.I. 21), is hereby GRANTED for the reasons articulated in that motion.

of information required by the BPCIA, and ultimately agreed in April 2017 that United States Patent No. 8,273,707 (the "'707 patent") should be included in an infringement action to be filed by Amgen pursuant to Section 262(*l*)(6)(A) of the BPCIA. (*Id.* at ¶¶ 12-13) Accordingly, on May 10, 2017, Amgen filed its Complaint, alleging that Coherus's process for manufacturing its biosimilar infringes the '707 patent. (*Id.* at ¶¶ 7, 18-20) Amgen seeks, *inter alia*, to enjoin Coherus from launching its pegfilgrastim biosimilar product. (*Id.* at ¶¶ 51, 56, 59, 65, 71)

The '707 patent is directed to a process for purifying proteins. Its specification explains that biologic drug products constitute therapeutic proteins that are manufactured inside living cells. (D.I. 1, ex. A, col. 1:19-25)[2] These proteins must then be separated from the source material. (*Id.*, col. 1:25-35) One such purification technique is known as hydrophobic interaction chromatography ("HIC"). (*Id.*, col. 1:36-51) With this process, a solution made up of the desired protein and associated impurities is poured onto a column filled with solid particles known as the "matrix." (*Id.*; *see also id.*, col. 3:53-61) The interaction between the matrix material and loading solution causes the proteins to adhere to the matrix as the solution flows through the matrix. (*Id.*, cols. 1:40-45, 3:53-61) This step in the HIC process is known as "loading" the mixture onto the column. (*Id.*, col. 1:40-41) More solution is then poured through the column to "wash" it. (*Id.*, col. 4:27-29) Finally, a different solution is then poured through the column to "elute" the desired proteins therefrom. (*Id.*, cols. 1:45-49, 4:29-30)

Amgen's claimed invention is a solution to a problem with HIC known as "breakthrough," in which significant amounts of protein are washed away with the impurities

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[2] The '707 patent is attached to the Complaint as Exhibit A. Hereafter, citation will be to the "'707 patent."

before the elution step begins. (*Id.*, cols. 3:37-41, 4:10-12) The specification explains that the claimed process increases the "'dynamic capacity'" of the column by "increas[ing] . . . the amount of protein that can be loaded onto a column without 'breakthrough[.]'" (*Id.*, col. 3:37-40) It does so by using "intermediate concentration[s]" of a combination of salts in the loading solution. (*Id.*, cols. 3:31-36, 4:24-27; *see also id.*, col. 2:39-42 ("The two salt buffers of the present invention result in an increase in dynamic capacity of an HIC column for a particular protein compared with the dynamic capacity achieved by single salts.")) As the patent summarizes: "The present invention is a process for purifying a protein comprising mixing a protein preparation with a buffered salt solution containing a first salt and a second salt, wherein each salt has a different lyotrophic value, and loading the protein salt mixture onto an HIC column." (*Id.*, col. 4:56-60)

The '707 patent contains two independent claims and 11 dependent claims. All 13 claims of the patent have at least two requirements. First, the combination of salts that is used in the loading solution must be one of three listed pairs of salts: "citrate and sulfate, citrate and acetate, [or] sulfate and acetate" (the "salt pairing limitation"). (*Id.*, cols. 15:15-16, 16:15-16) Second, the claims require that "the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0." (*Id.*, cols. 15:16-18, 16:16-18)[3]

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[3] More specifically, independent claim 1 recites:

> **1.** A process for purifying a protein on a hydrophobic interaction chromatography column such that the dynamic capacity of the column is increased for the protein comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, *wherein the first and second salts are selected from the group*

II. STANDARD OF REVIEW

The sufficiency of pleadings for non-fraud cases is governed by Federal Rule of Civil Procedure 8, which requires "a short and plain statement of the claim showing that the pleader is entitled to relief[.]" Fed. R. Civ. P. 8(a)(2). When presented with a Rule 12(b)(6) motion to dismiss for failure to state a claim, a court conducts a two-part analysis. *Fowler v. UPMC Shadyside*, 578 F.3d 203, 210 (3d Cir. 2009). First, the court separates the factual and legal elements of a claim, accepting "all of the complaint's well-pleaded facts as true, but [disregarding] any legal conclusions." *Id.* at 210-11. Second, the court determines "whether the facts alleged in the complaint are sufficient to show that the plaintiff has a 'plausible claim for relief.'" *Id.* at 211 (quoting *Ashcroft v. Iqbal*, 556 U.S. 662, 679 (2009)). A plausible claim does

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> *consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, respectively, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.*

('707 patent, col. 15:8-18 (emphasis added))

Independent claim 10 recites:

> **10.** A method of increasing the dynamic capacity of a hydrophobic interaction chromatography column for a protein, comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, and loading the mixture onto a hydrophobic interaction chromatography column, *wherein the first and second salts are selected from the group consisting of citrate and sulfate, citrate and acetate and sulfate and acetate, respectively, and wherein the concentration of each of the first and second salts in the mixture is between about 0.1 M and about 1.0 M.*

(*Id.*, col. 16:9-18 (emphasis added))

more than merely allege entitlement to relief; it must also demonstrate the basis for that "entitlement with its facts." *Id.* Thus, a claimant's "obligation to provide the 'grounds' of his 'entitle[ment] to relief' requires more than labels and conclusions, and a formulaic recitation of the elements of a cause of action will not do[.]" *Bell Atl. Corp. v. Twombly*, 550 U.S. 544, 555 (2007). In assessing the plausibility of a claim, the court must "'construe the complaint in the light most favorable to the plaintiff, and determine whether, under any reasonable reading of the complaint, the plaintiff may be entitled to relief.'" *Fowler*, 578 F.3d at 210 (quoting *Phillips v. Cnty. of Allegheny*, 515 F.3d 224, 233 (3d Cir. 2008)).[4]

III. DISCUSSION

Coherus asserts that it is entitled to dismissal of Amgen's Complaint for failure to state a claim because the accused manufacturing process described in Coherus's aBLA does not satisfy either of the two requirements of the '707 patent claims described above (i.e., that the combination of salts used must be either citrate/sulfate, citrate/acetate or sulfate/acetate, and that the concentration [redacted] must be between about 0.1 M and 1.0). (D.I. 10 at 2-3) Below, the Court need only evaluate the first of these requirements, as the Court agrees with Coherus that there is no plausible claim that its process satisfies the salt pairing limitation.[5]

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[4] In resolving a motion to dismiss, a court may consider not only the allegations in the Complaint, but also, *inter alia*, exhibits attached to the Complaint, documents integral to or explicitly relied upon in the Complaint, and matters of public record. *See, e.g.*, *In re Burlington Coat Factory Secs. Litig.*, 114 F.3d 1410, 1426 (3d Cir. 1997); *Oshiver v. Levin, Fishbein, Sedran & Berman*, 38 F.3d 1380, 1384-85 & n.2 (3d Cir. 1994); *Quest Integrity USA, LLC v. Clean Harbors Indus. Servs., Inc.*, C.A. No. 14-1482-SLR, Civ. No. 14-1483-SLR, 2015 WL 4477700, at *2 (D. Del. July 22, 2015).

[5] At the outset, the Court notes that Amgen seems to suggest in its answering brief that patent infringement actions brought under the BPCIA are not subject to Federal Rule of Civil Procedure 12(b)(6). To that end, Amgen notes that infringement here turns on whether

The relevant accused step of the manufacturing process set forth in Coherus's aBLA[6] is [redacted] Hence, the combination of salts [redacted] is thus not "selected from the group consisting of citrate and sulfate,

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Coherus's intended manufacturing process is covered by Amgen's claims, and states that it may learn additional facts in discovery "to support its infringement claim." (D.I. 17 at 2, 19-20) Amgen's position thus seems to be that even if it cannot not now make out a plausible claim of infringement, that doesn't matter, because it might well learn additional facts in the future in the litigation that *would* allow it to make out such a claim (and so, in the meantime, it should get to proceed forward). That is not the way that the federal pleading requirements normally work, as if a plaintiff cannot make out a plausible claim in its complaint, then the complaint is to be dismissed pursuant to Rule 12(b)(6). If there is some reason why an infringement claim brought pursuant to the BPCIA is not required to pass muster under *Twombly* and *Iqbal*, Amgen has not sufficiently explained it. Or if there is some case law that says that is so, Amgen has not cited it.

[6] The Court's decision here takes into account the contents of Coherus's aBLA, as well as portions of the '707 patent's prosecution history that Coherus attached to its opening brief. (D.I. 10) Amgen asserts that Coherus's Motion, which similarly relies on such materials, "improperly relies on documents outside the Complaint[.]" (D.I. 17 at 2 (emphasis in original)) Amgen is incorrect. With respect to the aBLA, it is the document that formed the basis for Amgen's patent infringement claims, and as such, is referenced throughout the Complaint. (*See, e.g.*, D.I. 1 at ¶¶ 9-20, 46) Thus, it is clearly a document that is integral to the Complaint and one that the Court can rely upon at this stage. *See, e.g.*, *AstraZeneca Pharms. LP v. Apotex Corp.*, 669 F.3d 1370, 1378 n.5 (Fed. Cir. 2012) (finding that the district court did not err in considering defendant's submissions to the FDA in resolving a motion to dismiss, as the complaints at issue "referenced and relied on" those submissions); (D.I. 10 at 10; D.I. 22 at 3). As for the prosecution history, a court may take judicial notice of a patent's prosecution history in resolving a motion to dismiss, as the prosecution history is a public record. *See, e.g., Purdue Pharma L.P. v. Mylan Pharms. Inc.*, Civil Action No. 15-1155-RGA-SRF, 2017 WL 784989, at *4, *6 (D. Del. Mar. 1, 2017); *Genetic Techs. Ltd. v. Bristol-Myers Squibb Co.*, 72 F. Supp. 3d 521, 526, 532 (D. Del. 2014) (citing *Hockerson-Halberstadt, Inc. v. Avia Grp. Int'l, Inc.*, 222 F.3d 951, 957 (Fed. Cir. 2000)); *see also Anchor Sales & Mktg., Inc. v. Richloom Fabrics Grp., Inc.*, No. 15-CV-4442 (RA), 2016 WL 4224069, at *1 n.1 (S.D.N.Y. Aug. 9, 2016). The Court will further address Amgen's objection to considering the prosecution history later in this Report and Recommendation.

citrate and acetate, and sulfate and acetate," as required by the claims of the '707 patent. It is not disputed, then, that Coherus's manufacturing process cannot literally infringe this limitation of the patent. (D.I. 10 at 11; D.I. 17 at 7, 13-14) As a result, Amgen's Complaint instead alleges that Coherus's process infringes the salt pairing limitation only pursuant to the doctrine of equivalents. (D.I. 1 at ¶ 50)

A product that does not literally infringe a patent claim may still infringe under the doctrine of equivalents if the differences between the claimed invention and the accused product are insubstantial. *Warner-Jenkinson Co., Inc. v. Hilton Davis Chem. Co.*, 520 U.S. 17, 21, 40 (1997); *Virnetx, Inc. v. Cisco Sys., Inc.*, 767 F.3d 1308, 1322 (Fed. Cir. 2014); *see also Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co., Ltd.*, 535 U.S. 722, 733 (2002) ("The doctrine of equivalents allows the patentee to claim those insubstantial alterations that were not captured in drafting the original patent claim but which could be created through trivial changes."). Under the doctrine of equivalents, the essential inquiry is whether there is equivalence between the elements of the accused process and the claimed elements of the patented invention. *Warner-Jenkinson Co.*, 520 U.S. at 21, 40; *MiiCs & Partners Am., Inc. v. Toshiba Corp.*, — F. Supp. 3d —, 2017 WL 4786426, at *3 (D. Del. Oct. 24, 2017).

Amgen's Complaint does not actually allege any facts that would support the notion that there is equivalence between [redacted] and one or more of the three recited salt pairs in the patent. It simply states the legal conclusion that there is such equivalence, nothing more. (D.I. 1 at ¶ 50 ("With respect to the use of dual salts, in the Coherus process, a preparation containing protein is mixed with a combination of a first salt and a second salt, *which combination is the equivalent of one or more of the recited salt pairs*." (emphasis

added)) And so, the Complaint is clearly insufficiently pleaded in that respect.

But Coherus further argues that there is no reason to allow re-pleading here. This is because Coherus asserts that, in light of the doctrine of prosecution history estoppel, as a matter of law there *can be no* infringement of the '707 patent claims' salt pairing limitation under the doctrine of equivalents. (D.I. 10 at 12) As the United States Court of Appeals for the Federal Circuit has explained, "[p]rosecution history estoppel applies as part of an infringement analysis to prevent a patentee from using the doctrine of equivalents to recapture subject matter surrendered from the literal scope of a claim during prosecution." *Trading Techs. Int'l, Inc. v. Open E Cry, LLC*, 728 F.3d 1309, 1322 (Fed. Cir. 2013). Whether prosecution history estoppel applies, and therefore whether a patentee may assert the doctrine of equivalents for a particular claim limitation, is a question of law. *Spectrum Pharms., Inc. v. Sandoz Inc.*, 802 F.3d 1326, 1337 (Fed. Cir. 2015); *Intellectual Ventures I LLC v. T-Mobile USA, Inc.*, C.A. No. 13-1632-LPS, 2017 WL 3723934, at *5 (D. Del. Aug. 29, 2017). Prosecution history estoppel can occur in two ways: (1) by making a narrowing amendment to a claim ("amendment-based estoppel"); or (2) by surrendering claim scope through argument to the patent examiner ("argument-based estoppel"). *Conoco, Inc. v. Energy & Envtl. Int'l, L.C.*, 460 F.3d 1349, 1363 (Fed. Cir. 2006).

Coherus relies on argument-based estoppel here as assertedly barring Amgen's infringement claim. (D.I. 10 at 11-12) To invoke argument-based estoppel, "the prosecution history must evince a clear and unmistakable surrender of subject matter." *Conoco, Inc.*, 460 F.3d at 1364 (internal quotation marks and citation omitted). The relevant inquiry is an objective test, which inquires "whether a competitor would reasonably believe that the applicant had surrendered the relevant subject matter." *Id.* (internal quotation marks and citation omitted); *see*

also AquaTex Indus., Inc. v. Techniche Sols., 419 F.3d 1374, 1382 (Fed. Cir. 2005). "[W]here a patent applicant sets forth multiple bases to distinguish between its invention and the cited prior art, the separate arguments [can] create separate estoppels as long as the prior art was not distinguished based on the combination of these various grounds." *PODS, Inc. v. Porta Stor, Inc.*, 484 F.3d 1359, 1367 (Fed. Cir. 2007) (internal quotation marks and citation omitted).[7]

Coherus contends that during prosecution of the '707 patent, Amgen distinguished a prior art reference ("Holtz") and overcame the patent examiner's ("Examiner") rejection, on the ground that Holtz did not teach or suggest the particular combinations of salts (citrate/sulfate, citrate/acetate and sulfate/acetate) claimed in the patent. As such, according to Coherus, Amgen is now estopped from asserting that a different salt combination [redacted] is infringing. (D.I. 10 at 12; D.I. 22 at 7-8)

To assess this issue, the Court turns to the prosecution history. In October 2010, the Examiner rejected the claims of the '707 patent as obvious over Holtz, a United States patent. (D.I. 10, ex. 2 at 4) Holtz was described by the Examiner as disclosing a method for purifying insulin-like growth hormone in which salts will be used to improve interaction between the protein and the matrix—[redacted] In rejecting the claims, the Examiner opined that:

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[7] Even where they are not necessary to secure allowance of the claim, statements that clearly and unmistakably surrender claim scope can preclude an assertion of equivalency. *See Bayer AG v. Elan Pharm. Research Corp.*, 212 F.3d 1241, 1252 (Fed. Cir. 2000) ("Unmistakable assertions made by the applicant to the . . . PTO . . . in support of patentability, whether or not required to secure allowance of the claim, . . . may operate to preclude the patentee from asserting equivalency[.]") (internal quotation marks and citation omitted).

> It would have been obvious to one of ordinary skill in the art at the time the claimed invention was made to purify a protein including an insulin-like growth hormone via the instantly claimed steps based upon the overall beneficial teachings provided by the cited reference. The adjustment of particular conventional working conditions (if not expressly taught) is deemed merely a matter of judicious selection and routine optimization which is well within the purview of the skilled artisan.

(*Id.* at 5)

In a January 26, 2011 Response to that Office Action, the patentee noted the Examiner's statement that Holtz "discloses the use of a number of salts . . . [redacted] The patentee then explained why it disagreed that its invention was obvious over Holtz: "Applicants point out that the pending claims recite a particular *combination* of salts. No combinations of salts is taught nor suggested in the Holtz et al. patent, nor is the *particular* combinations of salts recited in the pending claims taught nor suggested in this reference." (*Id.* (emphasis in original)) The patentee continued that "[t]he claimed subject matter is directed to use of combinations of salt that *increase the dynamic capacity* of the [HIC] columns." (*Id.* (emphasis in original)) In Holtz, meanwhile, "[t]here is no description or suggestion . . . for the use of any combination of salts to increase the dynamic capacity of a HIC[,]" (*id.*), nor any "suggestion in Holtz [] that any *particular* combinations of salts would have the result . . . of increasing dynamic capacity of a HIC[,]" (*id.* at 6 (emphasis added)).[8] The patentee also attached a Declaration of the patent's

[8] Coherus further points out that during prosecution of the parent patent, which claims recited a combination of "citrate and phosphate salts," (D.I. 10, ex. 7 at 3), the patentee explained that Holtz described an example containing a solution loaded onto a HIC column containing "16% saturated ammonium sulfate, 40 mM sodium acetate, 40 mM sodium

first-listed inventor, Anna Senczuk, to support its patentability position. (*Id.* at 5-7) In the Declaration, Ms. Senczuk explained that she performed experiments testing single salts and combination of salts, and that:

> [1] My co-inventor and I discovered that using *certain combinations* of salts will greatly improve the dynamic capacity of a . . . HIC[] column . . . . Previously, it was not known that *salt combinations* had anything to do with improving dynamic capacity of a HIC.
>
> [2] Increasing the dynamic capacity of the HIC is very significant in a commercial manufacturing setting, since this allows more protein to be purified per purification cycle. . . . I performed calculations illustrating the benefits for commercial manufacturing of using *a specific dual salt combination* to load protein onto a HIC[.]

(*Id.*, ex. 4 at ¶¶ 2-3 (emphasis added)) Ms. Senczuk then sets out the "benefits that result from the use of dual salts in the HIC column[,]" noting that the claimed sulfate/citrate and sulfate/acetate combinations allowed for fewer cycles of purification, and that these combinations, as well as the claimed acetate/citrate combination, reduced processing time. (*Id.* at ¶ 4) Ms. Senczuk then concluded her Declaration by noting that:

> The improvement resulting from the use of dual salts in HIC goes beyond merely optimizing a column to best suit a particular protein. *Use of this particular combination of salts* greatly improves the cost-effectiveness of commercial manufacturing by reducing the number of cycles required for each harvest and reducing the processing time for each harvest.

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phosphate, pH 4.5, and 0.4M NaCl [sodium chloride][,]" (*id.* at 6). (D.I. 22 at 8) The patentee distinguished its invention by explaining that Holtz did not "teach or suggest combining the protein to be purified with the particular *combination of two salts, citrate and phosphate salts* . . . . [i]nstead, [disclosed in Holtz is] a protein solution containing lower concentrations of sodium acetate and sodium phosphate, together with [sodium chloride] and a high concentration of ammonium sulfate (four salts, not a combination of two salts as recited in the claimed method)[.]" (D.I. 10, ex. 7 at 6 (emphasis in original))

(*Id.* (emphasis added))

In an April 2011 Office Action, the Examiner maintained its rejection of the claims as obvious over Holtz for the same reasons as described above. (*Id.*, ex. 1 at 1, 4) In August 2011, the patentee traversed the rejection. The patentee explained that the Examiner's rejection over Holtz overlooked "the use of a *combination* of salts" in the invention's HIC process (and also overlooked the "*enhancement of the dynamic capacity of a HIC column*" caused by the invention). (*Id.* at 5 (emphasis in original)) Holtz, the patentee explained, "does not teach each and every element of the claimed invention[,]" "namely" because it "simply does not disclose, suggest or contemplate any steps involving a combination of two salts for any purpose whatsoever." (*Id.*) And later in its statement, the patentee further emphasized that "merely adding a second salt to the traditional HIC process, as the Patent Office appears to suggest, will not produce applicants' claimed method." (*Id.* at 7)

In view of this prosecution history, the Court finds that the patentee clearly and unmistakably—and indeed, repeatedly—indicated to competitors that it surrendered processes using combinations of salts different from the "*particular* combinations of salts recited in the [] claims[.]" (*Id.*, ex. 3 at 5 (emphasis in original)) The Court acknowledges that if all that was in the prosecution history was the patentee's general focus on the fact that its invention disclosed the use of a *combination* of salts (in contrast to Holtz's disclosure of the use of a single salt) then its conclusion would not be warranted. But in order to overcome the Examiner's rejection of the claims over Holtz, the patentee distinguished its invention not only on that ground, but also for

the independent reason that the invention recited the use of *particular* combinations of salts.[9] And the patentee supported its position with an inventor declaration providing test results for those *particular claimed combinations*—one that touted the benefits of use of those specific combinations—in order to show how their use resulted in a process that improved the dynamic capacity of a HIC column. In sum, the patentee's arguments distinguishing its invention from Holtz clearly and unmistakably demonstrate that it limited its claims to a process using one of the *particular*, recited combinations of salts; thus, Amgen surrendered any claim to a process that used other, unrecited salt combinations. *See PODS, Inc.*, 484 F.3d at 1367-68 (finding that statements made by the patentee during prosecution barred it from asserting that defendant's device infringed pursuant to the doctrine of equivalents where the patentee, "in support of its assertion of patentability over [prior art reference] Dousset, clearly stated that its claimed frame was rectangular in shape [and] [a] competitor would reasonably believe that [the patentee] had surrendered any claim to a frame that was not rectangular or four-sided in shape, such as [the defendant's] three-sided, u-shaped device"); *see also Ottah v. VeriFone Sys., Inc.*, 524 F. App'x 627, 629-30 (Fed. Cir. 2013) (affirming the district court's holding that the patentee's claim was barred by prosecution history estoppel where, in response to a prior art rejection, the patentee "emphasized that the patentability of the '840 patent's claim was based on the removable nature of the mount [and accordingly] [h]e cannot now, under the doctrine of equivalents, seek to broaden the scope of his claim to include mounts that are fixed as well as those that are

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[9] Indeed, as if to highlight this point even further, so that the Examiner would not miss it, the patentee actually placed the word "particular" in the phrase "particular combination of salts" in italics. (D.I. 10, ex. 3 at 5 ("No combinations of salts is taught nor suggested in the Holtz [] patent, nor is the *particular* combinations of salts recited in the pending claims taught nor suggested in this reference.") (emphasis in original))

removable"); *Anchor Sales & Mktg., Inc.*, 2016 WL 4224069, at *5 (concluding that argument-based prosecution history estoppel barred plaintiff from now arguing infringement under the doctrine of equivalents for methods of forming scalloped configuration in curtains that do not involve sliding a bead up and down, where the patentee had argued in prosecution that unlike the cord stops or toggles described in the prior art, the essence of his invention was sliding the sphere up and down).[10]

Amgen's arguments to the contrary are not persuasive. As an initial matter, it is telling that nowhere in Amgen's answering brief does it actually grapple with the substance of the prosecution history upon which Coherus's argument relies. Thus, it never attempts to explain with any specificity *why* those statements fail to trigger prosecution history estoppel. Instead, it

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[10] Other courts examining the type of language that can give rise to a finding of argument-based prosecution history estoppel have observed that in arguing to overcome a rejection, while a patentee may "surrender what the invention is being differentiated from, one does not necessarily surrender all other equivalents, especially when the applicant does not discuss or limit the contents of the claimed invention itself." *AstraZeneca UK Ltd. v. Dr. Reddy's Labs., Ltd.*, Civil Action No. 08-3237 (MLC), 2010 WL 4721384, at *7 (D.N.J. Nov. 15, 2010) (citing cases). Oftentimes, courts find argument-based estoppel where the applicant has specifically disclaimed a feature found in the prior art (a feature that a plaintiff is then trying to accuse of infringement pursuant to the doctrine of equivalents). *See id.* (citing cases); *see also, e.g., Texas Instruments Inc. v. U.S. Int'l Trade Comm'n*, 988 F.2d 1165, 1175 (Fed. Cir. 1993) ("By expressly stating that claim 12 was patentable because of the opposite-side gating limitation, particularly in light of their previous admission that same-sided gating was known in the art, the inventors unmistakably excluded the same-side gating as an equivalent."). However, patentees need not "stress the disadvantages of other equivalents to clearly and unmistakably surrender them[,]" for courts have found "clear and unmistakable surrender when the patentee asserted the singularity or uniqueness of the claimed invention in arguing for its patentability." *AstraZeneca UK Ltd.*, 2010 WL 4721384, at *8 (citing cases); *see also Anchor Sales & Mktg.*, 2016 WL 4224069, at *5. Here, as explained above, a competitor examining the prosecution history would reasonably believe that the patentee clearly and unmistakably surrendered combinations other than the particular combinations recited in the claims, in light of the way that the patentee emphasized that its claims were patentable on the separate basis that they included those particular combinations. *See, e.g., PODS, Inc.*, 484 F.3d at 1368.

makes three more peripheral arguments that the Court will take up below.

First, Amgen asserts in conclusory fashion that Coherus's argument regarding the salt-pairing limitation can be "raised after claim construction and discovery, and not before." (D.I. 17 at 15) Amgen makes no attempt to explain how claim construction or discovery would shed light on the objective inquiry regarding whether argument-based prosecution history estoppel applies here. Nor is that clear to the Court. And so in the absence of such an explanation, the Court finds it appropriate to resolve this question of law at the pleading stage. *See, e.g.*, *Jenny Yoo Collection, Inc. v. Watters Design Inc.*, 16-CV-2205 (VSB), 2017 WL 4997838, at *9 (S.D.N.Y. Oct. 20, 2017) ("Although examination of the prosecution history is typically handled during the summary judgment stage, whether prosecution history estoppel applies may be determined on a motion to dismiss."); *cf. In re Bendamustine Consolidated Cases*, Civil Action No. 13-2046-GMS, 2015 WL 1951399, at *1-3 (D. Del. Apr. 29, 2015) (in resolving a Rule 12(c) motion, considering ANDA filings and prosecution history over plaintiff's objection, where the plaintiff's theory of infringement was based on the doctrine of equivalents, and the only issue was whether plaintiff's doctrine-of-equivalents-arguments were barred by the disclosure-dedication rule).

Second, Amgen suggests that prosecution history estoppel does not apply to "clarifying amendments." (D.I. 17 at 15) Amgen is correct that clarifying statements made during prosecution do not amount to the clear and unmistakable surrender of subject matter that is required for prosecution history estoppel. *See, e.g.*, *Deering Precision Instruments, L.L.C. v. Vector Distribution Sys., Inc.*, 347 F.3d 1314, 1326 (Fed. Cir. 2003) (finding no prosecution history estoppel where the examiner had objected to original claim 9 but had stated that the claim would be allowed if rewritten in independent form, and where the applicants, in response, noted

that original claim 9 was already written in such form and also restated that a particular limitation in the claim was not disclosed in the references of record; the applicants' statement was deemed "merely a clarification of the Examiner's mistake"). But here again, Amgen makes no attempt to explain how the relevant statements actually amount to mere clarification, as opposed to the clear surrender of claim scope.

Finally, Amgen argues that Coherus has not met the stringent standard for applying argument-based estoppel. In doing so, it merely points to Coherus's statement (found in Coherus's opening brief) that "'[h]aving saved its claims by highlighting the use of *specific* salt pairs, Amgen cannot now expand its patent coverage by saying that its claims equivalently cover processes with *other* salt pairs.'" (D.I. 17 at 15-16 (quoting D.I. 10 at 12) (emphasis in original)) Amgen then focuses on Coherus's use of the word "highlighting" in that statement, arguing that "'*[h]ighlighting*' the use of specific salt pairs is not a clear and unmistakable surrender of other salt pairs that are not discussed in the prosecution history statements relied on by Coherus." (*Id.* at 16 (emphasis added)) But this bit of wordplay ignores the meat of Coherus's position. As detailed above, Coherus did not argue that the patentee had merely "highlighted" the use of specific salt pairs. Rather, as Coherus pointed out, the patentee explicitly argued (at some length) to the Examiner, in order to overcome the rejection based on Holtz, that its claimed invention was distinguishable from Holtz because of the claims' use of specific salt pairs. (D.I. 10 at 6, 12; D.I. 22 at 7-8) The Court thus agrees with Coherus that the patentee clearly and unmistakably surrendered to the public claims using salt pairings other than those recited in the claims of the '707 patent. *See Pharmacia & Upjohn Co. v. Mylan Pharms., Inc.*, 170 F.3d 1373, 1376-78 (Fed. Cir. 1999) (concluding that prosecution history estoppel precluded the patentee

from asserting the doctrine of equivalents against a composition that did not contain spray-dried lactose, explaining that a competitor of plaintiff's would reasonably interpret the applicant's prosecution statements to mean that spray-dried lactose was an indispensable component of the claimed formulations and noting that the plaintiff's arguments to the contrary "fail[ed] to address" or to "explain away" the key portions of the prosecution history).

For these reasons, the Court concludes that prosecution history estoppel bars Amgen from now attempting to reassert surrendered ground involving other combinations of salts, and thus recommends that Coherus's motion to dismiss be granted. *See Anchor Sales & Mktg, Inc.*, 2016 WL 4224069, at *6 (granting defendant's motion to dismiss where argument-based prosecution history estoppel barred plaintiff from arguing infringement under the doctrine of equivalents); *cf. Advantek Mktg., Inc. v. Shanghai Walk-Long Tools Co.*, Case No. CV 16-3061-R, 2016 WL 9178079, at *2 (C.D. Cal. Nov. 3, 2016) (granting motion for judgment on the pleadings where plaintiff's infringement allegations with respect to a design patent were barred by the doctrine of prosecution history estoppel); *Cumberland Pharms. Inc. v. InnoPharma, Inc.*, C.A. No. 12-618-LPS, 2013 WL 5945794, at *1-3 (D. Del. Nov. 1, 2013) (granting a motion to dismiss for failure to state a claim of infringement in ANDA litigation where all claims of the asserted patent required a formulation "free from a chelating agent" and the complaint alleged that defendant's accused product contained a "chelating agent").[11]

IV. CONCLUSION

For the foregoing reasons, the Court recommends that the Motion be GRANTED

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[11] In light of the Court's conclusion, the Court need not consider Coherus's second argument that it cannot infringe because its manufacturing process [redacted] at the required concentration.

with prejudice.[12]

This Report and Recommendation is filed pursuant to 28 U.S.C. § 636(b)(1)(B), Fed. R. Civ. P. 72(b)(1), and D. Del. LR 72.1. The parties may serve and file specific written objections within fourteen (14) days after being served with a copy of this Report and Recommendation. Fed. R. Civ. P. 72(b)(2). The failure of a party to object to legal conclusions may result in the loss of the right to de novo review in the district court. *See Henderson v. Carlson*, 812 F.2d 874, 878–79 (3d Cir. 1987); *Sincavage v. Barnhart*, 171 F. App'x 924, 925 n.1 (3d Cir. 2006). The parties are directed to the Court's Standing Order for Objections Filed Under Fed. R. Civ. P. 72, dated October 9, 2013, a copy of which is available on the District Court's website, located at http://www.ded.uscourts.gov.

Because this Report and Recommendation may contain confidential information, it has been released under seal, pending review by the parties to allow them to submit a single, jointly proposed, redacted version (if necessary) of the Report and Recommendation. Any such redacted version shall be submitted no later than **December 12, 2017** for review by the Court, along with a motion for redaction that includes a clear, factually-detailed explanation as to why disclosure of any proposed redacted material would "work a clearly defined and serious injury to the party seeking closure." *Pansy v. Borough of Stroudsburg*, 23 F.3d 772, 786 (3d Cir. 1994) (internal quotation marks and citation omitted). The Court will subsequently issue a publicly-available version of its Report and Recommendation.

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[12] The Court recommends that the dismissal be with prejudice as Amgen did not make an argument that it should be granted leave to amend in the event of dismissal, (D.I. 17), and more importantly, because amendment would be futile in light of the Court's conclusion that Amgen's claim for relief fails as a matter of law, *see Anchor Sales & Mktg., Inc.*, 2016 WL 4224069, at *6 n.3.

Dated: December 7, 2017

Christopher J. Burke
UNITED STATES MAGISTRATE JUDGE

US008273707B2

(12) **United States Patent**
Senczuk et al.

(10) **Patent No.: US 8,273,707 B2**
(45) **Date of Patent: Sep. 25, 2012**

(54) **PROCESS FOR PURIFYING PROTEINS**

(75) Inventors: **Anna Senczuk**, Shoreline, WA (US); **Ralph Klinke**, Sammamish, WA (US)

(73) Assignee: **Amgen Inc.**, Thousand Oaks, CA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 55 days.

(21) Appl. No.: **12/822,072**

(22) Filed: **Jun. 23, 2010**

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US 2010/0311953 A1 Dec. 9, 2010

Related U.S. Application Data

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C07K 1/16 (2006.01)

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(58) **Field of Classification Search** ..... None
See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

5,231,178	A *	7/1993	Holtz et al.	530/399
5,395,856	A	3/1995	Haase	
5,928,915	A	7/1999	Warner et al.	

OTHER PUBLICATIONS

Perkins, et al., "Protein retention in hydrophobic interaction chromatography: modeling variation with buffer ionic strength and column hydrophobicity," *J. Chromatogr. A 766 1-14*, 1997.
International Search Report mailed Nov. 24, 2004.

* cited by examiner

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(57) **ABSTRACT**

The invention relates to a process for purifying a protein by mixing a protein preparation with a solution having a first salt and a second salt, wherein each salt has a different lyotropic value, and loading the mixture onto a hydrophobic interaction chromatography column. The dynamic capacity of the column for a protein using the two salt combination will be increased compared with the dynamic capacity of the column for either single salt alone.

13 Claims, 5 Drawing Sheets

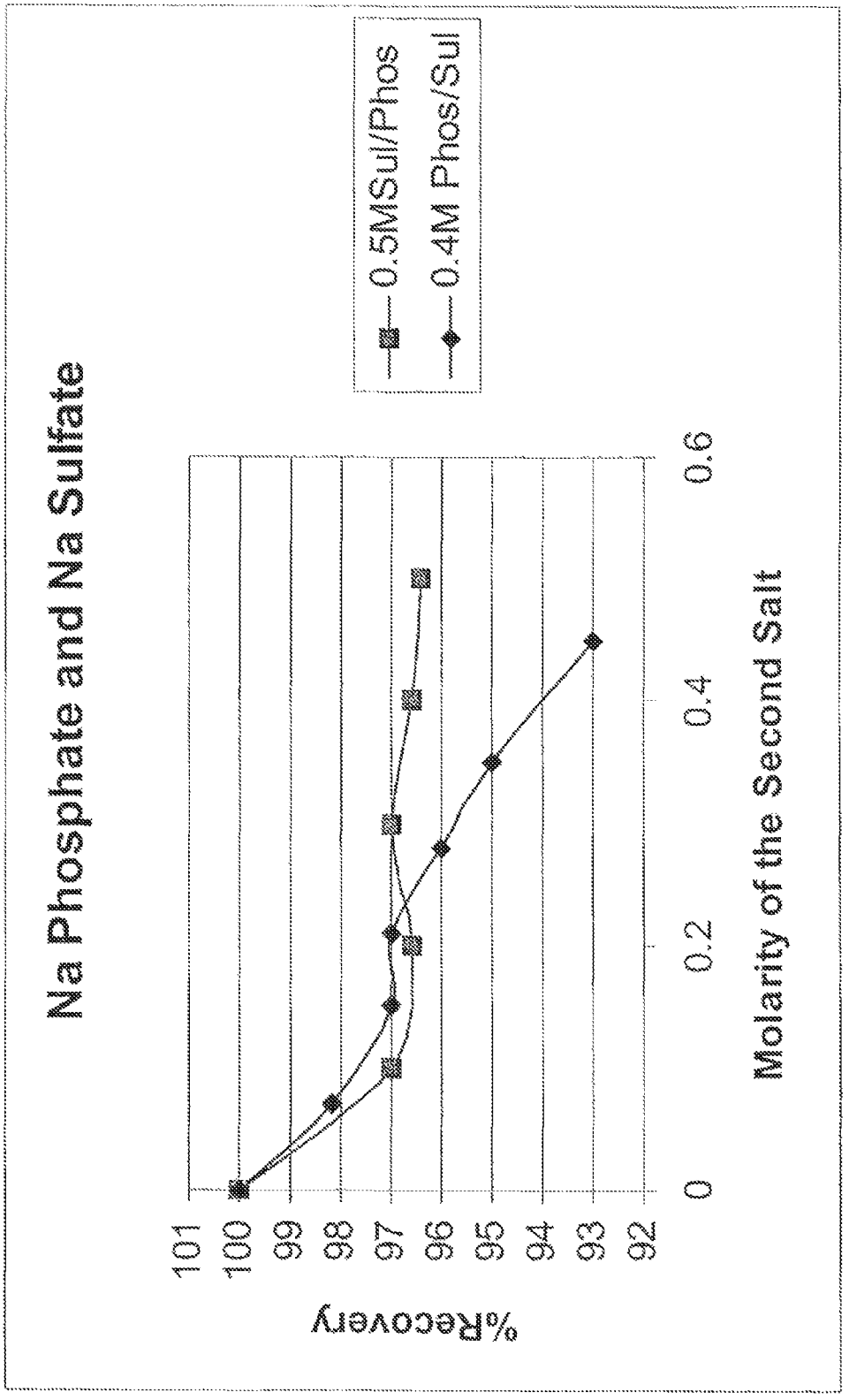

Figure 1A

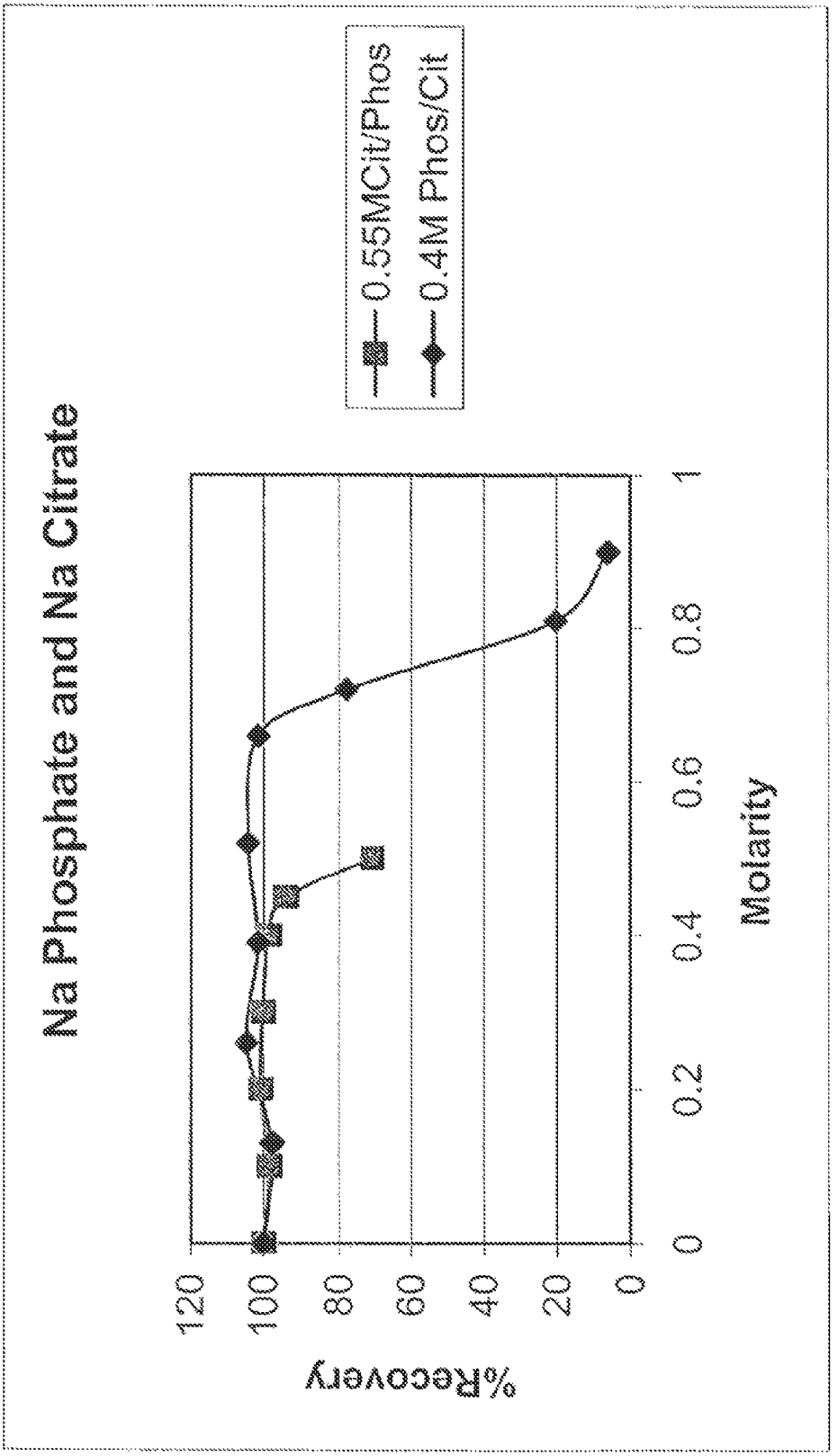

Figure 1B

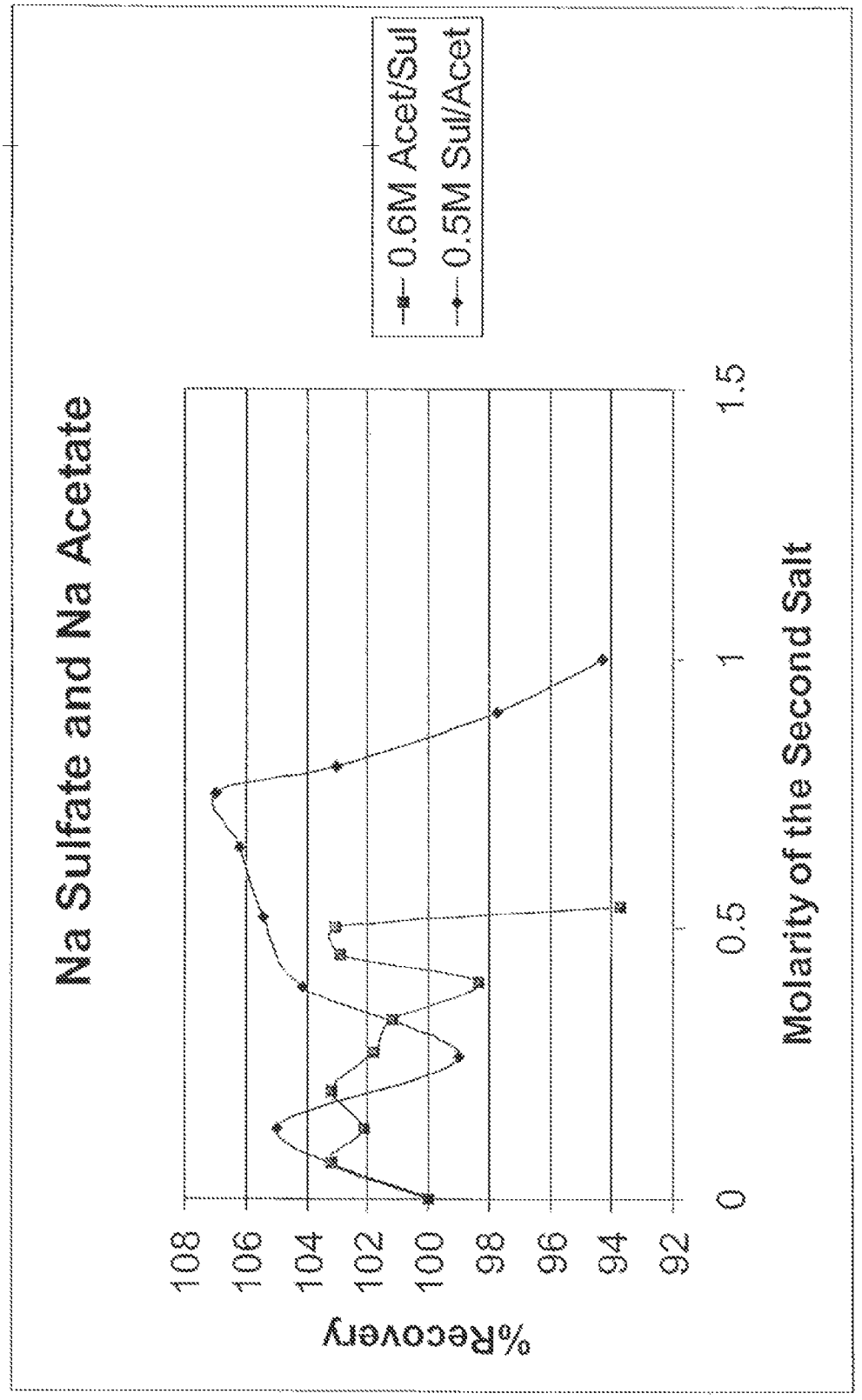

Figure 1C

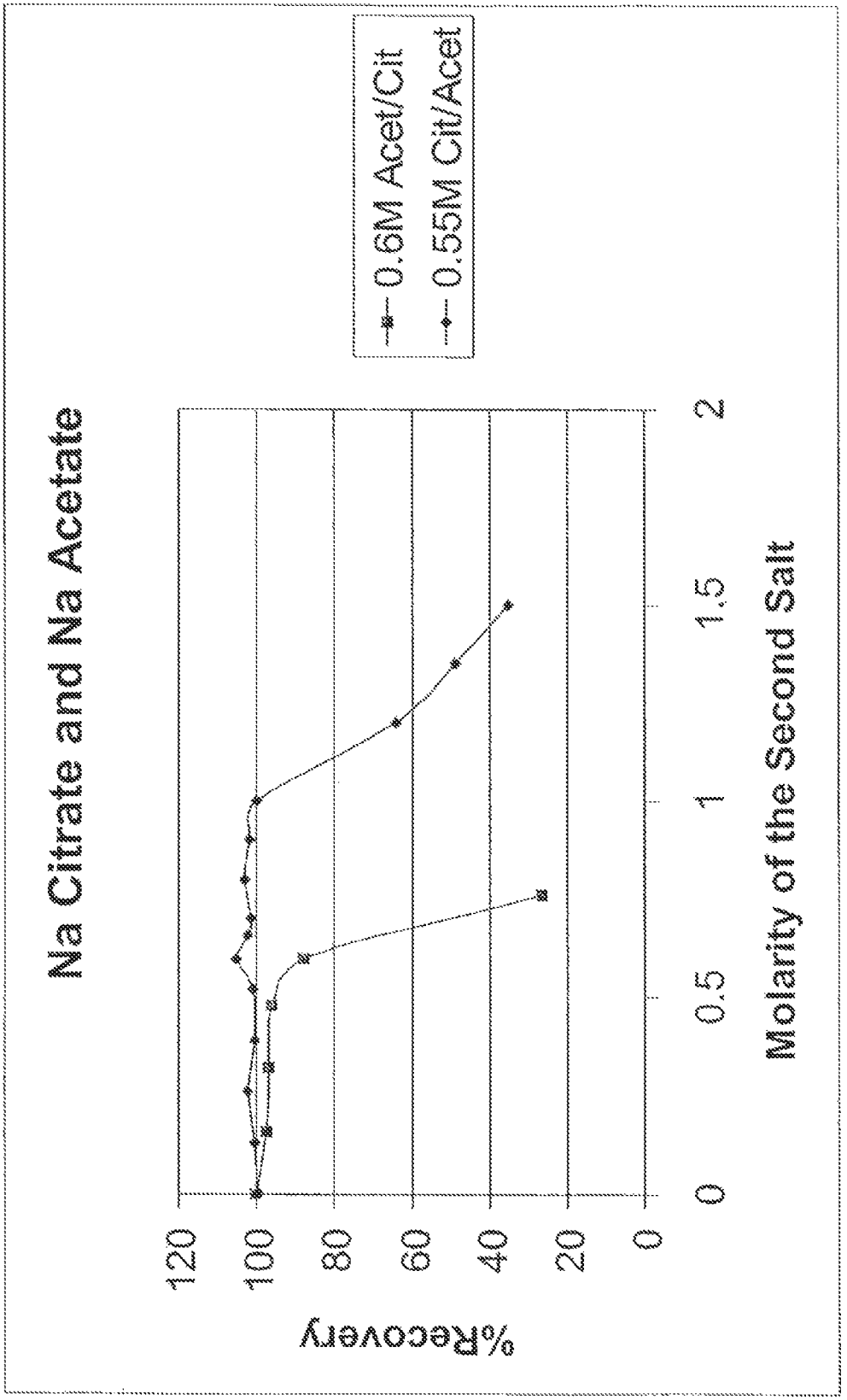

Figure 1D

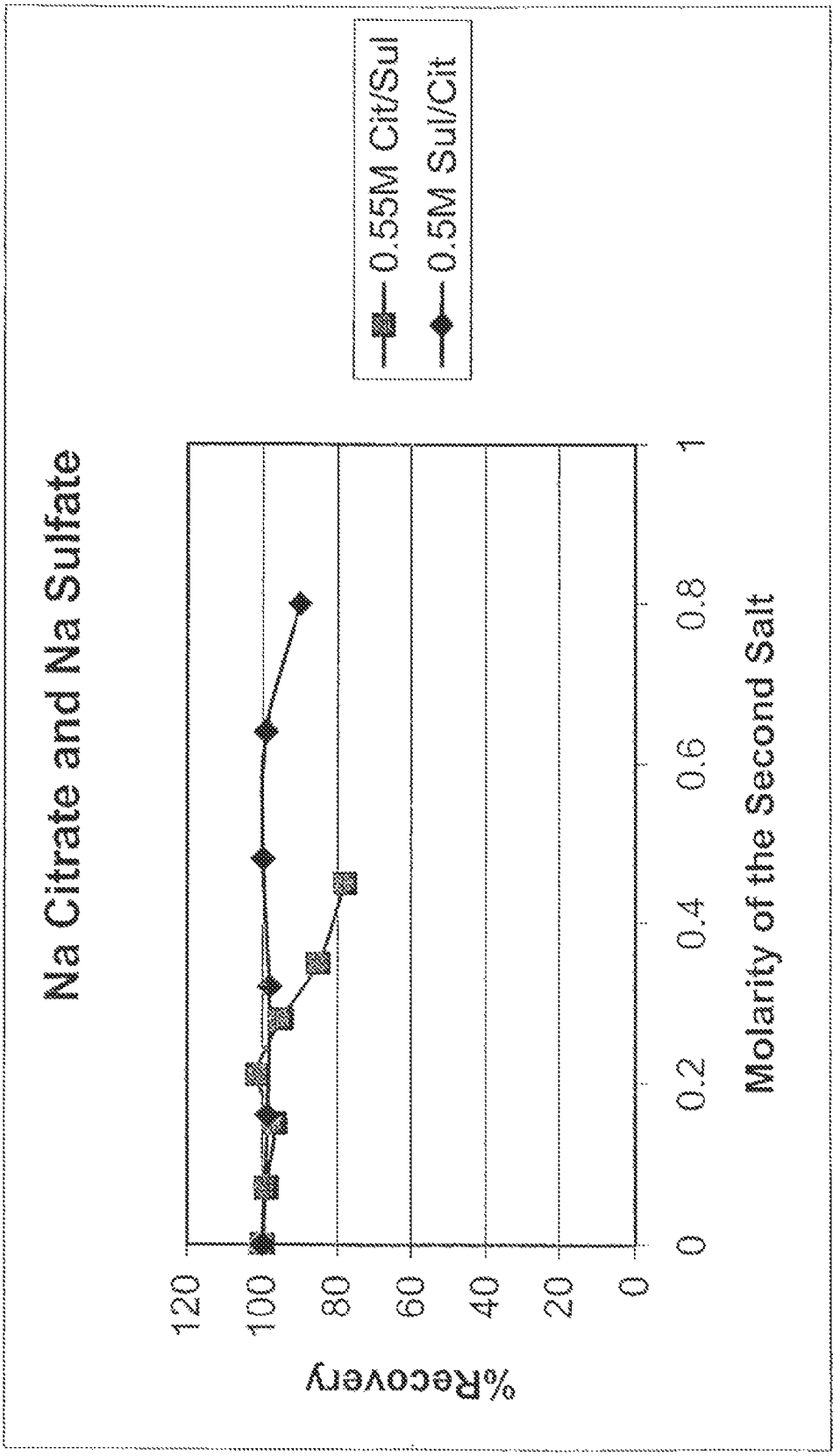

Figure 1E

PROCESS FOR PURIFYING PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a divisional of U.S. application Ser. No. 10/895,581, filed Jul. 21, 2004, now allowed, which claims the benefit of U.S. provisional application No. 60/540,587, filed Jan. 30, 2004, the entire disclosure of which is relied on and incorporated by reference.

FIELD OF THE INVENTION

This invention relates to protein purification and specifically to a process for protein purification using hydrophobic interaction chromatography.

BACKGROUND OF THE INVENTION

The purification of proteins for the production of biological or pharmaceutical products from various source materials involves a number of procedures. Therapeutic proteins may be obtained from plasma or tissue extracts, for example, or may be produced by cell cultures using eukaryotic or procaryotic cells containing at least one recombinant plasmid encoding the desired protein. The engineered proteins are then either secreted into the surrounding media or into the perinuclear space, or made intracellularly and extracted from the cells. A number of well-known technologies are utilized for purifying desired proteins from their source material. Purification processes include procedures in which the protein of interest is separated from the source materials on the basis of solubility, ionic charge, molecular size, adsorption properties, and specific binding to other molecules. The procedures include gel filtration chromatography, ion-exchange chromatography, affinity chromatography, and hydrophobic interaction chromatography.

Hydrophobic interaction chromatography (HIC) is used to separate proteins on the basis of hydrophobic interactions between the hydrophobic moieties of the protein and insoluble, immobilized hydrophobic groups on the matrix. Generally, the protein preparation in a high salt buffer is loaded on the HIC column. The salt in the buffer interacts with water molecules to reduce the solvation of the proteins in solution, thereby exposing hydrophobic regions in the protein which are then adsorbed by hydrophobic groups on the matrix. The more hydrophobic the molecule, the less salt is needed to promote binding. Usually, a decreasing salt gradient is used to elute proteins from a column. As the ionic strength decreases, the exposure of the hydrophilic regions of the protein increases and proteins elute from the column in order of increasing hydrophobicity. See, for example, *Protein Purification,* 2d Ed., Springer-Verlag, New York, 176-179 (1988).

When developing processes for commercial production of therapeutically important proteins, increasing the efficiency of any intermediate purification steps is highly desirable. One way of improving the ease and efficiency of manufacturing is to increase the load capacity of one or more of the intermediate steps of the purification process to the point that the number of cycles required to purify a batch of protein is reduced without compromising the quality of the protein separation. The present invention improves the process of protein purification by increasing the capacity and efficiency of an intermediate step.

SUMMARY OF THE INVENTION

The present invention provides a process of purifying a protein comprising mixing a protein preparation with a solution containing a first salt and a second salt, forming a mixture which is loaded onto a hydrophobic interaction chromatography column, wherein the first and second salts have different lyotropic values, and at least one salt has a buffering capacity at a pH at which the protein is stable. In one embodiment, the pH of the mixture and equilibrium buffer is between about pH 5 and about pH 7. The process further comprises eluting the protein.

The present invention provides combinations of salts useful for increasing the dynamic capacity of an HIC column compared with the dynamic capacity of the column using separate salts alone. These combinations of salts allow for a decreased concentration of at least one of the salts to achieve a greater dynamic capacity, without compromising the quality of the protein separation. The first and second salt combinations are selected for each particular protein through a process of establishing precipitation curves for each salt individually, and precipitation curves for the combination of salts holding one salt constant and varying the second. The concentrations of the salt combinations can be optimized further, for example, to ensure protein stability at room temperature and to prevent formation of aggregates in the protein preparation.

Preferred first salts are those which form effective buffers at a pH at which the protein is stable. In one embodiment, the first and second salts are selected from acetate, citrate, phosphate, sulfate, or any mineral or organic acid salt thereof. In one embodiment the pH of the mixture is between about pH 5 and about pH 7. In one embodiment, the final salt concentrations of the first salt and second salts in the mixture are each between about 0.1 M and 1.0 M, in another embodiment between about 0.3 M and about 0.7 M. The cations can be selected from any non-toxic cations, including NH_4^+, K^+, and Na^+. Preferred cations are those which do not tend to denature the protein or to cause precipitation in combination with other ions, including NH_4^+ and Na^+.

The two salt buffers of the present invention result in an increase in dynamic capacity of an HIC column for a particular protein compared with the dynamic capacity achieved by single salts. This results in decreased number of cycles required for purifying a batch of protein. Therefore, the present invention has special applicability to commercial manufacturing practices for making and purifying commercially important proteins.

BRIEF DESCRIPTION OF THE FIGURES

FIG. **1** shows dual salt precipitation curves for an antibody against EGFR performed as described in Example I below. FIG. **1**A shows the precipitation curve for 0.5 M sodium sulfate with increasing concentrations of sodium phosphate and the precipitation curve for 0.4 M sodium phosphate with increasing concentrations of sodium sulfate. FIG. **1**B shows the precipitation curves for 0.55 M sodium citrate with increasing concentrations of sodium phosphate, and 0.4 M sodium phosphate with increasing concentrations of sodium citrate. FIG. **1**C shows the precipitation curves for 0.6 M sodium acetate with increasing concentrations of sodium sulfate, and 0.5 M sodium phosphate with increasing concentrations of sodium sulfate. FIG. **1**D shows the precipitation curves for 0.6 M sodium acetate with increasing concentrations of sodium citrate, and 0.55 M sodium citrate with increasing concentrations of sodium acetate. FIG. **1**E shows the precipitation curves for 0.55 M sodium citrate with

increasing concentrations of sodium sulfate, and 0.5 M sodium sulfate with increasing concentrations of sodium citrate.

DETAILED DESCRIPTION OF THE INVENTION

Hydrophobic interaction chromatography (HIC) is now widely used as an important bioseparation tool in the purification of many types of proteins. The process relies on separation of proteins on the basis of hydrophobic interactions between non-polar regions on the surface of proteins and insoluble, immobilized hydrophobic groups on the matrix. The absorption increases with high salt concentration in the mobile phase and the elution is achieved by decreasing the salt concentration of the eluant (Fausnaugh et al. *J Chromatogr* 359, 131-146 (1986)). A protein preparation at any stage of purification is "conditioned" in preparation for HIC by mixing with high salt buffers to prepare the HIC "load" to be loaded onto the column. Generally, salt conditions are adjusted to individual proteins. Generally, requirements of between about 0.7 and about 2 M ammonium sulfate and between about 1.0 and 4.0 M NaCl salt concentration has been considered as useful for purifying proteins using HIC columns. The practice was to add a high concentration of salt to a low concentration buffer solution, such as, for example, 1.4 M NH_4SO_4 added to a 0.024 M phosphate buffer for the purification of monoclonal antibodies at pH 7.2 (Nau et al. *BioChromotography* 62 (5), 62-74 (1990)); or 1.7 M ammonium sulfate in 50 mM $NaPO_4$ for purifying yeast cell surface proteins (Singleton et al., *J. Bacteriology* 183 (12) 3582-3588 (2001)). The present invention differs from these practices in the use of an intermediate concentration of a buffering salt in combination with an intermediate concentration of a second buffering salt, or in combination with an intermediate concentration of a second non-buffering salt, to achieve increased dynamic capacity.

It has also been recognized that increasing salt concentrations can increase the "dynamic capacity" of a column, or the amount of protein that can be loaded onto a column without "breakthrough" or loss of protein to the solution phase before elution. At the same time, high salt can be detrimental to protein stability. High salt increases the viscosity of a solution, results in increased formation of aggregates, results in protein loss due to dilution and filtration of the protein after elution from the column, and can lead to reduced purity (Queiroz et al., *J. Biotechnology* 87:143-159 (2001), Sofer et al., *Process Chromatography*, Academic Press (1999)). The present invention, however, provides a process of purifying proteins that increases the dynamic capacity of an HIC column for a particular protein while reducing the concentration of the salts used, without reducing the quality of the protein separation or raising manufacturing issues.

As used herein, the term "hydrophobic interaction chromatography (HIC)" column refers to a column containing a stationary phase or resin and a mobile or solution phase in which the hydrophobic interaction between a protein and hydrophobic groups on the matrix serves as the basis for separating a protein from impurities including fragments and aggregates of the subject protein, other proteins or protein fragments and other contaminants such as cell debris, or residual impurities from other purification steps. The stationary phase comprises a base matrix or support such as a cross-linked agarose, silica or synthetic copolymer material to which hydrophobic ligands are attached.

As used herein the term "dynamic capacity" of a separation column such as a hydrophobic interaction chromatography column refers to the maximum amount of protein in solution which can be loaded onto a column without significant breakthrough or leakage of the protein into the solution phase of a column before elution. More formally, K' (capacity factor) =moles of solute in stationary phase divided by moles of solute in mobile phase=Vr−Vo/Vo, where Vr is the volume of the retained solute and Vo is the volume of unretarded solute. Practically, dynamic capacity of a given HIC column is determined by measuring the amount of protein loaded onto the column, and determining the resin load which is mg protein/column volume (mg/ml−r). The amount of protein leaving the column in the solution phase after the column is loaded ("breakthrough") but before elution begins can then be measured by collecting fractions during the loading process and first wash with equilibrium buffer. The load at which no significant breakthrough occurs is the dynamic capacity of the protein for those conditions.

As used herein, the term "buffer" or "buffered solution" refers to solutions which resist changes in pH by the action of its conjugate acid-base range. Examples of buffers that control pH at ranges of about pH 5 to about pH 7 include citrate, phosphate, and acetate, and other mineral acid or organic acid buffers, and combinations of these. Salt cations include sodium, ammonium, and potassium. As used herein the term "loading buffer" or "equilibrium buffer" refers to the buffer containing the salt or salts which is mixed with the protein preparation for loading the protein preparation onto the HIC column. This buffer is also used to equilibrate the column before loading, and to wash to column after loading the protein. The "elution buffer" refers to the buffer used to elute the protein from the column. As used herein, the term "solution" refers to either a buffered or a non-buffered solution, including water.

As used herein, the term "lyotropic" refers to the influence of different salts on hydrophobic interactions, more specifically the degree to which an anion increases the salting out effect on proteins, or for cations, increases the salting-in effect on proteins according to the Hofmeister series for precipitation of proteins from aqueous solutions (Queiroz et al. *J. Biotechnology* 87: 143-159 (2001), Palman et al. *J. Chromatography* 131, 99-108 (1977), Roe et al. *Protein Purification Methods: A Practical Approach*. IRL Press Oxford, pp. 221-232 (1989)). The series for anions in order of decreasing salting-out effect is: PO_4^{3-}>SO_4^{2-}>CH_3COO^->Cl^->Br^->NO_3^->ClO_4^->I^->SCN^-, while the series for cations in order of increasing salting-in effect: NH_4^+<Rb^+<K^+<Na^+<Li^+<Mg^{2+}<Ca^{2+}<Ba^{2+} (Queiroz et al., supra). According to the present invention, combining two different salts having different lyotrophic values with a protein preparation allows more protein to be loaded onto a column with no or negligible breakthrough compared with higher salt concentrations of each single salt.

It is an objective of the present invention to produce conditions for particular proteins which maximize the amount of protein which can be loaded and retained by an HIC column with little or no reduction in the quality of separation of the protein. The present invention is a process for purifying a protein comprising mixing a protein preparation with a buffered salt solution containing a first salt and a second salt, wherein each salt has a different lyotrophic value, and loading the protein salt mixture onto an HIC column.

It is now understood that several factors influence the hydrophobic interactions which control the retention of a native protein to the hydrophobic groups attached to the matrix. These include van der Waals forces, or electrostatic interactions between induced or permanent dipoles; hydrogen bonding, or electrostatic interactions between acidic donor and basic acceptor groups; the hydrophobicity of the

protein itself; and the influence of various salts on hydrophobic interactions. (Queiroz et al., *J Biotechnology* 87:143-159 (2001)). The Hofmeister ("lyotropic") series is an ordering of anions and cations in terms of their ability to precipitate proteins from aqueous solutions, as described above. The series for anions in order of decreasing salting-out effect is: PO_4^{3-}->SO_4^{2-}->CH_3COO^-->Cl^-->Br^-->NO_3^-->ClO_4^-->I^-->SCN^-, while the series for cations in order of increasing salting-in effect: NH_4^+<Rb^+<K^+<Na^+<Li^+<Mg^{2+}<Ca^{2+}<Ba^{2+} (Queiroz et al., supra)

The ions at the beginning of the series promote hydrophobic interactions and protein precipitation or salting out effects, and are called antichaotropic (Queiroz et al., supra). They are considered to be water structuring, whereas the ions at the end of the series are salting-in or chaotropic ions, and randomize the structure of water and tend to decrease the strength of hydrophobic interactions and result in denaturation (Porath et al., *Biotechnol Prog* 3: 14-21 (1987)). The tendency to promote hydrophobic interactions is the same tendency which promotes protein precipitation, and thus determining the salt concentration which causes a particular protein to begin to precipitate is a means of determining an appropriate concentration of that salt to use in an HIC column.

According to the present invention a first salt and a second salt are selected which have differing lyotropic values. This combination of salts acts together to increase the dynamic capacity of the HIC column for a particular protein. It has been found according to the present invention that each salt in combination can be provided at a lower concentration that the concentration of the salt alone to achieve a higher dynamic capacity for a protein compared with the dynamic capacity using a single salt. According to the present invention at least one salt has a buffering capacity at the desired pH.

According to the present invention, the appropriate concentrations of the salts are determined for a particular protein by generating precipitation curves for individual salts, then for combined salts. On the basis of individual salt precipitation curves, precipitation curves for combinations of salts are generated by holding one salt concentration constant, and varying the concentration of the second salt. Then the concentration of the second salt is held constant, and the concentration of the first salt is varied. From these two-salt precipitation curves, concentrations of salts useful for increasing the dynamic capacity of an HIC column can be determined. This is demonstrated in Examples 1 and 2 below, in which the concentrations of two salt combinations are determined using precipitation curves for each particular protein. In addition, the salt concentrations can be optimized to in order to confer additional stability on a protein at room temperature, for example, or to limit aggregate formation. Therefore, the present invention further provides a method of maximizing the dynamic capacity of a hydrophobic interaction chromatography column for a particular protein by selecting a combination of concentrations for a first and second salt having different lyotropic values by generating a series of precipitation curves for the salts alone, and then in combination holding a each salt constant while varying the second.

The salts of the present invention are selected from those having a buffering capacity at the pH at which the protein to be purified is stable. In one embodiment, salt combinations are chosen with a buffering capacity at between about pH 5 to about 7. These include, for example, citrate, phosphate, and acetate, and other mineral acid or organic acid buffers, and combinations of these. A second salt is selected from a salt which may or may not buffer at the desired pH, and can be added to the buffered solution, such as ammonium or sodium sulfate. Cations are selected from those which are non-toxic and non-denaturing. Preferred cations according to the present invention are sodium, potassium, and ammonium, with sodium being the most preferred for manufacturing purposes. Preferred salts for purifying proteins according to the present invention include combinations of sodium citrate, sodium phosphate, sodium acetate, and sodium sulfate.

The concentration of the salts used according to the present invention will depend on the characteristics of the particular salts. In one embodiment, the salts are used at concentrations from about 0.1 M to about 1.0 M in the final concentration of the mixture of salt solution and protein preparation depending on the salt and protein, in another embodiment is in the range between about 0.3 M and about 0.7 M. The pH of the buffered solution may be varied depending on requirements of the protein separation. In one embodiment, the pH varies between about pH 5 to about pH 7.

Hydrophobic Interaction Chromatography Column

The present invention can be used with any type of HIC stationary phase. Stationary phases vary in terms of ligand, ligand chain length, ligand density, and type of matrix or support. Ligands used for HIC include linear chain alkanes with and without an amino group, aromatic groups such as phenyl and N-alkane ligands including methyl, ethyl, propyl, butyl, pentyl, hexyl, heptyl and octyl (Queiroz et al, supra). Many types of HIC columns are available commercially. These include, but are not limited to, SEPHAROSE™ columns such as Phenyl SEPHAROSE™ (Pharmacia LCK Biotechnology, AB, Sweden), FAST FLOW™ column with low or high substitution (Pharmacia LKB Biotechnology, AB, Sweden); Octyl SEPHAROSE™ High Performance column (Pharmacia LKB Biotechnology, AB, Sweden); FRACTOGEL™ EMD Propyl or FRACTOGEL™, EMD Phenyl columns (E. Merck, Germany); MACRO-PREP™ Methyl or MACRO-PREP™ t-Butyl Supports (Bio-Rad, Calif.); WP HI-Propyl (C_3)™ column (J. T. Baker, N.J.); and TOYOPEARL™ ether, phenyl or butyl columns (TosoHaas, Pa.).

In one embodiment, TOYOPEAR™ BUTYL-M columns have been used for purifying proteins as described in Examples 1 and 2.

The mobile phase of HIC according to the present invention is the two salt solution. Commercial applications processes for purifying large quantities of proteins require that the exact ion concentrations of the two salt solution be constant and consistent. Therefore, the adjustment of the dissolved salt solution is made with the acid form of the salt, such as citric acid mixed with citrate to get an exact ion concentration. The salts of the present invention are all commercially available from a number of vendors. At least one salt in the two salt solution will have a buffering effect at the pH at which the protein to be purified is stable. In one embodiment, the buffering capacity of at least one salt is between pH 5 to about pH 7 according to the present invention.

The protocol for using an HIC column according to the present invention is generally as follows. The column is first regenerated with several column volumes of sodium hydroxide, 0.5 N NaOH, for example, then washed with water. The column is then equilibrated with several column volumes of equilibration buffer, which is the same buffer containing the protein preparation for loading onto the column. The protein preparation is prepared by "conditioning" or mixing with the two salt buffered solution. Generally the salt solution is added slowly with the protein preparation at a rate of about 1-2% volume per minute, to avoid protein destabilization. Next, the protein/buffered salt solution mixture is loaded onto the column, and the column washed with several column volumes of equilibrium buffer. The HIC column is then eluted. Elution

can preferably be accomplished by decreasing the salt concentration of the buffer using a salt gradient or isocratic elution. The gradient or step starts at equilibrium buffer salt concentration, and is then reduced as a continuous gradient, or as discrete steps of successively lower concentrations. The elution generally concludes with washing the column with a solution such as a no-salt buffer, such as low ionic strength MES buffer, for example. Elution of the subject protein can also be accomplished by changing the polarity of the solvent, and by adding detergents to the buffer. The protein when purified can be diafiltered or diluted to remove any remaining excess salts.

The method of purifying a protein according to the present invention applies to protein preparations at any stage of purification. Protein purification of recombinantly produced proteins typically includes filtration and/or differential centrifugation to remove cell debris and subcellular fragments, followed by separation using a combination of different chromatography techniques.

A wide range of concentrations of protein can be loaded onto an HIC column using the two salt system of the present invention. The protein preparation to be purified according to the present invention may be of any concentration, however preferably may be varied from about 0.1 mg/ml to about 100 mg/ml or more, more preferably between about 2.5 mg/ml to about 20 mg/ml in an aqueous solution. As used herein the term "protein" is used interchangeably with the term "polypeptide" and is considered to be any chain of at least ten amino acids or more linked by peptide bonds. As used herein, the term "protein preparation" refers to protein in any stage of purification in an aqueous solution. The concentration of a protein preparation at any stage of purification can be determined by any suitable method. Such methods are well known in the art and include: 1) colorimetric methods such as the Lowry assay, the Bradford assay, and the colloidal gold assay; 2) methods utilizing the UV absorption properties of proteins; and 3) visual estimation based on stained protein bands in gels relying on comparison with protein standards of known quantity on the same gel such as silver staining. See, for example, Stoschek *Methods in Enzymol.* 182:50-68 (1990).

For the purposes of the present invention a protein is "substantially similar" to another protein if they are at least 80%, preferably at least about 90%, more preferably at least about 95% identical to each other in amino acid sequence, and maintain or alter the biological activity of the unaltered protein. Amino acid substitutions which are conservative substitutions unlikely to affect biological activity are considered identical for the purposes of this invention and include the following: Ala for Ser, Val for Ile, Asp for Glu, Thr for Ser, Ala for Gly, Ala for Thr, Ser for Asn, Ala for Val, Ser for Gly, Tyr for Phe, Ala for Pro, Lys for Arg, Asp for Asn, Leu for Ile, Leu for Val, Ala for Glu, Asp for Gly, and the reverse. (See, for example, Neurath et al., *The Proteins*, Academic Press, New York (1979)).

The method of purifying proteins according to the present invention is directed to all types of proteins. The present invention is particularly suitable for purifying protein-based drugs, also known as biologics. Typically biologics are produced recombinantly, using procaryotic or eukaryotic expression systems such as mammalian cells or yeasts, for example. Recombinant production refers to the production of the desired protein by transformed host cell cultures containing a vector capable of expressing the desired protein. Methods and vectors for creating cells or cell lines capable of expressing recombinant proteins are described for example, in Ausabel et al, eds. *Current Protocols in Molecular Biology*, (Wiley & Sons, New York, 1988, and quarterly updates).

The method of purifying proteins according to the present invention is particularly applicable to antibodies. As used herein, the term "antibody" refers to intact antibodies including polyclonal antibodies (see, for example *Antibodies: A Laboratory Manual*, Harlow and Lane (eds), Cold Spring Harbor Press, (1988)), and monoclonal antibodies (see, for example, U.S. Pat. Nos. RE 32,011, 4,902,614, 4,543,439, and 4,411,993, and *Monoclonal Antibodies: A New Dimension in Biological Analysis*, Plenum Press, Kennett, McKearn and Bechtol (eds.) (1980)). As used herein, the term "antibody" also refers to a fragment of an antibody such as F(ab), F(ab'), $F(ab')_2$, Fv, Fc, and single chain antibodies which are produced by recombinant DNA techniques or by enzymatic or chemical cleavage of intact antibodies. The term "antibody" also refers to bispecific or bifunctional antibodies, which are an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. (See Songsivilai et al, *Clin. Exp. Immunol.* 79:315-321 (1990), Kostelny et al., *J. Immuno* 1.148:1547-1553 (1992)). As used herein the term "antibody" also refers to chimeric antibodies, that is, antibodies having a human constant antibody immunoglobin domain is coupled to one or more non-human variable antibody immunoglobin domain, or fragments thereof (see, for example, U.S. Pat. No. 5,595,898 and U.S. Pat. No. 5,693,493). Antibodies also refers to "humanized" antibodies (see, for example, U.S. Pat. No. 4,816,567 and WO 94/10332), minibodies (WO 94/09817), and antibodies produced by transgenic animals, in which a transgenic animal containing a proportion of the human antibody producing genes but deficient in the production of endogenous antibodies are capable of producing human antibodies (see, for example, Mendez et al., *Nature Genetics* 15:146-156 (1997), and U.S. Pat. No. 6,300,129). The term "antibodies" also includes multimeric antibodies, or a higher order complex of proteins such as heterdimeric antibodies. "Antibodies" also includes anti-idiotypic antibodies including anti-idiotypic antibodies against an antibody targeted to the tumor antigen gp72; an antibody against the ganglioside GD3; or an antibody against the ganglioside GD2.

One exemplary antibody capable of being purified according to the present invention is an antibody that recognizes the epidermal growth factor receptor (EGFR), referred to as "an antibody against EGFR" or an "anti-EGFR antibody", described in U.S. Pat. No. 6,235,883, which is herein incorporated by reference in its entirety. An antibody against EGFR includes but is not limited to all variations of the antibody as described in U.S. Pat. No. 6,235,883. Many other antibodies against EGFR are well known in the art, and additional antibodies can be generated through known and yet to be discovered means. A preferred antibody against EGFR is a fully human monoclonal antibody capable of inhibiting the binding of EGF to the EGF receptor. The purification of an antibody against EGFR using a dual salt HIC according to the present invention is described herein in Example 1.

Additional exemplary proteins are three IgG monoclonal antibodies having the following designations: mAb1, mAb2, and mAb3. Purification of these monoclonal antibodies according to the present invention is described herein in Example 2.

The invention is also particularly applicable to proteins, in particular fusion proteins, containing one or more constant antibody immunoglobin domains, preferably an Fc domain of an antibody. The "Fc domain" refers to the portion of the antibody that is responsible for binding to antibody receptors on cells. An Fc domain can contain one, two or all of the

following: the constant heavy 1 domain (C_H1), the constant heavy 2 domain (C_H2), the constant heavy 3 domain (C_H3), and the hinge region. The Fc domain of the human IgG1, for example, contains the C_H2 domain, and the C_H3 domain and hinge region, but not the C_H1 domain. See, for example, C. A. Hasemann and J. Donald Capra, Immunoglobins: Structure and Function, in William E. Paul, ed. *Fundamental Immunology*, Second Edition, 209, 210-218 (1989). As used herein the term "fusion protein" refers to a fusion of all or part of at least two proteins made using recombinant DNA technology or by other means known in the art.

An example of an Fc-containing protein capable of being purified according to the present invention is tumor necrosis factor receptor-Fc fusion protein (TNFR:Fc). As used herein the term "TNFR" (tumor necrosis factor receptor) refers to a protein having an amino acid sequence that is identical or substantially similar to the sequence of a native mammalian tumor necrosis factor receptor, or a fragment thereof, such as the extracellular domain. Biological activity for the purpose of determining substantial similarity is the capacity to bind tumor necrosis factor (TNF), to transduce a biological signal initiated by TNF binding to a cell, and/or to cross-react with anti-TNFR antibodies raised against TNFR. A TNFR may be any mammalian TNRF, including murine and human, and are described in U.S. Pat. Nos. 5,395,760, 5,945,397, and 6,201,105, all of which are herein incorporated by reference. TNFR:Fc is a fusion protein having all or a part of an extracellular domain of any of the TNFR polypeptides including the human p55 and p75 TNFR fused to an Fc region of an antibody. An exemplary TNFR:Fc is a dimeric fusion protein made of the extracellular ligand-binding portion of the human 75 kDa tumor necrosis factor receptor linked to the Fc portion of the human IgG1 from natural (non-recombinant) sources. The purification of the exemplary TNFR:Fc according to the present invention is described in Example 2 below.

Additional proteins capable of being purified according to the present invention include differentiation antigens (referred to as CD proteins) or their ligands or proteins substantially similar to either of these. Such antigens are disclosed in *Leukocyte Typing VI* (*Proceedings of the VIth International Workshop and Conference*, Kishimoto, Kikutani et al., eds., Kobe, Japan, 1996). Similar CD proteins are disclosed in subsequent workshops. Examples of such antigens include CD27, CD30, CD39, CD40, and ligands thereto (CD27 ligand, CD30 ligand, etc.). Several of the CD antigens are members of the TNF receptor family, which also includes 41BB ligand and OX40. The ligands are often members of the TNF family, as are 41BB ligand and OX40 ligand.

An exemplary ligand capable of being purified according to the present invention is a CD40 ligand (CD40L). The native mammalian CD40 ligand is a cytokine and type II membrane polypeptide, having soluble forms containing the extracellular region of CD40L or a fragment of it. As used herein, the term "CD40L" refers to a protein having an amino acid sequence that is identical or substantially similar to the sequence of a native mammalian CD40 ligand or a fragment thereof, such as the extracellular region. As used herein, the term "CD40 ligand" refers to any mammalian CD40 ligand including murine and human forms, as described in U.S. Pat. No. 6,087,329, which is herein incorporated by reference in its entirety. Biological activity for the purpose of determining substantial similarity is the ability to bind a CD40 receptor. A preferred embodiment of a human soluble CD40L is a trimeric CD40L fusion protein having a 33 amino acid oligomerizing zipper (or "leucine zipper") in addition to an extracellular region of human CD40L as described in U.S. Pat. No. 6,087,329. The 33 amino acid sequence trimerizes spontaneously in solution.

In addition, a number of other proteins are capable of purified according to the improved purification methods of the present invention include a number of proteins of commercial, economic, pharmacologic, diagnostic, or therapeutic value. Such proteins may be monomeric or multimeric. These proteins include, but are not limited to, a protein or portion of a protein identical to, or substantially similar to, one of the following proteins: a flt3 ligand, erythropoietin, thrombopoeitin, calcitonin, Fas ligand, ligand for receptor activator of NF-kappa B (RANKL), TNF-related apoptosis-inducing ligand (TRAIL), thymic stroma-derived lymphopoietin, granulocyte colony stimulating factor, granulocyte-macrophage colony stimulating factor, mast cell growth factor, stem cell growth factor, epidermal growth factor, RANTES, growth hormone, insulin, insulinotropin, insulin-like growth factors, parathyroid hormone, interferons, nerve growth factors, glucagon, interleukins 1 through 18, colony stimulating factors, lymphotoxin-β, tumor necrosis factor, leukemia inhibitory factor, oncostatin-M, and various ligands for cell surface molecules ELK and Hek (such as the ligands for eph-related kinases or LERKS). Descriptions of proteins that can be stabilized according to the inventive methods may be found in, for example, *Human Cytokines: Handbook for Basic and Clinical Research, Vol. II* (Aggarwal and Gutterman, eds. Blackwell Sciences, Cambridge, Mass., 1998); *Growth Factors: A Practical Approach* (McKay and Leigh, eds., Oxford University Press Inc., New York, 1993); and *The Cytokine Handbook* (A. W. Thompson, ed., Academic Press, San Diego, Calif., 1991).

Additional proteins capable of being purified according to the present invention are receptors for any of the above-mentioned proteins or proteins substantially similar to such receptors or a fragment thereof such as the extracellular domains of such receptors. These receptors include, in addition to both forms of tumor necrosis factor receptor (referred to as p55 and p75) already described: interleukin-1 receptors (type 1 and 2), interleukin-4 receptor, interleukin-15 receptor, interleukin-17 receptor, interleukin-18 receptor, granulocyte-macrophage colony stimulating factor receptor, granulocyte colony stimulating factor receptor, receptors for oncostatin-M and leukemia inhibitory factor, receptor activator of NF-kappa B (RANK), receptors for TRAIL, and receptors that comprise death domains, such as Fas or apoptosis-inducing receptor (AIR). Proteins of interest also includes antibodies which bind to any of these receptors.

Proteins of interest capable of being purified according to the present invention also include enzymatically active proteins or their ligands. Examples include polypeptides which are identical or substantially similar to the following proteins or portions of the following proteins or their ligands: metalloproteinase-disintegrin family members, various kinases, glucocerebrosidase, superoxide dismutase, tissue plasminogen activator, Factor VIII, Factor IX, apolipoprotein E, apolipoprotein A-I, globins, an IL-2 antagonist, alpha-1 antitrypsin, TNF-alpha Converting Enzyme, ligands for any of the above-mentioned enzymes, and numerous other enzymes and their ligands. Proteins of interest also include antibodies that bind to the above-mentioned enzymatically active proteins or their ligands.

Additional proteins of interest capable of being purified according to the present invention are conjugates having an antibody and a cytotoxic or luminescent substance. Such substances include: maytansine derivatives (such as DM1); enterotoxins (such as a Staphlyococcal enterotoxin); iodine

isotopes (such as iodine-125); technium isotopes (such as Tc-99m); cyanine fluorochromes (such as Cy5.5.18); and ribosome-inactivating proteins (such as bouganin, gelonin, or saporin-S6). Examples of antibodies or antibody/cytotoxin or antibody/luminophore conjugates contemplated by the invention include those that recognize the following antigens: CD2, CD3, CD4, CD8, CD11a, CD14, CD18, CD20, CD22, CD23, CD25, CD33, CD40, CD44, CD52, CD80 (B7.1), CD86 (B7.2), CD147, IL-4, IL-5, IL-8, IL-10, IL-2 receptor, IL-6 receptor, PDGF-β, VEGF, TGF, TGF-β2, TGF-β1, VEGF receptor, C5 complement, IgE, tumor antigen CA125, tumor antigen MUC1, PEM antigen, LCG (which is a gene product that is expressed in association with lung cancer), HER-2, a tumor-associated glycoprotein TAG-72, the SK-1 antigen, tumor-associated epitopes that are present in elevated levels in the sera of patients with colon and/or pancreatic cancer, cancer-associated epitopes or proteins expressed on breast, colon, squamous cell, prostate, pancreatic, lung, and/or kidney cancer cells and/or on melanoma, glioma, or neuroblastoma cells, the necrotic core of a tumor, integrin alpha 4 beta 7, the integrin VLA-4, B2 integrins, TNF-α, the adhesion molecule VAP-1, epithelial cell adhesion molecule (EpCAM), intercellular adhesion molecule-3 (ICAM-3), leukointegrin adhesin, the platelet glycoprotein gp IIb/IIIa, cardiac myosin heavy chain, parathyroid hormone, rNAPc2 (which is an inhibitor of factor VIIa-tissue factor), MHC I, carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), tumor necrosis factor (TNF), CTLA-4 (which is a cytotoxic T lymphocyte-associated antigen), Fc-γ-1 receptor, HLA-DR 10 beta, HLA-DR antigen, L-selectin, IFN-γ, Respiratory Syncitial Virus, human immunodeficiency virus (HIV), hepatitis B virus (HBV), *Streptococcus mutans*, and *Staphlycoccus aureus*.

The present invention is particularly useful in the context of commercial production and purification of proteins, especially recombinantly produced proteins. By increasing the capacity of one step in the overall purification scheme of a commercially important protein, the present invention can reduce the number of cycles required to purify a batch of protein. The present invention therefore increases the efficiency of protein purification, without reducing the quality of the protein product. For large-scale production of commercially important biologics, for example, this represents a significant savings in cost and time.

The invention having been described, the following examples are offered by way of illustration, and not limitation.

Example I

Various combinations of salt solutions were tested for their ability to increase the dynamic capacity of an HIC column used for purifying an antibody against epidermal growth factor receptor (antibody against EGFR).

First the range of effective concentrations for single salts ("salts") and two salt buffers for the antibody against EGFR was determined by plotting precipitation curves for single salts and their combinations. The following salts were used: sodium citrate, sodium phosphate, sodium acetate, and sodium phosphate. All buffers were made by weighing out the appropriate chemicals, dissolving at approximately 80% of the final volume, and adjusting the pH using 11.2 N HCl or 10 NaOH to pH 6.0, at room temperature (21-23° C.), and bringing up to volume. For commercial applications, however, the buffered salts are prepared by mixing a salt with its acid form, such as sodium citrate with citric acid, to achieve an exact ion concentration, rather than adjusting to a pH with other acids or bases.

The antibody preparation used for testing was a partially purified eluant from a previous column having a concentration of approximately 5 mg/ml protein. Precipitation studies of this antibody using individual buffers were performed as follows: the antibody preparation was mixed with the buffer stock to make between 0 and 1.2 M final concentration of salt. The samples incubated for 20 minutes, centrifuged for 10 minutes at approximately 6000×g, filtered, and the supernatant assayed for protein. The control sample was diluted with water, and its supernatant reading was taken as 100% recovery. A salting out or precipitation curve was generated for the antibody by plotting amount of protein in the supernatant (percent recovery, compared with the control) versus salt molarity. The percent recovery decreased significantly at greater than about 0.6 M for sodium citrate, while the percent recovery decreased significantly at greater than about 0.8 M for sodium phosphate buffer, at greater than about 1.2 M for sodium acetate, and at greater than about 0.6 M for sodium sulfate. Using this information, a second series of salting out curves for two salt combinations was generated in which the concentration of the first salt was kept constant, while the concentration of the second salt was increased. The precipitation curves were generated by incubating the antibody and two salt mixture for twenty minutes and centrifuging as described for the single salts solutions. For example, sodium citrate was kept at 0.55 M while the concentration of sodium phosphate was increased, and the percent recovery of the antibody in the supernatant was measured and compared with that of the control. The reverse test was also performed keeping 0.4 M sodium phosphate constant while varying the concentration of sodium sulfate. The results are shown in FIG. **1**A through E. These results show that reduced concentrations of the salts together compared with a salt alone could precipitate the protein. This indicated that reduced concentrations of each salt in combination produced equivalent hydrophobic effects compared with higher concentrations of each salt alone.

The results of the single and two salt precipitations provided a range of single and combined salt concentrations for the determination of dynamic capacity for an HIC column for the antibody against EGFR. The dynamic capacity was determined according to the following protocol. An approximately 5 mg/ml antibody preparation was "conditioned" by diluting 1:1 with the appropriate buffered salt stock solution (2×). The salt stock was added to the antibody preparation at a rate of 1-2% volume per minute with stirring. Further salt dilution was performed as necessary to provide a range of salt concentrations, and the mixture of antibody preparation and salt buffer was filtered on a 0.2 um cellulose filter. This mixture was the hydrophobic interaction chromatography (HIC) load. The HIC column used to determine dynamic capacity for single and two salt combinations was a Millipore (Bellerica, Mass.) VANTAGE column having 1.1 cm diameter and packed to 8.5 mL column volume (CV) (9 cm bed height) with TOYOPEARL™ BUTYL 650 M resin (TosoHaas). The column was prepared by regenerating with 0.5N sodium hydroxide at 180 cm/hr for 3 column volumes (CV), washing for 3 CV at 180 cm/hr with water, then equilibrating the column at 180 cm/hr with the appropriate salt buffer or salt combination. Then the load mixture was loaded at 90 cm/hr and washed at 90 cm/hr with 3 CV of the same salt buffer (equilibrium buffer). For determining dynamic capacity, the columns were overloaded with protein, so that fractions were collected during the loading ("flow-through") and washing

steps. Protein content was determined by absorption at 280 nm, or by SDS-PAGE gels. The load concentration in mg/ml-resin at which the % breakthrough is zero is considered to be the dynamic capacity of the antibody at that salt concentration. The dynamic capacity was determined from plotting HIC load versus percent breakthrough (BT) (flow-through concentration/load concentration).

The antibody was then eluted at 180 cm/hr using a step elution or step gradient starting with the equilibrium conditions to a concentration of 0.2 M salt. Fractions were collected and SDS-PAGE analysis was performed on 4-20% Tris/Glycine Novex gels using silver stain (Pharmacia One-Plus™ kit) to visualize protein bands.

Two salt concentrations were optionally further modified in order to stabilize the monomer antibody preparation at room temperature, rather than 4-8° C., and also to minimize the formation of aggregates in the antibody sample. For example, the dynamic capacity of the column for the antibody using 0.4 M sodium phosphate buffer was 43/ml-r (ml-resin); the dynamic capacity of 0.35 M sodium phosphate was 40 mg/ml-r, and the dynamic capacity of 0.3 M sodium phosphate was 38 mg/ml-r. However, 25% protein loss was found to occur at 0.5 M phosphate at room temperature, while only 8% loss was found in 0.4 M for up to six days at room temperature. In addition, it was found that material that precipitated out between 0.3M and 0.4 M salt concentrations included almost all of the high molecular weight aggregates (HMW).

In addition, the rate at which the salt stock was mixed with the antibody preparation influenced the stability of the antibody. At a rate of 2% volume/minute, only about 2% of the antibody was lost as fragments of the monomer, as opposed to 12% lost at 10% volume/minute.

The dynamic capacities of the HIC column for the antibody against EGFR for the various single and combination salts were determined as described above and are shown in Table 1 below.

TABLE 1

Dynamic capacities of antibody against EGFR with four salts and their combinations. Only anions are listed; the cations were sodium for every salt

Experimental Conditions	Dynamic Capacity (mg/ml-r)
0.55M Citrate	24
0.5M Phosphate	12
0.8M Sulfate	24
1.2 M Acetate	5
0.55M Citrate/0.3M Sulfate	30
0.6M Acetate/0.5M Citrate	29
0.35M Phosphate/0.6M Citrate	39
0.6M Acetate/0.7M Sulfate	27
0.5M Citrate/1M Acetate	34
0.5M Sulfate/1M Acetate	33
0.4M Phosphate/0.3M Sulfate	15
0.5M Sulfate/0.3M Citrate	33
0.5M Sulfate/0.3M Phosphate	17
0.3M Citrate/0.6M Phosphate	35

Table 1 shows that the combinations of citrate/sulfate, acetate/citrate, phosphate/citrate, acetate/sulfate, citrate/acetate, sulfate/acetate, sulfate/citrate, and citrate/phosphate increased the dynamic capacity of the HIC column for the antibody by factors varying from approximately 1.5 to 2 times or more that of each salt alone. The phosphate/sulfate combination did not increase the dynamic capacity for the following reasons: sulfate in combination with phosphate resulted in a precipitate, so that lower concentrations of sulfate were required to prevent precipitation. These low concentrations proved too low to improve dynamic capacity. In addition, phosphate and acetate did not prove to be an effective combination due to the precipitation which resulted when the two salts were mixed.

Example 2

Using the same procedures as described in Example 1 the dynamic capacities of four additional proteins was determined for the single salts sodium phosphate and sodium citrate, and two salt combination 0.55 M sodium citrate with phosphate concentration varied. The additional proteins were the fusion protein TNFR:Fc described above, and three monoclonal antibodies designated mAb1, mAb2, and mAb3. The three monoclonal antibodies were partially purified and obtained as eluants from other types of chromatography columns. The TNFR:Fc fusion protein was obtained as a fully purified protein. The concentrations of the proteins used was between 4-5 mg/ml, for this particular experiment.

The precipitation curves for sodium citrate and sodium phosphate alone were first determined for each protein, and then a two salt precipitation curve for 0.55M sodium citrate with sodium phosphate varied was determined. The concentration at which each protein begins to precipitate is given in Table 2 below.

TABLE 2

Salt concentrations at which protein begins to precipitate (taken from the precipitation curves.)

Protein	Conc. Sodium Citrate	Conc. Sodium Phosphate	Combination Salt
mAb1	0.6M	0.9M	0.55M NaCitrate/ 0.4M Na Phosphate
mAb2	0.7M	1.1M	0.55M Na Citrate/ 0.4M Na Phosphate
mAb3	0.7M	1.0M	0.55M Na Citrate/ 0.2M Na Phosphate
TNFR:Fc	0.55M	1.0M	0.4M Na Citrate/ 0.2M Na Phosphate

It is clear from Table 2 that the combination of salts precipitated the proteins at lower concentrations compared to the concentrations of each salt alone.

The dynamic capacities of these proteins on TOYOPEARL™ BUTYL 650M (TosoHaas) gels was determined for the salt concentrations shown in Table 2, using the same procedure described above for the antibody against EGFR. The results are given in Table 3 below.

TABLE 3

Dynamic capacities under the salt conditions listed in Table 2.

Protein	Na Citrate	Na Phosphate	Combination
mAb1	37	20	49
mAb2	36	30	44
mAb3	21	12	25
TNFR:Fc	17	18	25

Again, it is clear that the combination of salts increased the dynamic capacity for all four proteins over that achieved using the single salts by 1.5 to 2 times.

The present invention is not to be limited in scope by the specific embodiments described herein, which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are

within the scope of the invention. Indeed, various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

What is claimed is:

1. A process for purifying a protein on a hydrophobic interaction chromatography column such that the dynamic capacity of the column is increased for the protein comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, loading the mixture onto a hydrophobic interaction chromatography column, and eluting the protein, wherein the first and second salts are selected from the group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, respectively, and wherein the concentration of each of the first salt and the second salt in the mixture is between about 0.1 M and about 1.0.

2. The process of claim **1** wherein the pH of the mixture loaded onto the column is between about pH 5 and about pH 7.

3. The process of claim **1** wherein the column is eluted with a solution having a pH between about pH 5 and pH 7.

4. The process of claim **1** wherein the first and second salts are selected from the group consisting of sodium, potassium and ammonium salts.

5. The process of claim **1** wherein the protein is a fusion protein or an antibody.

6. The process of claim **1**, further comprising diluting the protein.

7. The process of claim **1**, further comprising filtering the protein.

8. The process of claim **1**, further comprising formulating the protein.

9. The process of claim **1**, further comprising lyophilizing the protein.

10. A method of increasing the dynamic capacity of a hydrophobic interaction chromatography column for a protein, comprising mixing a preparation containing the protein with a combination of a first salt and a second salt, and loading the mixture onto a hydrophobic interaction chromatography column, wherein the first and second salts are selected from the group consisting of citrate and sulfate, citrate and acetate and sulfate and acetate, respectively, and wherein the concentration of each of the first and second salts in the mixture is between about 0.1 M and about 1.0 M.

11. The method of claim **10** wherein the pH of the mixture loaded onto the column is between about pH 5 and about pH 7.

12. The method process of claim **10**, wherein the first and second salts are selected from the group consisting of sodium, potassium and ammonium salts.

13. The method of claim **10** wherein the protein is a fusion protein or an antibody.

* * * * *

CERTIFICATE OF SERVICE

I hereby certify that on this 20th of August, 2018, I caused the BRIEF FOR PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING, LIMITED (CONFIDENTIAL AND NON-CONFIDENTIAL) to be filed with the Clerk of the Court using the NextGen System. I also caused a true and correct copy of the BRIEF FOR PLAINTIFFS-APPELLANTS AMGEN INC. AND AMGEN MANUFACTURING, LIMITED (CONFIDENTIAL AND NON-CONFIDENTIAL) to be electronically served, pursuant to agreement of the parties, on Defendant-Appellee Coherus Biosciences Inc.'s counsel of record as follows:

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CERTIFICATE OF COMPLIANCE

This brief complies with the type-volume limitation of Federal Circuit Rule 32(a). The brief contains 11,985 words, excluding parts of the brief exempted by Fed. R. App. P. 32(f) and Federal Circuit Rule 32(b). The word count includes the words counted by the Microsoft Word 2016 function. This brief also complies with the typeface requirements of Fed. R. App. P. 32(a)(5) and the type style requirements of Fed. R. App. P. 32(a)(6). The brief has been prepared in a proportionally spaced typeface using Microsoft Word 2016 in 14-point font of Times New Roman.

Dated: August 20, 2018

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UNITED STATES COURT OF APPEALS FOR THE FEDERAL CIRCUIT

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(Date)