IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF DELAWARE

AMGEN INC. and AMGEN MANUFACTURING, LIMITED,	)	
Plaintiffs,	)	
v.	)	Case No. 17-546 (LPS) (CJB)
COHERUS BIOSCIENCES, INC.	)	[redacted]
Defendant.	)	**PUBLIC VERSION**

COHERUS BIOSCIENCES INC.'S MEMORANDUM OF LAW IN SUPPORT OF MOTION TO DISMISS PURSUANT TO FED. R. CIV. P. 12(B)(6)

Dated: June 1, 2017

OF COUNSEL:
Bradford P. Lyerla
Louis E. Fogel
JENNER & BLOCK LLP
353 N. Clark Street
Chicago, IL 60654
(312) 222-9350

Kenneth L. Dorsney (#3726)
Richard K. Herrmann(#405)
MORRIS JAMES LLP
500 Delaware Avenue, Suite 1500
Wilmington, DE 19801
(302) 888-6800
kdorsey@morrisjames.com

TABLE OF CONTENTS

TABLE OF AUTHORITIES

Page(s)

CASES

STATUTES

OTHER AUTHORITIES

I. INTRODUCTION

Defendant Coherus BioSciences, Inc. ("Coherus") moves for immediate dismissal of this patent infringement action because there is no plausible interpretation under which Coherus would infringe the patent-in-suit. As explained below, this suit involves an alleged artificial act of infringement—namely, that Coherus has filed an abbreviated Biologic License Application ("aBLA") seeking FDA approval to market a generic (or "biosimilar") version of Amgen's Neulasta pharmaceutical product. There is no dispute about what Coherus does in its accused manufacturing step, for it is spelled out in the aBLA. The sole dispute is a legal one: can the patent's claims be read broadly enough to cover the accused manufacturing step disclosed in Coherus' aBLA?

The answer is no, for two reasons. *First*, the patent requires that a manufacturing process use one of three listed pairs of salts: citrate and sulfate, citrate and acetate, or acetate and sulfate. [redacted]

Second, even setting aside that glaring deficiency, the patent requires that *each* salt be present in a concentration of at least "about 0.1 M."[1] And there is no dispute that Coherus's accused process uses [redacted]

Non-infringement is so clear that Coherus surmises Amgen is pursuing this case not for its merits, but rather to throw a costly and artificial wrench into Coherus's plans to launch a biosimilar of Neulasta. This motion should be granted to prevent that from happening.

[1] "M" refers to "moles per liter," a measure of concentration. One M is equivalent to 1000 millimoles per liter, abbreviated "mM."

II. NATURE AND STAGE OF THE PROCEEDINGS

Plaintiffs Amgen Inc. and Amgen Manufacturing, Limited (collectively, "Amgen") filed this action against Coherus on May 10, 2017, alleging infringement of U.S. Patent No. 8,273,707 ("the '707 patent"). The complaint, based on the Biologics Price Competition and Innovation Act ("BPCIA"), attempts to block the entry of biosimilar competition to Amgen's biologic drug product Neulasta. Coherus moves to dismiss the case for failure to state a claim.

III. SUMMARY OF THE ARGUMENT

Coherus is entitled to dismissal because, as a matter of law, its accused manufacturing process does not infringe any claim of the '707 patent. All of those claims are directed to a column chromatography process that requires, among other things, (1) loading onto the column a protein mixture that includes one of three specified pairs of salts, where (2) each of the salts is present in this mixture at concentrations of at least "about 0.1 M." As a matter of law, the process described in the Coherus aBLA, and already disclosed to Amgen pursuant to the BPCIA, satisfies neither of these limitations.

1. Coherus's accused chromatography process does not load onto the column a protein mixture containing any of the salt pairs listed in the claims (citrate and sulfate, citrate and acetate, or acetate and sulfate). Instead, Coherus's process uses [redacted] Because this is not one of the three listed pairs, there can be no literal infringement. Nor can there be infringement under the doctrine of equivalents. During prosecution, Amgen overcame a rejection on the ground that "the *particular* combination of salts recited in the pending claims" was not taught or suggested by the prior art—which disclosed using [redacted] the very ones that Coherus's process uses.

2. [redacted] The claims re-

quire that the loading mixture (the solution poured onto the chromatography column) contain each salt in a concentration of at least "about 0.1 M." [redacted] And during prosecution, Amgen disclaimed a prior art concentration of 0.04 M, [redacted]

3. The salt concentrations used in the "elution" step of Coherus's accused process is irrelevant to infringement. In the claimed process, the salt pair must increase the amount of protein that can be loaded onto the chromatography column without being washed away "before elution." Any salts used in the elution step therefore cannot bear on infringement—as the claims, the specification, and the prosecution history all make clear.

4. This case's distinctive posture makes dismissal appropriate even at this early stage. As the BPCIA envisions, Coherus has supplied Amgen with its full aBLA, and the parties have exchanged detailed infringement and invalidity contentions—all before Amgen commenced this litigation. The accused process is, by definition, the one described in the aBLA, and no amount of additional detail concerning that process could be material to the question of infringement. Nor could the claims plausibly be construed in any manner that encompasses the accused process. These circumstances warrant immediate dismissal.

IV. FACTUAL BACKGROUND

The BPCIA provides a streamlined process for an applicant (here, Coherus) to obtain FDA approval to market a "biosimilar" drug product—*i.e.*, one that is "highly similar" to an already-approved biologic product, known as the "reference product." *See* 42 U.S.C. § 262(i), (k). In August 2016, Coherus filed an abbreviated Biologic License Application ("aBLA") seeking authorization to market a biosimilar of Amgen's pegfilgrastim product, Neulasta. Compl. ¶ 10.

The BPCIA establishes a patent dispute resolution regime that requires the parties to exchange a significant amount of technical information and litigation contentions prior to district court proceedings. *See* 42 U.S.C. § 262(*l*). Here, Coherus provided Amgen with its entire aBLA, which details the product that Coherus plans to market and the manufacturing process that Coherus plans to use. *See id.* § 262(*l*)(2)(A); Compl. ¶¶ 12-13. After Amgen identified the patents for which it believed infringement "could reasonably be asserted" against Coherus, the parties exchanged detailed infringement and validity contentions, with Coherus's running to nearly 500 pages of narrative and claim charts. 42 U.S.C. § 262(*l*)(3)(A)-(C); Compl. ¶ 36. Ultimately, because the BPCIA treats the filing of an aBLA as an artificial act of patent infringement, *see* 25 U.S.C. § 271(e)(2)(C)(i), Amgen sued Coherus in this Court, alleging that Coherus's process for manufacturing its pegfilgrastim biosimilar infringes the '707 patent.

A. The '707 Patent

Biologic drug products, such as those at issue here, are made by producing therapeutic proteins inside living cells. Those proteins must then be separated from the cell debris and other impurities, a multi-step process known as protein purification. '707 Patent at 1:19-35.

One technique used in protein purification is column chromatography. *See id.* at 1:36-51, 3:53-54 (describing hydrophobic interaction chromatography ("HIC"), which is a form of column chromatography). In column chromatography, a column filled with solid particles, called the resin or the "matrix," has a solution containing the desired protein and the impurities poured onto it. *Id.* at 1:36-45. With an appropriate selection of matrix material and loading solution, the desired protein's chemical properties cause it to adhere to the matrix as the solution flows through the column. *Id.* at 1:40-45, 3:53-61. This step is referred to as "loading the mixture" onto the column. *Id.; see also* 15:12-13. Then, any unbound impurities remaining in the column are flushed (or "washed") away by pouring more solution through the column. *Id.* at 4:27-29.

Finally, molecules of the desired protein remaining on the matrix are detached (or "eluted") by pouring a different solution (one that disrupts the interactions between the protein and the matrix) through the column. *Id.* at 1:45-49. The solution flowing off the column in this final "elution" step contains the desired protein, but with far fewer impurities than the solution initially loaded onto the column.

Sometimes, there is too much of the desired protein for all of it to stick to the matrix when loaded. As a result, significant amounts of the protein can be washed away with the impurities, and lost before elution begins—a problem known as "breakthrough." *Id.* at 3:37-41. The '707 patent is directed at increasing "the amount of protein that can be loaded onto a column without 'breakthrough' or loss of protein to the solution phase before elution." *Id.* The patent refers to this amount as the column's "dynamic capacity." *Id.* at 4:10-14.

Amgen's claimed invention purports to increase the column's dynamic capacity by using a pair of salts in the loading solution (which the patent calls the "loading buffer" or "equilibrium buffer"). '707 Patent at 4:24; *see id.* at 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 . . . ."). The patent explains: "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.* at 4:56-60.

During prosecution, Amgen made clear that increasing a column's dynamic capacity was what saved the claimed invention from being ruled unpatentable in light of prior art. Responding to an office action, Amgen argued that the prior art did not "disclose each and every element of the claimed method." Ex. 1, August 22, 2011 Response to Office Action at 5. Amgen elaborat-

ed: "Applicants strenuously disagree with the Patent Office's conclusion and submit that the Patent Office's argument again overlooks two elements of the claimed method—the use of a *combination* of salts in the HIC operation, and the *enhancement of the dynamic capacity of a HIC column* imparted by applicants' method." *Id.* (emphasis in original); *see* '707 Patent at 3:38-41 (defining "dynamic capacity" as "the amount of protein that can be loaded onto a column without 'breakthrough' or loss of protein to the solution phase before elution").

Amgen also left no doubt that its claimed invention was limited to processes using *particular* salt pairs in loading. The examiner rejected the claims as obvious in view of U.S. Patent No. 5,231,178 to Holtz, which disclosed using salts including "sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate and the like." *See* Ex. 2, Oct. 13, 2010 Office Action at 4. In response, Amgen argued that "[n]o combinations of salts is taught or suggested in the Holtz et al. patent, nor is the *particular* combinations of salts recited in the pending claims taught or suggested in this reference." Ex. 3, Jan. 26, 2011 Response to Office Action at 5 (emphasis in original). Amgen also submitted an inventor declaration that discussed the advantages of three particular salt pairs: "sulfate/citrate," "sulfate/acetate," and "acetate/citrate." The declaration stated that "[u]se of *this particular combination of salts* greatly improves the cost-effectiveness of commercial manufacturing." *Id*. at 6-7; Ex. 4, Jan. 20, 2011 Dec'l of Anna Senczuk ¶ 4, (emphasis added).

Amgen also made clear, in prosecution of the '707 patent's parent, that the respective concentrations of the two salts mattered. As relevant here, the parent application included claims that set "about 0.1 M" as the lower limit of the concentration of each salt. Ex. 5, U.S. Application No. 10/895,581, Nov. 16, 2007 Response to Office Action and Amendment at 3. The Examiner rejected the claims as anticipated over prior art that used acetate and phosphate salts at a

concentration of 40 mM, or 0.04 M. Ex. 6, U.S. Application No. 10/895,581, Feb. 14, 2008 Office Action at 2-3; Ex. 7, U.S. Application No. 10/895,581, July 14, 2008, Response to Office Action and Amendment at 6. In response, Amgen argued that the concentration of 0.04 M was lower than "about 0.1 M." Amgen explained:

> The precipitated protein is then resuspended . . . in a solution of 16% saturated ammonium sulfate, 40 mM sodium acetate, 40 mM sodium phosphate, pH 4.5, and 0.4M NaCl, and this solution is then loaded onto the HIC column . . . . Again Holtz et al. . . . does not teach or suggest combining the protein to be purified with the particular *combination of two salts, citrate and phosphate salts* at concentrations of between about *0.1 M and 1.0M* before loading the protein on the HIC column. Instead, a protein solution containing lower concentrations of sodium acetate and sodium phosphate, together with NaCl and a high concentration of ammonium sulfate (four salts, not a combination of two salts as recited in the claimed method), is loaded onto the HIC column.

Ex. 7 at 6. (Emphasis in original). Thus, to avoid a prior art reference, Amgen argued that 40 mM, or 0.04 M, was a "lower concentration[]" than "about 0.1 M"—the language demarcating the lower bound of the required salt concentration. *Id.* The parent claims issued with that language. *See* Ex. 8, U.S. Patent No. 7,781,395.

The patent-in-suit, meanwhile, ultimately issued with thirteen claims, of which claims 1 and 10 are the only independent claims. Claim 1 reads as follows:

> 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 15:8-18 (emphasis added). Claim 10 reads as follows:

> 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 mix-

> ture 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. at 16:9-18 (emphasis added). All other claims depend from claims 1 and 10. Thus, every claim in the '707 patent requires the use of pairs of salts—in the loading solution—chosen from among citrate, acetate, or sulfate. Moreover, every claim requires that each salt be present at a concentration of "between about 0.1 M and about 1.0 M."

B. Coherus' Manufacturing Process

[redacted]

[redacted]

V. ARGUMENT

This Court should dismiss this case at the pleading stage because it does not state a plausible claim of infringement. Taking into account the prosecution history and Coherus's aBLA—both of which the Court can consider in deciding this motion—Coherus's accused process does not infringe as a matter of law.

A. Governing Law

To withstand a motion to dismiss under Rule 12(b)(6), a complaint must contain "enough facts to state a claim to relief that is plausible on its face." *Bell Atl. Corp. v. Twombly*, 550 U.S. 544, 570 (2007). This standard "demands more than an unadorned, the-defendant-unlawfully-harmed-me accusation" or "[t]hreadbare recitals of the elements of a cause of action, supported by mere conclusory statements." *Ashcroft v. Iqbal*, 556 U.S. 662, 678 (2009) (quoting *Twombly*, 550 U.S. at 555). Rather, to survive a motion to dismiss, a complaint must contain enough factual "heft" to "nudge" claims "across the line from conceivable to plausible" in showing that the plaintiff "is entitled to relief." *Twombly*, 550 U.S. at 557, 570 (internal quotation marks omitted). Both the Federal Circuit and this Court have applied these standards to dismiss infringement complaints. *See, e.g.*, *AstraZeneca Pharms. LP v. Apotex Corp.*, 669 F.3d 1370, 1381 (Fed. Cir. 2012); *Cumberland Pharmaceuticals Inc. v. Sagent Agila LLC*, No. 12-825, 2013 WL 5913742 (D. Del. Nov. 1, 2013) (Stark, J.); *Cumberland Pharmaceuticals Inc. v. InnoPharma, Inc.*, No. 12-618, 2013 WL 5945794 (D. Del. Nov. 1, 2013) (Stark, J.).

Importantly, a court ruling on a Rule 12(b)(6) motion may consider not just the complaint itself, but also documents integral thereto. *See, e.g.*, *AstraZeneca*, 669 F.3d at 1378 n.5; *In re Burlington Coat Factory Sec. Litig.*, 114 F.3d 1410, 1426 (3d Cir. 1997). Thus, when those documents establish facts that negate the plaintiff's claim for relief, a Rule 12(b)(6) motion may be granted on that basis. *See, e.g.*, *Cumberland Pharms.*, 2013 WL 5913742. Here, this rule means that the Court may consider the contents of Coherus's aBLA, which forms the basis for the complaint's accusations of infringement. *See* Compl. ¶¶ 46, 56, 62, 68. The Federal Circuit has reached the same conclusion in the parallel setting of Hatch-Waxman Act litigation, where the filing of an Abbreviated New Drug Application ("ANDA") provides the predicate for a patent infringement lawsuit. *See AstraZeneca*, 669 F.3d at 1378 n.5. Reasoning that "the district court was entitled to examine documents integral to or explicitly relied upon in the complaint in evaluating [the] motions to dismiss," the Federal Circuit concluded: "[The] complaints referenced and relied upon Appellees' FDA filings, and the parties do not dispute the authenticity of the documents that were before the court. We therefore see no error in the district court's decision to consider these documents." *Id.*

A court ruling on a Rule 12(b)(6) motion may also consider the prosecution history of the patent-in-suit. *See Genetic Techs. Ltd. v. Bristol-Myers Squibb Co.*, 72 F. Supp. 3d 521, 526 (D. Del. 2014) (Stark, J.) (court deciding motion "may . . . take judicial notice of the prosecution histories, which are 'public records'"); *Int'l Business Machines Corp. v. Priceline Group Inc.*, No. 15-137-LPS-CJB, 2016 WL 626495, *20 n.18 (Feb. 16, 2016); *Quest Integrity USA, LLC v. Clean Harbors Indus. Servs., Inc.*, Nos. 14-1482-SLR, 14-1483-SLR, 2015 WL 4477700, *1 n.4 (D. Del. Jul. 22, 2015) (prosecution history "is a public document that the court may rely upon in deciding this motion to dismiss").

B. The Complaint Fails To State A Claim For Infringement.

The '707 patent is directed to purifying a protein with chromatography using a HIC column. The asserted claims require mixing together "a preparation containing the protein" (here, filgrastim) "with a combination of a first salt and a second salt," and then loading this mixture onto the column. The first and second salts are "selected from the group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate, respectively," and each salt in the pair must be present at a concentration of at least "about 0.1 M."

As explained above, Amgen accuses Coherus's [redacted] of infringement. For that process to infringe, Coherus must load a protein solution containing one of the listed pairs of salts (as well as filgrastim) onto the chromatography column, and each salt must be present in a concentration of at least "about 0.1 M." But the aBLA makes clear that [redacted] Therefore, Coherus' accused process cannot infringe the '707 patent.

1. Coherus' Process Does Not Use Any Of The Required Salt Pairs.

Coherus' process cannot literally infringe because [redacted] is not "selected from the group consisting of citrate and sulfate, citrate and acetate, and sulfate and acetate"—a limitation of both asserted independent claims. '707 Patent at 15:14-16; *id.* at 16:14-16. Instead, Coherus uses [redacted]. For that reason alone, Coherus's process cannot literally infringe any asserted claim. And indeed, Amgen's complaint does not even contend that any of these salt pairs is used. Instead, it alleges only that Coherus uses a salt pair that "is *equivalent* of one or more of the recited salt

pairs." Compl. ¶ 50 (emphasis added). Consequently, Amgen's case is limited to infringement under the doctrine of equivalents.

As a matter of law, Amgen cannot make out a doctrine-of-equivalents claim with respect to the salt pair that Coherus uses. That is because during prosecution of the '707 patent, Amgen distinguished prior art—and overcame the examiner's rejection—on the ground that the prior art did not teach or suggest the particular combinations of salts recited in the claims. The Holtz patent, Amgen explained, did not teach or suggest these combinations. At the same time, Amgen submitted a declaration that specifically touted the advantages of these three specific pairs, and no others. *See supra*.

Having 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. The doctrine of prosecution history estoppel prevents this result by barring Amgen from asserting a range of equivalents that includes subject matter surrendered during prosecution. *See, e.g.*, *Trading Techs. Int'l, Inc. v. Open E Cry, LLC*, 728 F.3d 1309, 1323 (Fed. Cir. 2013); *Texas Instruments, Inc. v. United States ITC*, 988 F.2d 1165, 1174-75 (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-side gating was known in the art, the inventors unmistakably excluded the same-side gating as an equivalent."); *Conoco, Inc. v. Energy & Envt'l Int'l, LC*, 460 F.3d 1349, 1363 (Fed. Cir. 2006) (explaining that prosecution history estoppel can arise either through an amendment to the claim or through argument to the examiner); *see also Festo Corp. v. Shoketsu Kinzoku Kogyo Kabushiki Co.*, 535 U.S. 722 (2002).

Resort to the doctrine of equivalents is barred for a further reason, too—namely, the dedication-disclosure rule. Under that rule, when a patentee "discloses but declines to claim subject

matter," it necessarily "dedicates that unclaimed subject matter to the public" and places it beyond the reach of the doctrine of equivalents. *Johnson & Johnston Assoc., Inc. v. R.E. Service Co.*, 285 F.3d 1046, 1055-56 (Fed. Cir. 2002). [redacted]

2. Coherus' Process Does Not [redacted]

Even if Coherus's [redacted] were somehow deemed sufficient to satisfy the salt-pair limitation of the claims, there still can be no infringement because, in the Coherus process, the salts are not loaded in the proper concentrations. The claims require that the concentration of each of the loading mixture's two salts "is between about 0.1 M and about 1.0 M." '707 Patent at 15:18; *id.* at 16:18. Thus, the minimum concentration required for infringement is "about 0.1 M." [redacted]

No plausible construction of "about 0.1 M" could stretch the minimum concentration to encompass [redacted] Still, there is no need to determine exactly how much flexibility "about" provides—because Amgen surrendered any claim to processes that use salt concentrations as low as Coherus's. As explained above, the parent application of the '707 patent used claim language that set "about 0.1 M" as the lower limit of each salt's concentration in the loading mixture. Ex. 10, U.S. Application No. 10/895,581, April 13, 2007, Response to Restriction Requirement at 3. The Examiner rejected the claims as anticipated by prior art that used

acetate and phosphate salts at a concentration of 0.04 M. Ex. 7 at 6. Amgen overcame this rejection by arguing that the prior art's concentration—0.04 M, [redacted]—was below the claimed range of "about 0.1 M to about 1.0 M." *Id.* at 6.

By arguing that a concentration of 0.04 M was below the range of "about 0.1 M to about 1.0 M," Amgen necessarily disclaimed processes using even lower concentrations. When an applicant secures a patent by arguing that the claims do not encompass certain subject matter, it cannot later assert the contrary. *See, e.g., Chimie v. PPG Indus.*, 402 F.3d 1371, 1384 (Fed. Cir. 2005). Here, because Amgen secured its patent by arguing that 0.04 M was below "about 0.1 M," it has disclaimed any argument that even lower concentrations literally infringe.

The fact that Amgen made this argument in prosecuting the parent of the '707 patent—rather than the '707 patent itself—does not undermine this conclusion. It is well-settled that "prosecution disclaimer may arise from disavowals made during the prosecution of ancestor patent applications." *Ormco Corp. v. Align Tech., Inc.*, 498 F.3d 1307, 1314 (Fed. Cir. 2007) (quoting *Omega Eng'g, Inc. v. Raytek Corp.*, 334 F.3d 1314, 1333 (Fed. Cir. 2003)). The question is whether the statements from the prosecution "relat[e] to the same subject matter as the claim language at issue in the patent being construed." *Id*. Here, the statements do not just relate to the same subject matter—the claim language at issue in prosecution is *identical* to the language of the '707 patent's claims.[3]

[3] *See, e.g.*, Ex. 7 at 3 (claim for "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, where 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 [M]*" (emphasis added)); *id.* at 4 (claim for "A method of increasing the dynamic capacity of a hydrophobic interaction chromatography column for a particular 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 hy-

Nor can Amgen resort to the doctrine of equivalents and argue [redacted] "is equivalent to a concentration within the claimed range." D.I. 1, ¶ 50. For one thing, Amgen's arguments during prosecution (described immediately above) estop it from arguing that concentrations below 0.04 M are equivalent to the claimed lower limit of "about 0.1 M," no less than they bar Amgen from claiming literal infringement. *See, e.g.*, *Trading Techs.*, 728 F.3d at 1323; *Festo*, 535 U.S. at 734.

3. Coherus' Elution Buffer Is Irrelevant To Infringement.

[redacted]

First, the salt in the elution buffer is irrelevant because all of the claims require that the two-salt process increase the "dynamic capacity" of the column. That language is present in the claims themselves. *See* '707 Patent at 15:9-10 (process "such that the dynamic capacity of the column is increased"); *Id.* at 16:9 ("method of increasing the dynamic capacity"). In addition, and as explained above, Amgen distinguished prior art during prosecution for *not* providing any increase in dynamic capacity. *See supra*. [redacted]

drophobic chromatography column, wherein the first and second salts are citrate and phosphate salts, 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*" (emphasis added)).

[redacted]

Dynamic capacity, the patent makes clear, is an attribute that the column must possess *before* elution. The specification defines "dynamic capacity" as "the amount of protein that can be loaded onto a column without 'breakthrough' or loss of protein to the solution phase *before elution*." '707 Patent at 3:37-41 (emphasis added). As a logical matter, salts added as part of the elution buffer cannot decrease the amount of protein lost before the elution step, and hence can have no impact on the column's dynamic capacity. Rather, to accomplish the required increase in dynamic capacity, the salt pairs recited by claims 1 and 10 must be present in the column in the correct concentrations *before* elution begins.

Second, the salt in the elution buffer is irrelevant because—consistent with the requirement to increase dynamic capacity—the claims list the mixing step before the step in which the protein is loaded. Claim 1 states that the process comprises "*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." '707 Patent at 15:11-14 (emphasis added). Similarly, Claim 10 states that the process comprises "*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." *Id.* at 16:11-14 (emphasis added). In each instance, the salt pair must be mixed with the protein preparation before the mixture is loaded onto the column.[4] It would make no sense, therefore, to treat salts added during elution—

[4] Consistent with this common-sense understanding, the complaint alleges that "the Coherus mixture containing protein and dual salts is loaded onto a hydrophobic interaction chromatography column." Compl. ¶ 50.

after the protein has *already* been mixed and loaded, and bound to the column—as satisfying the salt pair limitation.

Third, Amgen cannot overcome these problems by resorting to the doctrine of equivalents. For one thing, as noted above, any salt present in the elution buffer necessarily cannot perform the function of the salts in Amgen's claimed invention—namely, to increase the column's dynamic capacity—because the protein has *already* been loaded and bound onto the column. *See, e.g.*, *Akzo Nobel Coatings, Inc. v. Dow Chem. Co.* 811 F.3d 1334, 1341-1342 (Fed. Cir. 2016) (equivalent element must "perform[] *the same function* in substantially the same way to reach the same result" (emphasis added)). Indeed, the patent defines dynamic capacity as the amount of the desired protein that can be introduced into the column without washing away *before* elution begins. For another thing, prosecution history estoppel (which is a question of law) bars Amgen from treating the salt concentration in Coherus's elution buffer as equivalent to the salt concentration in the loading solution recited in the claims. Amgen overcame the Examiner's rejection by arguing that the invention increased dynamic capacity, whereas the prior art did not. *See supra*. Amgen is therefore barred from contending that salt concentrations that *cannot possibly increase dynamic capacity*—such as any salt added during elution—infringe under the doctrine of equivalents.

Finally, even if the elution salts could somehow be relevant, [redacted]

[redacted] Once again, the claims all require one of three listed salt pairs: acetate and sulfate, acetate and phosphate, or sulfate and phosphate. [redacted]

The absence of any listed pair means that Amgen cannot show literal infringement; for the rea-

sons stated above, moreover, it cannot show infringement by equivalents either. *See supra* § V.B.1.

C. The Court Can Grant Coherus's Motion Now.

Amgen will likely maintain that the arguments herein are better suited to a later stage of the case. Not so: Dismissal at this early stage is entirely appropriate, in view of the parties' exchange of information and the clarity of the issues.

First, this case comes to the Court in a different posture from the typical patent case, and even from the typical Hatch-Waxman case. The BPCIA envisions that the parties will exchange a significant amount of information before any lawsuit is filed, including detailed infringement and invalidity contentions. *See* 42 U.S.C. § 262*(l)*. The parties here have done just that—and, as a result, have considerably narrowed the issues for litigation. *See* Compl. ¶ 36.

Second, no amount of discovery could change Amgen's (or the Court's) understanding of the accused process in any way material to this motion. Coherus's aBLA describes the process in sufficient detail to establish, as a matter of law, that there can be no infringement. [redacted] And it is the aBLA that controls the infringement inquiry: that document is itself the predicate for Amgen's infringement action, as well as the foundation for the parties' exchange of detailed infringement contentions. *See*, *e.g.*, 42 U.S.C. § 262(*l*)(3)(A)(i) (addressing possible infringement through the unlicensed "making, using, offering to sell, selling, or importing into the United States of *the biological product that is the subject of the subsection (k) application*" (emphasis added)); 25 U.S.C. §§ 271(e)(2), 271(e)(2)(C)(i) (filing an aBLA "shall be an act of infringement"); Compl. ¶ 15 ("Co-

herus committed an act of infringement with respect to the '707 Patent under 35 U.S.C. § 271(e)(2)(C)(i) when it submitted its aBLA for the purpose of obtaining FDA approval to engage in the commercial manufacture, use, or sale of the Coherus Pegfilgrastim Product."). Meanwhile, marketing of pegfilgrastim manufactured in a manner *different* from what the aBLA describes would be outside the scope of FDA approval sought by Coherus. And regardless of whether it would be lawful for Coherus to market pegfilgrastim made with a non-aBLA process, the complaint provides no reason to think that such an eventuality is more than hypothetical.

Third, there is no need to wait for a formal process of claim construction. No construction of the relevant claim terms could possibly encompass the process described in the aBLA. That much is clear not only from the claim language, but from Amgen's arguments during prosecution—as described in detail above. Waiting until a *Markman* ruling would merely saddle Coherus with the costs of defending against Amgen's meritless infringement suit.

VI. CONCLUSION

For the foregoing reasons, the motion to dismiss should be granted.

Dated: June 1, 2017

OF COUNSEL:
Bradford P. Lyerla
Louis E. Fogel
JENNER & BLOCK LLP
353 N. Clark Street
Chicago, IL 60654
(312) 222-9350

/s/ Kenneth L. Dorsney
Kenneth L. Dorsney (#3726)
Richard K. Herrmann(#405)
MORRIS JAMES LLP
500 Delaware Avenue, Suite 1500
Wilmington, DE 19801
(302) 888-6800
kdorsey@morrisjames.com

Exhibit 1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Anna Seneczuk and Ralph Klinke

Serial No: 12/822,072

Filed: June 23, 2010

For: PROCESS FOR PURIFYING PROTEINS

Docket No.: 3470-US-DIV

Group Art Unit: 1654

Examiner: Teller, Roy R.

Confirmation No.: 5094

AMENDMENT AFTER FINAL REJECTION UNDER 37 CFR 1.116

Mail Stop AF
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

This paper is being filed in response to the Office Action dated April 7, 2011, having a term that expired on July 7, 2011. An authorization to charge a two month extension fee to Deposit Account 01-0519 in the name of Amgen Inc. is enclosed herewith.

Applicants respectfully request that the subject amendment be entered and that the outstanding rejections be reconsidered in light of the following amendments and remarks.

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks/Arguments begin on page 4 of this paper.

CERTIFICATE OF EFS-WEB TRANSMISSION

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted electronically through EFS-WEB to the Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313-1450, on the date appearing below.

Aug 22, 2011
Date

Lynne Buchsbaum
Lynne Buchsbaum

Remarks

I. Status of Claims

Claims 1-13 are currently pending in the application and have been examined.

Claims 12 and 13 were objected to as depending from a non-existent claim.

Claims 1-13 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Holtz et al., US Patent No. 5,231,178.

Claims 9 and 11-13 have been amended in the instant Response. The amendments merely correct typographical errors and revise dependencies, and therefore do not incorporate any new matter.

II. Response to the Objection to Claims 12 and 13

The Patent Office objected to claims 12 and 13 as depending from a non-existent claim, claim 20. Applicants have amended claims 12 and 13 to depend from claim 10.

III. Declarant Senczuk's Declaration Under 35 U.S.C. §132 Filed With the Response of January 10, 2011

Upon review of the record, it has come to applicants' attention that Exhibit A of Declarant Senczuk's Declaration under 35 U.S.C. §132 filed with applicants' Response of January 10, 2011 appears to have been inadvertently omitted from the submission, although the data contained in Exhibit A is referenced in paragraphs 3 and 4. As a component of the instant Response, applicants resubmit Declarant Senczuk's Declaration in its entirety and regret any confusion this inadvertent omission may have caused the Patent Office.

IV. Response to the Rejection of Claims 1-13 Under 35 U.S.C. §103(a)

The Patent Office maintained its rejection of claims 1-13 under 35 U.S.C. §103(a) as obvious over the single cited reference, Holtz et al., US Patent No. 5,231,178 ("Holtz et al."), for the reasons presented in the previous Office Action. The Patent Office again contends that Holtz et al. discloses a method for purifying insulin-like growth hormone wherein "prior to contacting the eluate with the first hydrophobic interaction chromatography matrix, the initial eluate is buffered to a pH between 4.0-7.0. Salts contemplated for such use are those salts which improve

the hydrophobic interaction of IGF-1 and the hydrophobic interaction chromatography matrix, e.g., sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate and the like. The salt content will fall in the ranges of about 0.2 up to 2.0 M; with salt content of about 0.4 up to 1M being preferred." *Office Action*, page 3. The Patent Office again concludes that "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." *Office Action*, page 3. The Patent Office appears to base its conclusion on its belief that "The adjustment of particular working 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." *Office Action*, page 3-4. Applicants again traverse the rejection and submit the following comments.

IV.A. Holtz et al. Does not Disclose Each and Every Element of the Claimed Method

Applicants strenuously disagree with the Patent Office's conclusion and submit 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 applicants' method.

IV.A.1 Holtz et al. Does not Disclose a Combination of Salts

Applicants previously argued that Holtz et al. does not teach each and every element of the claimed invention, namely the use of a *combination* of salts in a HIC separation, and consequently cannot render the claimed invention obvious. Applicants reiterate their position and again urge that a reading of Holtz et al. indicates that Holtz et al. merely teaches a standard step in HIC chromatography--adding a high concentration of ammonium sulfate to a low concentration of a buffer solution to prepare a protein for a HIC column. Holtz et al. simply does not disclose, suggest or contemplate any steps involving a combination of two salts for any purpose whatsoever.

In this regard, applicants also submit that Holtz et al does not even make reference a "single salt system. Applicants submit that this is due to the fact that until applicants' disclosure highlighting a dual salt system the very term "single salt system" was redundant in the traditional

HIC process. Indeed, applicants selected the term "dual salt system" to differentiate their disclosed method from the traditional (Holtz') HIC process.

IV.A.2 Holtz et al. Does not Disclose Enhancing the Dynamic Capacity of a HIC Column

Applicants again note that, to the extent Holtz et al applied a salt in a HIC operation, it was a single salt and it was used in a traditional and well-established capacity, namely to alter the hydrophobic interactions in a buffered salt-containing solution so as to induce the target protein to associate with the HIC column matrix. As the data provided by Declarant Senczuk in her Declaration under 35 U.S.C. §132 demonstrates, the use a single salt—even a "judiciously selected" salt--will not enhance the dynamic capacity of a HIC column. Holtz et al. did not, however, consider, recognize or solve the issue of enhancing the dynamic capacity of the HIC column matrix; applicants' method solves this problem as demonstrated by the examples and as underscored by Declarant Senczuk's Declaration. Applicants submit that, while it may be argued that Holtz et al.'s single salt method is effective in enhancing adsorption of IFG-1 to a HIC column matrix in the context of a separation, Holtz et al.'s method does not disclose the idea of enhancing the dynamic capacity of the HIC column, as recited in the pending claims. In fact, applicants cannot identify any point in the cited reference at which the dynamic capacity of a HIC column is mentioned, nor can applicants find any disclosure of enhancing the dynamic capacity of a HIC column.

As stated in MPEP 2143.03, "All words in a claim must be considered in judging the patentability of that claim against the prior art." *In re Wilson*, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970). Since the cited reference fails to disclose (a) a combination of salts or (b) any enhancement of the dynamic capacity of a HIC column, which are recited in applicants' pending claims, Holtz et al. cannot render the pending claims obvious.

IV.B. "Routine Optimization" Does not Lead to the Claimed Invention

The Patent Office is of the position that the leap from Holtz et al. to applicants' claimed invention is merely a matter of "adjustment of particular conventional working conditions" and "is deemed merely a matter of judicious selection and routine optimization which is well within

the purview of the skilled artisan." Office Action, pages 3-4. Applicants respectfully submit this sweeping conclusion is unsupported and ignores the lengthy development path presented in the Examples of the pending application. As the Examples clearly demonstrate, arriving at the claimed invention was a lengthy and in-depth exercise; it was not merely a matter of "judicious selection and routine optimization." Development of the claimed method required making a determination of the optimum concentration range for the individual salt solutions by generating "salting out" or precipitation curves for each protein. A second series of salting out curves was subsequently generated for two salt combinations in which the first salt concentration was kept constant and the second salt concentration was increased. In a second series of experiments the second salt was kept constant and the first salt varied. The dynamic capacities were determined for the salts alone at the previously determined optimum concentrations, and then for the combinations of salts at the previously determined optimum concentrations, in order to determine what combinations of salts would increase the dynamic capacity for the proteins on the HIC column. Applicants submit this focused and time-consuming development process represents a significantly more in-depth development process than the "judicious selection and routine optimization" urged by the Patent Office.

Underscoring the above, applicants further submit that merely adding a second salt to the traditional HIC process, as the Patent Office appears to suggest, will not produce applicants' claimed method. In fact, merely adding a second salt to the traditional HIC process will not even provide a working method; in this scenario the protein to be purified will precipitate out of solution and it will not be possible to load the protein onto the HIC column.

IV.C. Applicants' Secondary Evidence of Non-obviousness

Applicants also direct attention to secondary evidence of non-obviousness. The Declaration of Anna Senczuk under 35 U.S.C. §132, which is of record. As Declarant Senczuk states in paragraph 3 of her Declaration, she tested several single salts for their ability to enhance dynamic breakthrough on a HIC column and subsequently tested several pairs of salts for the same property. She observed that the combination of salts markedly enhanced dynamic breakthrough on the HIC column, while the single salts performed as expected. In the case of the dual salt combination of sodium sulfate and sodium citrate, this provided a 38% increase in the dynamic capacity of the column over the single salt, sodium sulfate. As Declarant Senczuk

states in paragraph 4 of her Declaration, this unexpected observation translates in a significant cost savings over using a single salt as described by Holtz et al., namely a cost savings of $1297 whena sulfate/citrate or sulfate acetate combination is used instead of a single sulfate salt, $12972 when a sulfate/acetate combination is used instead of a single acetate salt and $11675 when an acetate/citrate salt combination is used instead of a single acetate salt. Thus, the data provided by Declarant Senczuk provides yet further evidence of non-obviousness, namely the commercial value of employing applicants' claimed method in a commercial setting, which is one of the *Graham* factors.

Applicants respectfully submit that the sole cited reference, Holtz et al., cannot support the Patent Office's rejection of claims 1-13 under 35 U.S.C. §103(a) as obvious and respectfully request that the rejection of claim 1-13 under 35 U.S.C. §103(a) be reconsidered and withdrawn.

V. Conclusions

Applicants submit that the claims are in condition for allowance. Accordingly, applicants respectfully request that the rejections of record be reconsidered and withdrawn, and a Notice of Allowance issued.

If any small matter remains outstanding after the Examiner has reviewed the amendments and remarks presented herein, the Examiner is respectfully requested to telephone the undersigned attorney at the telephone number provided below to resolve any such matter.

The Commissioner is hereby authorized to charge any fees which may be required or credit any overpayment to Deposit Account No. 01-0519 in the name of Amgen Inc.

Respectfully submitted,

John A. Lamerdin
Attorney/Agent for Applicant(s)
Registration No.: 44,858
Phone: (805) 313-6398
Date: August 22, 2011

Please send all future correspondence to:

US Patent Operations/JAL
Dept. 10200, M/S 28-2-C
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Anna Senczuk and Ralph Klinke

Docket No.: 3470-US-DIV

Group Art Unit: 1654

Serial No: 12/822,072

Examiner: Teller, Roy R.

Filed: June 23, 2010

For: PROCESS FOR PURIFYING PROTEINS

DECLARATION OF ANNA SENCZUK UNDER 37 C.F.R § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Anna Senczuk, do hereby declare as follows that:

1. I, Anna Senczuk, am one of the inventors in the above-referenced patent application. I have been employed as a senior associate scientist at Amgen and Immunex since December 1, 2000. Prior to Amgen, I worked at the University of Calgary, Alberta, Canada. I received my B.S. and M.S. degrees at University of Calgary in Cell Molecular and Microbial Biology. I have approximately 13 years of experience in the field of protein purification.

2. I have performed the experiments testing single salts and combination of salts (dual salts) described in the above-referenced patent application. My co-inventor and I discovered that using certain combinations of salts will greatly improve the dynamic capacity of a hydrophobic interaction chromatography (HIC) column, or the amount of protein that can be loaded onto the column without "breakthrough" or leakage of protein into the solution phase before elution. 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. Previously, it was not known that salt combinations had anything to do with improving dynamic capacity of a HIC.

3. 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. This greatly improves the efficiency and reduces the cost of manufacturing a therapeutic protein. I performed calculations illustrating the benefits for commercial manufacturing of using a specific dual salt combination to load protein onto a HIC compared with a single salt for a commercial purification of an IgG2 antibody.

The single sulfate salt, 0.8 M sodium sulfate at pH 6.0, was calculated to have a dynamic capacity of 24 g/L-r for the particular IgG2 monoclonal antibody, whereas the dual salt combination, 0.5M sodium sulfate plus 0.3 M sodium citrate at pH 6.0 was calculated to have a dynamic capacity of 33 g/L-r for the same IgG2 monoclonal antibody. This represents an increase of 38% for the dynamic capacity of this particular dual salt combination compared with a single salt for the same antibody.

The single acetate salt, 1.2 M sodium acetate at pH 6.0, was calculated to have a dynamic capacity of 5 g/L-r for the particular IgG2 monoclonal antibody, whereas the dual salt combination, 1.0 M sodium acetate plus 0.5 M sodium sulfate at pH 6.0 was calculated to have a dynamic capacity of 33 g/L-r for the same IgG2 monoclonal antibody. This represents an increase of 550% for the dynamic capacity of this particular dual salt combination compared with a single salt for the same antibody.

4. The benefits that result from the use of dual salts in the HIC column are presented in Exhibit A. The increase in dynamic capacity for the HIC resulting from the use of the dual salt combination in the HIC for an estimated harvest of 1.5 g/L in a 2 kL bioreactor allows for 2 instead of 3 cycles of purification for each bioreactor harvest in the case of a single sulfate salt versus the sulfate/citrate and sulfate/acetate combination, and for 2 instead of 12 cycles for each bioreactor harvest in the case of a single acetate salt versus the acetate/sulfate and acetate citrate combination.

Additionally, the increase in dynamic capacity for the HIC resulting from the use of the dual salt combination in the HIC for an estimated harvest of 1.5 g/L in a 12 kL bioreactor reduces the processing time from 10 hours to 7 hours in the case of a single sulfate salt versus the sulfate/citrate combination and the sulfate/acetate combination, and from 32 hours to 10 hours in the case of a single acetate salt versus an acetate/citrate combination.

As a consequence the use of salt combinations reduces the estimated cost/kg product produced from an estimated $3,961/kg for a single sulfate salt to $2,664/kg when the sulfate/citrate or sulfate/acetate salt combination is used, and from

$15,636/kg for a single acetate salt to $2,664/kg when the sulfate/acetate combination is used and from $15,636/kg to $3,961/kg when the acetate/citrate salt combination is used, thus reducing the cost of purifying a therapeutic protein on a commercial scale.

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.

5. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Jan 20, 11 — Date

A Seneczuk — Anna Seneczuk

Exhibit A

	Sulfate (0.5M)	Acetate (1.2M)	Sulfate 0.5M with Citrate 0.3M or Sulfate 0.5M with acetate 1.0M	Acetate 0/6M with Citrate 0.5M	Citrate 0.7M with Sulfate 0.28M
Cycles	3	12	2	2	2
Processing time	10	32	7	10	7
$/Kg product	3,961	15,636	2,664	3,961	2,664

Assumptions: 12 kL bioreactor, 1.5g/L, 20 batches per year.

Each saved cycle contributes to saving $470,000/year.

Exhibit 2

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
12/822,072	06/23/2010	Anna Senczuk	3470-US-DIV	5094

21069 7590 10/13/2010

AMGEN INC.
MAIL STOP 28-2-C
ONE AMGEN CENTER DRIVE
THOUSAND OAKS, CA 91320-1799

EXAMINER	
TELLER, ROY R	
ART UNIT	**PAPER NUMBER**
1654	

MAIL DATE	DELIVERY MODE
10/13/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	12/822,072	SENCZUK ET AL.	
	Examiner	Art Unit	
	ROY TELLER	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 June 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *6/23/10*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claims 1-13 are under examination.

Information Disclosure Statement

The information disclosure statement, received 6/23/10, is acknowledged. A signed copy is enclosed hereto.

Double Patenting

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting

ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

Claims 1-13 are rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1- 13 of U.S. Patent No.7,781,395. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims are drawn to 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, wherein the first and second salts in combination with the protein are selected from the group consisting of sodium, potassium and ammonium salts. The '395 claims are drawn to 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, wherein the first and second salts in combination with the protein are selected from the group consisting of sodium, potassium and ammonium salts.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Holtz et al. (USPN 5,231,178).

The instant invention is drawn to a process for purifying a protein on a hydrophobic interactive 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 column, and eluting the protein, wherein the first and second salt 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 0.1M and about 1.0M, wherein the column is eluted with a solution onto the column is between about pH 5 and 7. The instant specification reads on insulin-like growth factors as one of the proteins to be purified (see, e.g., instant specification, page 15, line 6).

Holtz et al. beneficially discloses a method of purification of insulin-like growth hormone, in which prior to contacting the eluate with the first hydrophobic interaction chromatography matrix, the initial eluate is buffered to a pH between 4.0- 7.0. Salts contemplated for such use are those salts which improve the hydrophobic interaction of IGF-1 and the hydrophobic interaction chromatography matrix, e.g., sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate and the like. The salt content will fall in the ranges of about 0.2 up to 2.0m; with salt content of about 0.4 up to 1M being preferred. See entire document including, for example, columns 11-13, 26-27 and 32.

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.

Thus, the invention as a whole is *prima facie* obvious over the reference, especially in the absence of evidence to the contrary.

Conclusion

All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROY TELLER whose telephone number is (571)272-0971. The examiner can normally be reached on Monday-Friday from 5:30 am to 2:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang, can be reached on 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished

applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

RT
1654

/Christopher R. Tate/
Primary Examiner, Art Unit 1655

Exhibit 3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Anna Senczuk and Ralph Klinke

Serial No: 12/822,072

Filed: June 23, 2010

For: PROCESS FOR PURIFYING PROTEINS

Docket No.: 3470-US-DIV

Group Art Unit: 1654

Examiner: Teller, Roy R.

Confirmation No.: 5094

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22131-1450

RESPONSE TO OFFICE ACTION

Sir:

This paper, and the accompanying Declaration under 37 C.F.R. §1.132 is being filed in response to the Office Action dated October 13, 2010, having a term that expired on January 13, 2011. An authorization to charge any extension fee to Deposit Account 01-0519 in the name of Amgen Inc., is enclosed herewith.

Applicants respectfully request that the subject amendment be entered and that the outstanding rejections be reconsidered in light of the following amendments and remarks.

Pending Claims begin on page 2 of this paper.

Remarks and arguments begin on page 4 of this paper.

CERTIFICATE OF EFS-WEB TRANSMISSION

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted electronically through EFS-WEB to the Commissioner for Patents, P.O. Box 1450 Alexandria, VA 22313-1450, on the date appearing below.

January 26, 2011
Date

Christina Tidy
Name

PENDING CLAIMS

What is claimed is:

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.

2. (Original) 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. (Original) The process of claim 1 wherein the column is eluted with a solution having a pH between about pH 5 and pH 7.

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

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

6. (Original) The process of claim 1, further comprising diluting the protein.

7. (Original) The process of claim 1, further comprising filtering the protein.

8. (Original) The process of claim 1, further comprising formulating the protein.

9. (Original) The process of claim 1, further comprising lyopholizing the protein.

10. (Original) 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, and wherein the concentration of each of the first and second salts in the mixture is between about 0.1M and about 1.0 M.

11. (Original) The process of claim 10 wherein the pH of the mixture loaded onto the column is between about pH 5 and about pH 7.

12. (Original) The process of claim 20, wherein the citrate and phosphate salts are selected from the group consisting of sodium, potassium and ammonium salts.

13. (Original) The process of claim 20 wherein the protein is a fusion protein or an antibody.

REMARKS

I. Status of Claims

Claims 1-13 are currently pending in the application and have been examined.

Claims 1-13 stand rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-13 of U.S. Patent No. 7,781,395.

Claims 1-13 stand rejected under 35 U.S.C. §103(a) as being unpatentable over Holtz et al., US Patent No. 5,231,178.

No claims have been amended in the instant Response.

II. Response Claim Rejections Under Obviousness-type Double Patenting

Claims 1-13 were rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-13 of U.S. Patent No. 7,781,395. The Patent Office states "[a]lthough the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims are drawn to 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, wherein the first and second salts in combination with the protein are selected from the group consisting of sodium, potassium and ammonium salts. The '395 claims are drawn to 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, wherein the first and second salts in combination with the protein are selected from the group consisting of sodium, potassium and ammonium salts." Office Action, page 3. Applicants traverse the rejection and submit the following comments.

Applicants refer to the voicemail provided by Examiner Teller to applicants' attorney on January 26, 2011. In his voicemail, Examiner Teller stated that, upon further review of the outstanding Office Action, he is withdrawing the rejection of claims 1-13 as unpatentable over claims 1-13 of U.S. Patent No. 7,781,395. Examiner Teller indicated that he will send an Interview Summary to this effect.

Applicants thank Examiner Teller for his voicemail and for his withdrawal of the Obviousness-type Double Patenting rejection. As this rejection is now moot, applicants do not address it in the instant response.

III. Response to Claim Rejections Under 35 U.S.C. § 103(a)

Claims 1-13 have been rejected under 35 U.S.C. §103(a) as allegedly *prima facie* obvious over the single reference of U.S. Patent 5,231,178 to Holtz et al. Applicants traverse the rejection and submit the following comments.

It is the Patent Office's position that the claimed subject matter is obvious over the disclosure in the Holtz et al., in particular, columns 11-13, 26-27 and 32. The Patent Office states that Hotz et al. discloses the use of a number of salts between 0.2 M and 2.0M concentration, preferably between 0.4 and 1 M concentration, including sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate and the like on a hydrophobic interaction chromatography (HIC) matrix. It is the Patent Office's position that it would have been obvious to one of ordinary skill in the art to purify a protein including IGF-1 based on the cited reference, 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." (page 3 of Office Action). Applicants do not agree.

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.

To that point, applicants provide the attached Declaration of Anna Senczuk under 37 C.F.R. § 1.132. As stated in paragraph 2 of that Declaration, the use of dual salts to increase dynamic capacity was not previously known based on "any information on HIC available from the scientific literature or other sources at the time" of the invention, and "it was not know that salt combinations had anything to do with improving dynamic capacity of a HIC." As pointed out in paragraph 4 of the Declaration, "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."

The United States Supreme Court, in its decision in *KSR v. Teleflex*, 550 U.S. 398 (2007), reaffirmed that a proper determination of obviousness requires an objective analysis of the factors set forth in *Graham v. Deere*, which include:

1) the scope and content of the prior art;
2) the differences between the prior art and the claims;
3) the level of ordinary skill in the pertinent art resolved, and
4) secondary evidence of non-obviousness, such as commercial success, long felt but unsolved needs, failure of others, etc.,

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.

Further, the Declaration of Anna Senczuk under 37 C.F.R. § 1.132 and the accompanying Exhibit A also provide secondary evidence of non-obviousness, that is,

the advantages of the use of the dual salts as claimed in a commercial setting. The calculations presented demonstrate that the use of dual salts in HIC result in an increase in cost-effectiveness of purifying an IgG2 antibody in a commercial setting. This provides evidence of commercial success or value, one of the *Graham v. Deere* factors.

Based on the accompanying Declaration of Anna Senczuk under 37 C.F. R. § 1.132 and Exhibit A, and the arguments presented above, applicants request reconsideration and withdrawal of the rejection of claims 1-13 on the basis of 35 U.S.C. §103(a) as allegedly *prima facie* obvious over U.S. Patent 5,231,178 to Holtz et al.

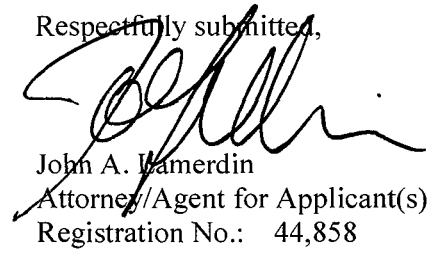
IV. Conclusions

Applicants submit that the claims are in condition for allowance. Accordingly, applicants respectfully request that the rejections of record be reconsidered and withdrawn, and a Notice of Allowance issued.

If any small matter remains outstanding after the Examiner has reviewed the amendments and remarks presented herein, the Examiner is respectfully requested to telephone the undersigned attorney at the telephone number provided below to resolve any such matter.

The Patent Office is authorized to charge any additional fees due or credit any fee owed to Deposit Account 01-0519 in the name of Amgen Inc.

Respectfully submitted,



John A. Hamerdin
Attorney/Agent for Applicant(s)
Registration No.: 44,858
Phone: (805) 313-6398
Date: January 26, 2011

Please send all future correspondence to:
US Patent Operations/ JAL
Dept. 10200, M/S 28-2-C
AMGEN INC.
One Amgen Center Drive
Thousand Oaks, California 91320-1799

Exhibit 4

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Anna Senczuk and Ralph Klinke

Docket No.: 3470-US-DIV

Group Art Unit: 1654

Serial No: 12/822,072

Examiner: Teller, Roy R.

Filed: June 23, 2010

For: PROCESS FOR PURIFYING PROTEINS

DECLARATION OF ANNA SENCZUK UNDER 37 C.F.R § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Anna Senczuk, do hereby declare as follows that:

1. I, Anna Senczuk, am one of the inventors in the above-referenced patent application. I have been employed as a senior associate scientist at Amgen and Immunex since December 1, 2000. Prior to Amgen, I worked at the University of Calgary, Alberta, Canada. I received my B.S. and M.S. degrees at University of Calgary in Cell Molecular and Microbial Biology. I have approximately 13 years of experience in the field of protein purification.

2. I have performed the experiments testing single salts and combination of salts (dual salts) described in the above-referenced patent application. My co-inventor and I discovered that using certain combinations of salts will greatly improve the dynamic capacity of a hydrophobic interaction chromatography (HIC) column, or the amount of protein that can be loaded onto the column without "breakthrough" or leakage of protein into the solution phase before elution. 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. Previously, it was not known that salt combinations had anything to do with improving dynamic capacity of a HIC.

3. 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. This greatly improves the efficiency and reduces the cost of manufacturing a therapeutic protein. I performed calculations illustrating the benefits for commercial manufacturing of using a specific dual salt combination to load protein onto a HIC compared with a single salt for a commercial purification of an IgG2 antibody.

The single sulfate salt, 0.8 M sodium sulfate at pH 6.0, was calculated to have a dynamic capacity of 24 g/L-r for the particular IgG2 monoclonal antibody, whereas the dual salt combination, 0.5M sodium sulfate plus 0.3 M sodium citrate at pH 6.0 was calculated to have a dynamic capacity of 33 g/L-r for the same IgG2 monoclonal antibody. This represents an increase of 38% for the dynamic capacity of this particular dual salt combination compared with a single salt for the same antibody.

The single acetate salt, 1.2 M sodium acetate at pH 6.0, was calculated to have a dynamic capacity of 5 g/L-r for the particular IgG2 monoclonal antibody, whereas the dual salt combination, 1.0 M sodium acetate plus 0.5 M sodium sulfate at pH 6.0 was calculated to have a dynamic capacity of 33 g/L-r for the same IgG2 monoclonal antibody. This represents an increase of 550% for the dynamic capacity of this particular dual salt combination compared with a single salt for the same antibody.

4. The benefits that result from the use of dual salts in the HIC column are presented in Exhibit A. The increase in dynamic capacity for the HIC resulting from the use of the dual salt combination in the HIC for an estimated harvest of 1.5 g/L in a 2 kL bioreactor allows for 2 instead of 3 cycles of purification for each bioreactor harvest in the case of a single sulfate salt versus the sulfate/citrate and sulfate/acetate combination, and for 2 instead of 12 cycles for each bioreactor harvest in the case of a single acetate salt versus the acetate/sulfate and acetate citrate combination.

Additionally, the increase in dynamic capacity for the HIC resulting from the use of the dual salt combination in the HIC for an estimated harvest of 1.5 g/L in a 12 kL bioreactor reduces the processing time from 10 hours to 7 hours in the case of a single sulfate salt versus the sulfate/citrate combination and the sulfate/acetate combination, and from 32 hours to 10 hours in the case of a single acetate salt versus an acetate/citrate combination.

As a consequence the use of salt combinations reduces the estimated cost/kg product produced from an estimated $3,961/kg for a single sulfate salt to $2,664/kg when the sulfate/citrate or sulfate/acetate salt combination is used, and from

$15,636/kg for a single acetate salt to $2,664/kg when the sulfate/acetate combination is used and from $15,636/kg to $3,961/kg when the acetate/citrate salt combination is used, thus reducing the cost of purifying a therapeutic protein on a commercial scale.

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.

5. I further declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon.

Jan 20, 11 — A. Senczuk

Date — Anna Senczuk

Exhibit 5

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Anna Senczuk and Ralph Klinke

Docket No.: 3470-US-NP

Group Art Unit: 1654

Serial No: 10/895,581

Examiner: Teller, Roy R.

Filed: July 21, 2004

For: PROCESS FOR PURIFYING PROTEINS

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22131-1450

RESPONSE TO OFFICE ACTION AND AMENDMENT

Dear Sir:

In response to the Office Action dated 7/17/2007, please consider the following response to the Office Action and amendment of the claims. This response is submitted with a request for a one month extension of time and the appropriate fee.

Amendments to the Specification begin on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks begin on page 5 of this paper.

CERTIFICATE OF FACSIMILE TRANSMISSION
I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is being facsimile transmitted to the United States Patent and Trademark Office on the date indicated below.

Signed: Kathleen F. Prindle

Date: November 16, 2007

AMENDMENT TO THE SPECIFICATION

Please replace the paragraph on page 25, under the heading "ABSTRACT", with the following amended paragraph.

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~~ the dynamic capacity of the column for either single salt alone.

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AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application. Entry of the amendments of the claims is respectfully requested.

What is claimed is:

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.

2. (original) 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.-5. (canceled)

6. (original) The process of claim 1 wherein the column is eluted with a solution having a pH between about pH 5 and about pH 7.

7. (currently amended) The process of claim 1 wherein the ~~first salt and second~~ citrate and phosphate salts are selected from the group consisting of ~~citrate and sulfate; citrate and acetate; citrate and phosphate; acetate and sulfate; and sulfate and phosphate~~ sodium, potassium and ammonium salts.

8. (original) The process of claim 1 wherein the protein is a fusion protein or an antibody.

9. (original) The process of claim 1, further comprising diluting the protein.

10. (original) The process of claim 1, further comprising filtering the protein.

11. (original) The process of claim 1, further comprising formulating the protein.

12. (original) The process of claim 1, further comprising lyopholizing the protein.

13.- 19. (canceled)

20. (currently amended) A method of ~~maximizing~~ increasing the dynamic capacity of a hydrophobic interaction chromatography column for a particular protein ~~at a desired pH comprising selecting a combination of concentrations for a first salt and a second salt wherein the first salt and the second salt have different lyotropic values, and least one salt has a buffering capacity at the desired pH, and wherein the concentrations of the first salt and the second salt are determined using precipitation curves for the salts individually and for the combination of salts,~~ 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 citrate and phosphate salts, and wherein the concentration of each of the first and second salts in the mixture is between about 0.1M and about 1.0 M.

21. (new) The process of claim 20 wherein the pH of the mixture loaded onto the column is between about pH 5 and about pH 7.

22. (new) The process of claim 20, wherein the citrate and phosphate salts are selected from the group consisting of sodium, potassium and ammonium salts.

23. (new) The process of claim 20 wherein the protein is a fusion protein or an antibody.

REMARKS

The specification has been amended to correct a typographical error, as shown above.

Claims 1-2, 6-12, 20, and new claims 21-23 are currently pending in the application. Claims 3-5 and 13-19 have been canceled without prejudice to future filing. Claims 1, 7, and 20 are currently amended.

Claims 1 and 20 are currently amended in response to the previously issued restriction requirement. In addition, claims 1 and 20 are amended to more clearly recite the subject matter considered to be the invention. Support for these amendments is found in the application as filed, for example, page 3, page 8, and page 9, line 30 to page 11, line 3. Claim 7 is amended to recite the types of citrate and phosphate salts. Support for this amendment is found in the application, for example, page 3, lines 9-11, page 5, line 30 to page 6, line 7. Support for claims 21-23 is found in the specification, pages 8, and 9-13, for example. Therefore, no new matter is added by the amendments to the claims or new claims 21-23. Entry of the amendments to the specification and claims is respectfully requested.

REJECTIONS UNDER 35 U.S.C. § 112, FIRST PARAGRAPH

Claims 1-2, 4-14, and 16-20 are rejected under 35 U.S.C. § 112, first paragraph as allegedly lacking enablement for the claimed processes. This rejection is respectfully traversed.

35 U.S.C. §112, first paragraph requires that the specification shall contain a written description of the invention and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, ...to make and use the same.

The Examiner has alleged that the Applicants have only provided guidance for the use of citrate, acetate, phosphate and sulfate salts, and have provided no guidance for any other salt that could be used to purify a protein. Applicants do not agree. First, Applicants point out that the claimed invention is directed to the use of the combination of an intermediate concentration of a buffering salt in combination with an intermediate concentration of a second buffering or non-buffering salt for purifying proteins on a HIC column (page 4, lines 22-26). This combination of salts offers advantages of previous methods of preparing and using HIC columns to purify proteins, by increasing the dynamic capacity of the column (page 6, lines 23-29, for

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example). The instant application teaches how to select combinations of salts and their concentrations for use in the improved methods of purifying proteins on HIC columns. This is described in detail in the specification, for example, page 7, line 29, to page 8, and Examples 1 and 2, pages 17-22, as illustrated in Figures 1A-1E. Potential salts are described in the application on page 6, lines 9-21, and line 31 to page 7 of the specification. Therefore, according to the *In re Wands* factors listed by the Examiner in the Office Action, Applicants have provided both specific guidance and direction in the specification, and actual working examples. Applicants therefore submit that on the basis of this specific guidance and the working examples, together with the high level of those skilled in the art, and the state of the art at the filing date of the application, the claims previously presented were in fact enabled by the specification.

In accordance with the restriction requirement, the claims have now been amended to recite citrate and phosphate salts, as shown above. The Examiner has stated in the Office Action, page 3, that the specification is enabling for the combination of citrate and phosphate salts. Therefore, on the basis of the arguments presented above, in addition to the amendments to the claims, Applicants respectfully request reconsideration and withdrawal of the rejection of the claims on the basis of 35 U.S.C. § 112, first paragraph.

Applicants' attorney invites the Examiner to call her at the number given below if it would be helpful in advancing the prosecution of this application.

Respectfully submitted,

Christine M. Bellas

Christine M. Bellas
Registration No. 34,122
Direct Dial No. (206) 265-8294
Date: November 16, 2007

Immunex Corporation
Law Department
1201 Amgen Court West
Seattle, WA 98119
Telephone (206) 265-7000

ik161901 11/16/07

Exhibit 6

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/895,581	07/21/2004	Anna Senczuk	3470-US-NP	7878

37500 7590 02/14/2008

AMGEN INC.
LAW DEPARTMENT
1201 AMGEN COURT WEST
SEATTLE, WA 98119

EXAMINER	
TELLER, ROY R	
ART UNIT	**PAPER NUMBER**
1654	

MAIL DATE	DELIVERY MODE
02/14/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/895,581	SENCZUK ET AL.	
	Examiner	Art Unit	
	ROY TELLER	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *16 November 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,2,6-12 and 20-23* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,2,6-12,20-23* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

This office action is in response to the amendment, received 11/16/07. Applicant has amended claims 1, 7 and 20; cancelled claims 3-5 and 13-19; and added new claims 21-23.

Claims 1, 2, 6-12 and 20-23 are under examination.

Response to Amendments/ Arguments

Applicant's amendments and arguments filed 11/16/07 are acknowledged and have been fully considered. Any rejection and/or objection not specifically addressed is herein withdrawn.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 2, 6-12 and 20-23 are rejected under 35 U.S.C. 102(b) as being anticipated by Holtz et al. (USPN 5,231,178).

The instant invention is drawn to a process for purifying a protein on a hydrophobic interactive 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 column, and eluting the protein, wherein the first and second salt 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 0.1M and about 1.0M, wherein the column is eluted with a solution onto the

column is between about pH 5 and 7. The instant specification reads on insulin-like growth factors as one of the proteins to be purified (see, e.g., instant specification, page 15, line 6).

Holtz et al. discloses a method of purification of insulin-like growth hormone, in which prior to contacting the eluate with the first hydrophobic interaction chromatography matrix, the initial eluate is buffered to a pH between 4.0- 7.0. Salts contemplated for such use are those salts which improve the hydrophobic interaction of IGF-1 and the hydrophobic interaction chromatography matrix, e.g., sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate and the like. The salt content will fall in the ranges of about 0.2 up to 2.0m; with salt content of about 0.4 up to 1M being preferred. See entire document including, for example, columns 11-13, 26-27 and 32. This reads on instant claims 1, 2, 6-12 and 20-23.

Therefore, the cited prior art is deemed to anticipate the instant claims.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1, 2, 6-12 and 20-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Holtz et al. (USPN 5,231,178).

Holtz et al. beneficially discloses a method of purification of insulin-like growth hormone, in which prior to contacting the eluate with the first hydrophobic interaction chromatography matrix, the initial eluate is buffered to a pH between 4.0- 7.0. Salts contemplated for such use are those salts which improve the hydrophobic interaction of IGF-1 and the hydrophobic interaction chromatography matrix, e.g., sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate and the like. The salt content will fall in the ranges of about 0.2 up to 2.0m; with salt content of about 0.4 up to 1M being preferred. See entire document including, for example, columns 11-13, 26-27 and 32.

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.

Thus, the invention as a whole is *prima facie* obvious over the reference, especially in the absence of evidence to the contrary.

Conclusion

All claims are rejected.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROY TELLER whose telephone number is (571)272-0971. The examiner can normally be reached on Monday-Friday from 5:30 am to 2:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang, can be reached on 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Roy Teller/
Examiner, Art Unit 1654
2/12/08

/Christopher R. Tate/
Primary Examiner, Art Unit 1655

Notice of References Cited	Application/Control No. 10/895,581	Applicant(s)/Patent Under Reexamination SENCZUK ET AL.	
	Examiner ROY TELLER	Art Unit 1654	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,231,178	07-1993	Holtz et al.	530/399
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Exhibit 7

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Anna Senczuk and Ralph Klinke

Docket No.: 3470-US-NP

Group Art Unit: 1654

Serial No: 10/895,581

Examiner: Teller, Roy R.

Filed: July 21, 2004

For: PROCESS FOR PURIFYING PROTEINS

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22131-1450

RESPONSE TO OFFICE ACTION AND AMENDMENT

Dear Sir:

In response to the Office Action dated 2/14/1008, please consider the following Response to the Office Action and Amendment of the claims. This response is submitted with a request for a two month extension of time and the appropriate fee.

Amendments to the Specification are shown on page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks and arguments begin on page 5 of this paper.

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this paper (along with any referred to as being attached or enclosed) is being transmitted to the United States Patent and Trademark Office via facsimile to facsimile number 571-273-8300 on the date indicated below, and is addressed to Mail Stop Amendment, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Signed: Kathleen B. Prindle

Date: July 14, 2008

07/15/2008 VBUI11 00000027 090089 10895581
01 FC:1252 460.00 DA

AMENDMENTS TO THE SPECIFICATION

Please replace lines 1-6 on page 21, and Table 2, lines 12-15, on page 21, with the following amended lines and Table 2.

--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~~3~~.

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	1.0 M	0.55M Na Citrate/ 0.2M Na Phosphate
TNFR:Fc	0.55M	1.0 M	0.4M Na Citrate/ 0.2M Na Phosphate

Table 2. Salt concentrations at which protein begins to precipitate (taken from the precipitation curves.)--

On page 22, please replace Table 3 with the following amended Table 3.

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

~~Table3~~ **Table 3.** Dynamic capacities under the salt conditions listed in Table 2.

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application. Entry of the amendments of the claims is respectfully requested.

What is claimed is:

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.

2. (original) 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.-5. (canceled)

6. (original) The process of claim 1 wherein the column is eluted with a solution having a pH between about pH 5 and about pH 7.

7. (previously presented) The process of claim 1 wherein the citrate and phosphate salts are selected from the group consisting of sodium, potassium and ammonium salts.

8. (original) The process of claim 1 wherein the protein is a fusion protein or an antibody.

9. (original) The process of claim 1, further comprising diluting the protein.

10. (original) The process of claim 1, further comprising filtering the protein.

11. (original) The process of claim 1, further comprising formulating the protein.

12. (original) The process of claim 1, further comprising lyopholizing the protein.

13.- 19 (canceled)

20. (previously presented) A method of increasing the dynamic capacity of a hydrophobic interaction chromatography column for a particular 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 citrate and phosphate salts, and wherein the concentration of each of the first and second salts in the mixture is between about 0.1M and about 1.0 M.

21. (previously presented) The process of claim 20 wherein the pH of the mixture loaded onto the column is between about pH 5 and about pH 7.

22. (previously presented) The process of claim 20, wherein the citrate and phosphate salts are selected from the group consisting of sodium, potassium and ammonium salts.

23. (previously presented) The process of claim 20 wherein the protein is a fusion protein or an antibody.

REMARKS

The specification has been amended as shown above to correct "TNF:Fc" to "TNFR:Fc", and to correct two typographical mistakes. TNFR:Fc was correctly presented in the application on page 13, paragraph 3, which describes tumor necrosis factor receptor-Fc fusion protein (TNFR:Fc). Page 13, paragraph 3 states "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)." Therefore, amending the specification on pages 21 and 22 to correct TNF:Fc to TNFR:Fc does not represent new matter. Entry of the amendments to the specification is respectfully requested.

Claims 1-2, 6-12, and 20-23 are currently pending in the application. Claims 3-5 and 13-19 have been canceled without prejudice to future filing. Claim 1 is currently amended to correct a typographical error by changing "interactive" to "interaction". No new matter is presented by this amendment, and entry of the amendment to claim 1 is respectfully requested.

REJECTION UNDER 35 U.S.C. § 102(b)

Claims 1-2, 6-12, and 20-23 have been rejected under 35 U.S.C. § 102 (b), as allegedly anticipated by U.S. Patent No: 5,231,178 (Holtz et al.). This rejection is respectfully traversed.

The Examiner has alleged that the claimed invention is disclosed throughout the entire Holtz et al. document, for example, columns 11-13, 26-27, and 32. Applicants do not agree.

A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference. *Verdegall Bros* v. *Union Oil Co. of California*, 814 F.2d 628, 631, 2 USPQ 2d 1051, 1053 (Fed. Cir. 1987).

Claim 1 of the instant application recites 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, *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* (emphasis added).

Holtz et al. describes a detailed process for recovery and purification of monomeric, intact, correctly folded IGF-1 through the use of a series of columns. Holtz et al., column 11, lines 42 to 62, describes mixing the eluate of a first cation exchange column with a buffered salt solution pH 4.0 to 7.0, preferably 4.5 to 5.0, before loading onto a hydrophobic interaction column, where the salts are in the range of 0.2 to 2.0M, preferably .4 to 1.0M, and where the salts are selected from "sodium sulfate, potassium sulfate, ammonium sulfate, potassium phosphate, sodium acetate, ammonium acetate, sodium chloride, sodium citrate, and the like. Broadly, the salt content employed will fall in the range of about 0.2 up to 2.0M, with a salt content of about 0.4 up to 1M being presently preferred. An especially preferred salt is ammonium sulfate, at a concentration of about 0.4 to 0.8M." (column 11, lines 55-62). Holtz et al. on column 11 does not describe or suggest combining the protein to be purified with the particular *combination* of two salts, citrate and phosphate salts, as recited in the claimed process, at concentrations of *each of* the first and second salt being between about 0.1M and 1.0M, before loading the protein on the HIC column.

Holtz et al., columns 26 and 27, describe methods of preparing and loading IGF-1 eluant from a cation exchange column onto a HIC column. Column 26, line 60 to column 27, line 16 describes diluting IGF-1 eluant from the cation exchange column into a buffer containing .5M sodium chloride, 0.5 M sodium acetate, and 0.5 sodium phosphate, pH 4.0, and then adding 80% saturated solution of ammonium sulfate until IGF-1 protein precitates. The precipitated protein is then resuspended to a concentration of 425 mg/5 liters (85 mg/l), in a solution of 16% saturated ammonium sulfate, 40 mM sodium acetate, 40 mM sodium phosphate, pH 4.5, and 0.4M NaCl, and this solution is then loaded onto the HIC column (column 26, line 61 to column 27, line 10). Again Holtz et al. column 26 and 27 does not teach or suggest combining the protein to be purified with the particular *combination of two salts, citrate and phosphate salts* at concentrations of between about *0.1M and 1.0M* before loading the protein on the HIC column. Instead, a protein solution containing lower concentrations of sodium acetate and sodium phosphate, together with NaCl and a high concentration of ammonium sulfate (four salts, not a combination of two salts as recited in the claimed method), is loaded onto the HIC column. Further, Holtz et al., column 27, lines 17 to 31, describes a second method of preparing IGF-1 for a HIC column, comprising collecting the IGF-1 eluant from the cation exchange column, diluting into sodium acetate/phosphate buffer in addition to adding ammonium sulfate

to 15% of saturation levels (three salt combination instead of two salts). Again Holtz et al. does not disclose the *particular combination of two salts, citrate and phosphate salts*, at concentrations of between about 0.1M to 1.0M, at a pH of between about 5.0 to about 7.0.

Holtz et al., column 32, lines 19 to 31, describes solutions of the IGF-1 protein in 50mM sodium acetate and 50 mM sodium phosphate buffer, pH 4.5, with added ammonium sulfate (up to 10% saturation) to prepare and load onto a second HIC column. Again Holtz. et al. does not describe the *combination of two salts, citrate and phosphate*, at concentrations of between about 0.1M to 1.0M., at a pH of between about 5.0 to about 7.0 (claim 2).

Therefore, because the reference to Holtz et al. does not describe all of the elements of the claimed process for purifying a protein comprising mixing the protein with a combination of two salts only, citrate and phosphate, at concentrations of between about 0.1M and 1.0M, and loading this mixture onto the column, Applicants submit that Holtz et al. does not anticipate the claimed subject matter. Reconsideration and withdrawal of the rejection on the basis of 35 U.S.C. § 102 (b) is respectfully requested.

REJECTION UNDER 35 U.S.C. § 103(a)

Claims 1, 2, 6-12 and 20-23 have been rejected under 35 U.S.C. §103(a) as allegedly *prima facie* obvious over U.S. Patent 5,231,178 to Holtz et al. This rejection is respectfully traversed.

103(a) states: "A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains."

The Examiner has alleged that it would have been obvious to one of ordinary skill in the art at the time the invention was made to purify a protein using the steps set forth in the claimed invention based on the teachings of Holtz et al. The Examiner has alleged that adjustment of the "particular conventional working conditions (if not expressly taught) is deemed merely a matter of judicious selection and routine optimization which is well within the view of the skilled artisan." (page 4 of Office Action). Applicants do not agree for the following reasons.

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.

Further, Applicants submit that it would require more than "routine optimization" to bridge the gap between what is disclosed in Holtz et al. and the instant claimed method. The instant application describes how the claimed process was derived, in Examples 1 and 2. First, the optimum concentration range for the individual salt solutions was determined by preparing salting out or precipitation curves for each protein. Then a second series of salting out curves was prepared for two salt combinations in which the first salt concentration was kept constant and the second salt concentration was increased. Then the second salt was kept constant and the first salt varied (see page 18, Example 1, of the instant application). Finally, the dynamic capacities were determined for the salts alone, at the previously determined optimum concentrations, and then for the combinations of salts, at the previously determined optimum concentrations, in order to determine what combinations of salts would increase the dynamic capacity for the proteins on the HIC column. This was performed for four antibodies and TNFR:Fc (See pages 18-21, Examples 1 and 2). Applicants submit that the work described in Examples 1 and 2 of the instant application represents more than "routine optimization", but rather a lengthy series of experiments leading to a new approach for the selection of combinations of salts for optimizing the dynamic capacity of a protein on a hydrophobic interaction chromatography column. Therefore, for these reasons, Applicants submit that the claimed processes are not in fact *prima facie* obvious over Holtz et al.

Further, the United States Supreme Court in its recent decision in *KSR v. Teleflex*, 127 S.Ct. 1727 (2007), has indicated that an analysis of obviousness should focus on whether an improvement represented by a claimed invention is "more than the *predictable* use of prior art elements according to their established functions." *Id.* at 13. There must, in addition, be some technical or logical basis for asserting that the advantages of the claimed process would have been *predictable* based on the prior art reference.

Applicants point out that the Examiner has not provided a technical or logical basis for asserting that the advantages of the particular combination of salts of the claimed process would have been *predictable* based on Holtz et al. Thus, Holtz et al. 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 et al.

Therefore, based on the arguments presented above, Applicants request that the rejection on the basis of 35 U.S.C. §103(a) as allegedly *prima facie* obvious over U.S. Patent 5,231,178 to Holtz et al. be reconsidered and withdrawn.

Applicants submit that the claims are currently in form for allowance. Applicants' attorney invites the Examiner to call her at the number given below if it would be helpful in advancing the prosecution of this application.

Respectfully submitted,

Christine M. Bellas

Christine M. Bellas
Registration No. 34,122
Direct Dial No. (206) 265-8294
Date: July 14, 2008

Immunex Corporation
Law Department
1201 Amgen Court West
Seattle, WA 98119
Telephone (206) 265-7000

jg141901 7/14/08

Exhibit 8

US007781395B2

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

(10) **Patent No.: US 7,781,395 B2**
(45) **Date of Patent: Aug. 24, 2010**

(54) **PROCESS FOR PURIFYING PROTEINS**

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

(73) Assignee: **Immunex Corporation**, 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 229 days.

(21) Appl. No.: **10/895,581**

(22) Filed: **Jul. 21, 2004**

(65) **Prior Publication Data**

US 2006/0229437 A1 Oct. 12, 2006

Related U.S. Application Data

(60) Provisional application No. 60/540,587, filed on Jan. 30, 2004.

(51) **Int. Cl.**
C07K 16/00 (2006.01)

(52) **U.S. Cl.** **514/2**; 530/387.1

(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.	

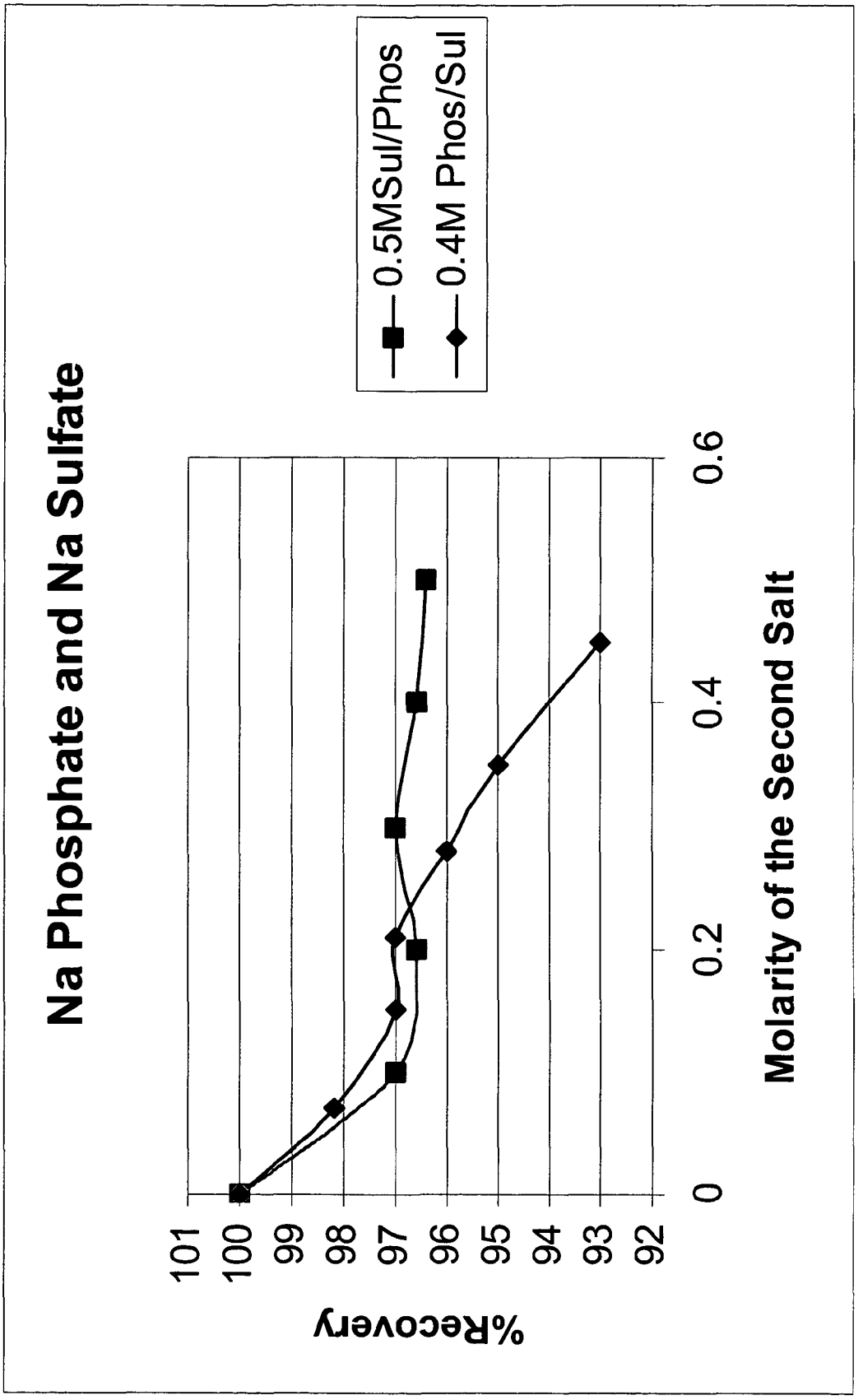
* cited by examiner

Primary Examiner—Christopher R. Tate
Assistant Examiner—Roy Teller
(74) *Attorney, Agent, or Firm*—Christine M. Bellas

(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


Na Phosphate and Na Sulfate
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0.4M Phos/Sul
%Recovery
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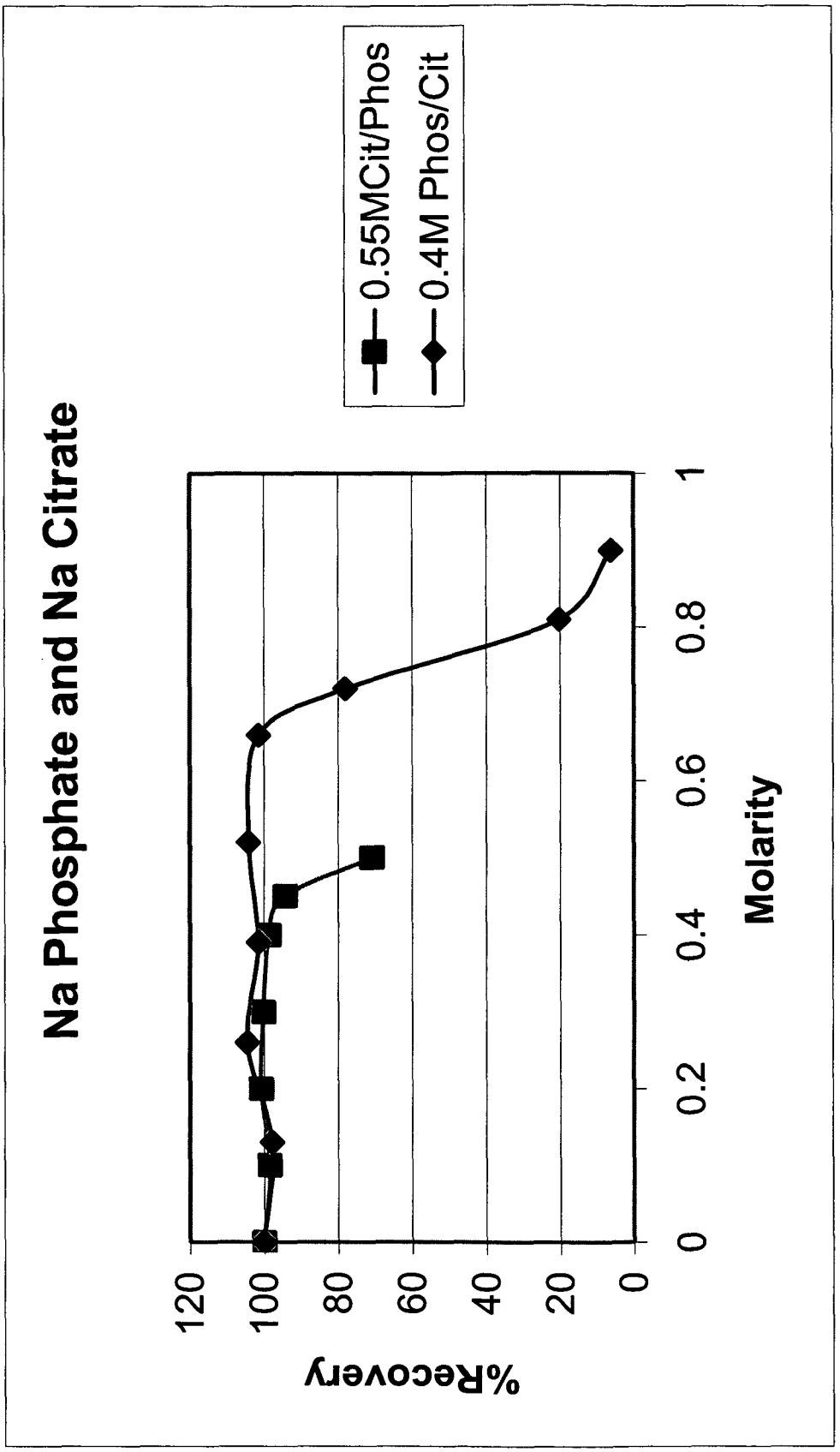
95
94
93
92
0
0.2
0.4
0.6
Molarity of the Second Salt

Figure 1A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0.55MCit/Phos
0.4M Phos/Cit
%Recovery
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60
40
20
0
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0.2
0.4
0.6
0.8
1
Molarity

Figure 1B

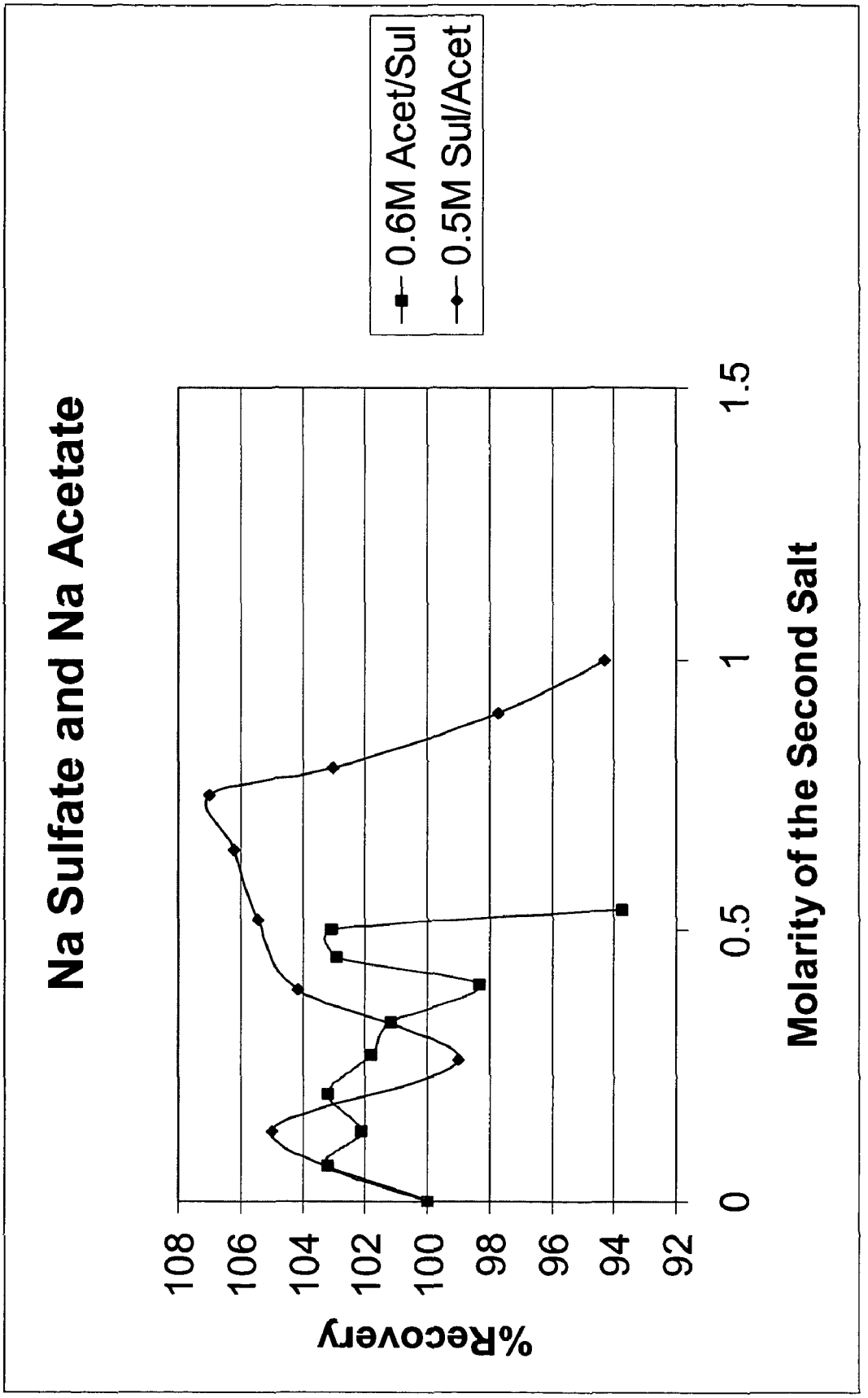
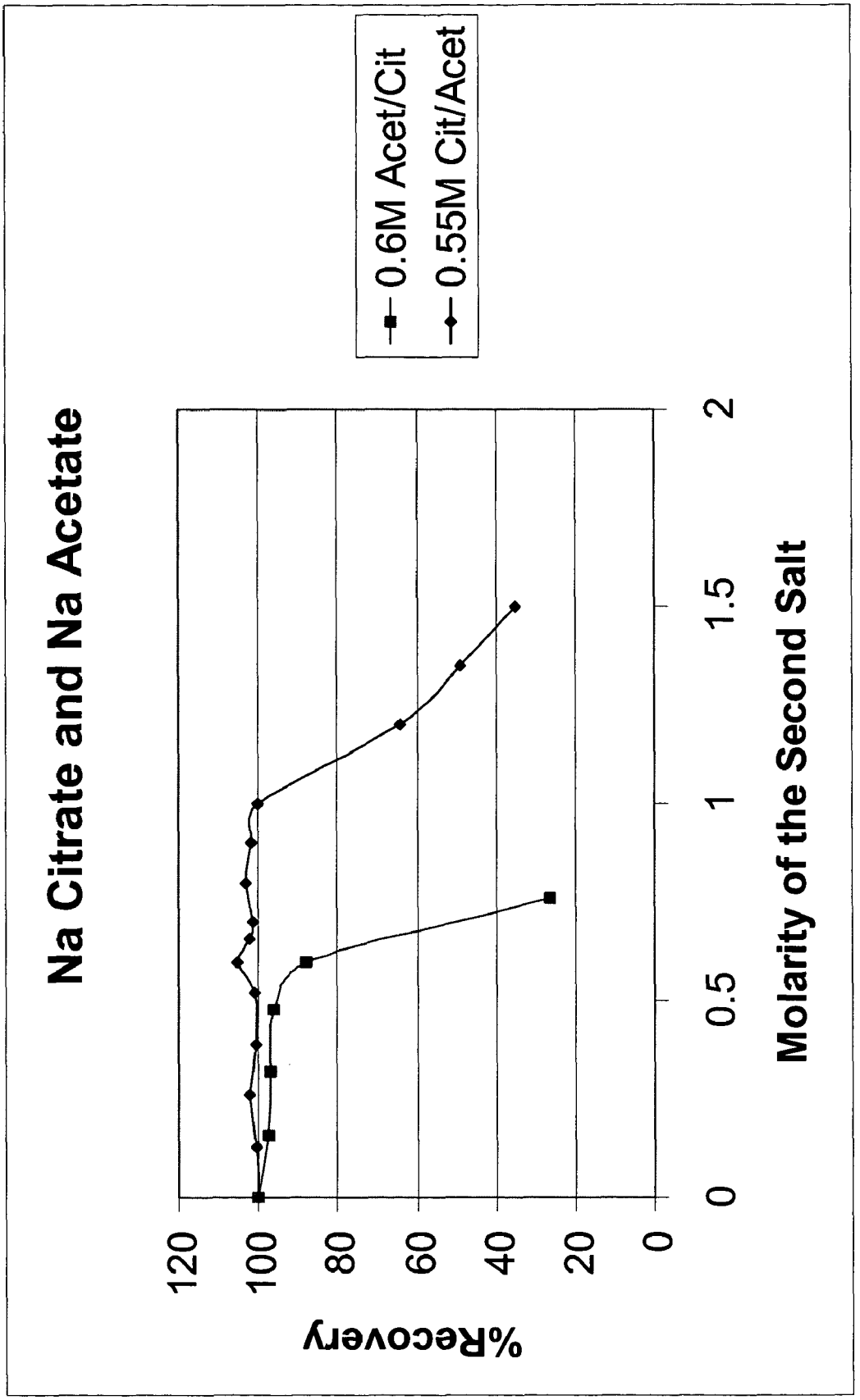

Na Sulfate and Na Acetate
0.6M Acet/Sul
0.5M Sul/Acet
%Recovery
108
106
104
102
100
98
96
94
92
0
0.5
1
1.5
Molarity of the Second Salt

Figure 1C


Na Citrate and Na Acetate
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0.55M Cit/Acet
%Recovery
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100
80
60
40
20
0
0
0.5
1
1.5
2
Molarity of the Second Salt

Figure 1D

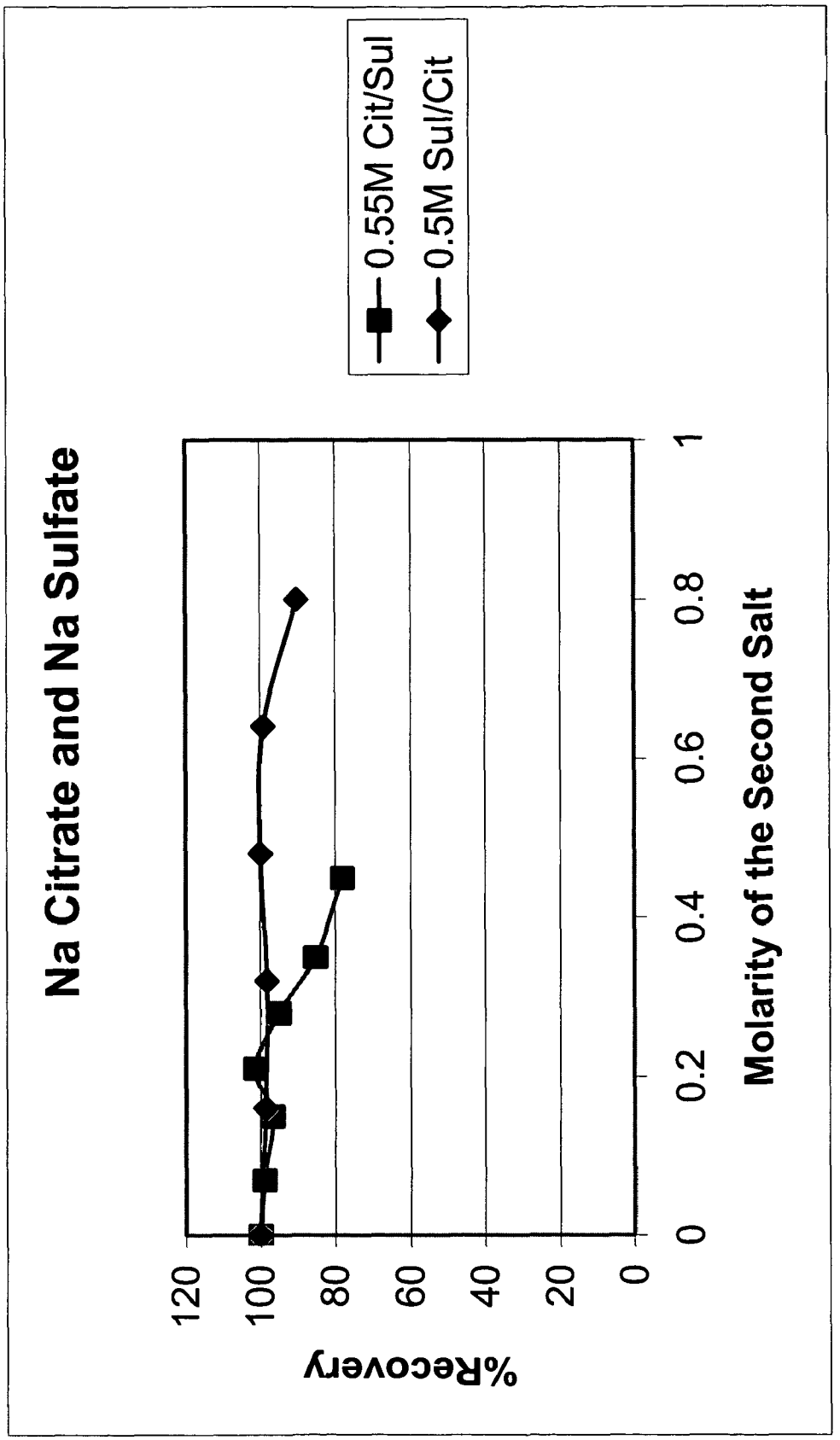

Na Citrate and Na Sulfate
0.55M Cit/Sul
0.5M Sul/Cit
%Recovery
120
100
80
60
40
20
0
0
0.2
0.4
0.6
0.8
1
Molarity of the Second Salt

Figure 1E

PROCESS FOR PURIFYING PROTEINS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application 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 0.1M 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 concentra-

tions of sodium sulfate. FIG. 1D 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. 1E 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 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 lyotropic 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, California); WP HI-Propyl (C_3)™ column (J. T. Baker, New Jersey); and TOYOPEARL™ ether, phenyl or butyl columns (TosoHaas, Pennsylvania).

In one embodiment, TOYOPEARL™ 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. Immunol.* 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. No. 5,395,760, U.S. Pat. No. 5,945,397, and U.S. Pat. No. 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, thrombopoietin, 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), CD 147, 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 *Staphylococcus 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	1.0 M	0.55M Na Citrate/ 0.2M Na Phosphate
TNFR:Fc	0.55M	1.0 M	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 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.

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 about pH 7.

4. The process of claim **1** wherein the citrate and phosphate 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 process of increasing the dynamic capacity of a hydrophobic interaction chromatography column for a particular 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 citrate and phosphate salts, and wherein the concentration of each of the first and second salts in the mixture is between about 0.1M and about 1.0 M.

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

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

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

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

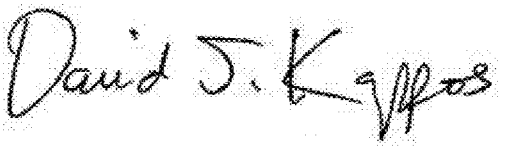
PATENT NO. : 7,781,395 B2
APPLICATION NO. : 10/895581
DATED : August 24, 2010
INVENTOR(S) : Anna Senczuk et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Title page item (73)

The Assignee for the above-referenced Patent is:
AMGEN INC., Thousand Oaks, California

Signed and Sealed this
Twenty-second Day of February, 2011



David J. Kappos
Director of the United States Patent and Trademark Office

Exhibit 9

Redacted in its Entirety

Exhibit 10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the Application of:
Anna Senczuk and Ralph Klinke

Docket No.: 3470-US-NP

Group Art Unit: 1654

Serial No: 10/895,581

Examiner: Teller, Roy R

Filed: July 21, 2004

For: PROCESS FOR PURIFYING PROTEINS

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22131-1450

RESPONSE TO RESTRICTION REQUIREMENT AND PRELIMINARY AMENDMENT OF THE CLAIMS

Dear Sir:

In response to the Office Action dated 12/14/2006, please consider the following response to the restriction requirement, and preliminary amendment of the claims. This response is submitted with a request for a three month extension of time and the appropriate fee.

Response to Restriction Requirement begins on page 2 of this paper.

Preliminary Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks begin on page 6 of this paper.

CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is being facsimile transmitted to the United States Patent and Trademark Office on the date indicated below.

Signed: Kathleen P. Fundle
Kathleen P. Fundle

Date: April 13, 2007

04/16/2007 TL0111 00000015 090089 10895581

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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).

In compliance with the Office Action, Applicants point out that claims 1 and 20 as set forth in the listing of claims attached herein represents the generic claims, claim 13 represents a sub-generic claim, and that the elected species are represented in claims 4, 5, 7, and 13.

Claims 1, 13, and 20 are preliminarily amended as set forth below. Applicants have deferred amending claims 4, 5, 7 and 13 to be limited to the elected species until a later time if deemed necessary.

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application. Entry of the amendments of the claims is respectfully requested.

CLAIMS

What is claimed is:

1. (currently amended) 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, 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.

2. (original) 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. (canceled)

4. (original) The process of claim 1 wherein the first salt is selected from the group consisting of citrate, acetate, phosphate, and sulfate salts.

5. (original) The process of claim 1 wherein the second salt is selected from the group consisting of citrate, acetate, phosphate, and sulfate salts, and wherein the second salt is not identical to the first salt.

6. (original) The process of claim 1 wherein the column is eluted with a solution having a pH between about pH 5 and about pH 7.

7. (original) The process of claim 1 wherein the first salt and second salt are selected from the group consisting of citrate and sulfate; citrate and acetate; citrate and phosphate; acetate and sulfate; and sulfate and phosphate.

8. (original) The process of claim 1 wherein the protein is a fusion protein or an antibody.

9. (original) The process of claim 1, further comprising diluting the protein.

10. (original) The process of claim 1, further comprising filtering the protein.

11. (original) The process of claim 1, further comprising formulating the protein.

12. (original) The process of claim 1, further comprising lyopholizing the protein.

13. (currently amended) 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 are selected from the group consisting of citrate and sulfate; citrate and acetate; citrate and phosphate; acetate and sulfate; and sulfate and phosphate, and wherein the concentration of each of the first and second salt in the mixture is between about 0.1M and about 1.0 M.

14. (original) The process of claim 13 wherein the pH of the mixture loaded onto the column is between about pH 5 and about pH 7.

15. (canceled)

16. (original) The process of claim 13 wherein the salts are sodium salts.

17. (original) The process of claim 13 wherein the column is eluted with a solution having a pH of between about pH 5 and about pH 7.

18. (original) The process of claim 13 wherein the protein is a fusion protein or an antibody.

19. (original) The process of claim 13 wherein the protein is a monoclonal antibody or an Fc fusion protein

20. (currently amended) A method of maximizing the dynamic capacity of a hydrophobic interaction chromatography column for a particular protein at a desired pH comprising selecting a combination of concentrations for a first salt and a second salt wherein the first salt and the second salt have different lyotropic values, and least one salt has a buffering capacity at the desired pH, and wherein the concentrations of the first salt and the second salt are determined using precipitation curves for the salts individually and for the combination of salts, and wherein the concentration of each of the first and second salt in the mixture is between about 0.1M and about 1.0 M.

REMARKS

Claims 1-2, 4-14 and 16-20 are currently pending in the application. Claims 1, and 13 have been amended to incorporate the limitations of claims 3 and 15 respectively. Claim 20 has also been amended to incorporate the same limitations. Claims 3 and 15 have been canceled. Basis for the amendments to the claims are found in the specification and the claims as originally filed, and therefore, no new matter is presented by the amendments. Amendments to limit claims 4, 5, 7, and 13 to a single species, if needed, will be deferred until a later response and amendment.

Applicants' attorney invites the Examiner to call her at the number given below if it would be helpful in advancing the prosecution of this application.

Respectfully submitted,

Christine M. Bellas
Registration No. 34,122
Direct Dial No. (206) 265-8294
Date: April 13, 2007

Immunex Corporation
Law Department
1201 Amgen Court West
Seattle, WA 98119
Telephone (206) 265-7000

Exhibit 11

Redacted in its Entirety