Internal distribution code:

(A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 10 January 2018

Case Number: T 1558/13 - 3.3.04

Application Number: 04796067.9

Publication Number: 1687328

IPC: C07K14/00, C12P21/06

Language of the proceedings: EN

Title of invention:
Process for purifying proteins in a hydrophobic interaction chromatography flow-through fraction

Patent Proprietor:
Amgen, Inc.

Opponent:
Franke, Andreas

Headword:
Process for purifying target proteins from contaminants/AMGEN

Relevant legal provisions:
EPC Art. 54, 56, 111(1), 123(2)
RPBA Art. 12(4), 13(1)

Keyword:
Main request - requirements of the EPC met (yes)
Late-filed lines of arguments - admission into the appeal proceedings
Remittal to the department of first instance - (no)

Decisions cited:
T 1067/08

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0
Fax +49 (0)89 2399-4465

Case Number: T 1558/13 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 10 January 2018

Appellant I: (Patent Proprietor)	Amgen, Inc. One Amgen Center Drive Thousand Oaks, CA 91320-1799 (US)
Representative:	Dörries, Hans Ulrich df-mp Dörries Frank-Molnia & Pohlman Patentanwälte Rechtsanwälte PartG mbB Theatinerstrasse 16 80333 München (DE)
Appellant II: (Opponent)	Franke, Andreas Salbeiweg 24 76149 Karlsruhe (DE)
Representative:	Schrell, Andreas Gleiss Große Schrell und Partner mbB Patentanwälte Rechtsanwälte Leitzstrasse 45 70469 Stuttgart (DE)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 4 June 2013 concerning maintenance of the European Patent No. 1687328 in amended form.**

Composition of the Board:

Chairwoman	G. Alt
Members:	M. Montrone
	M. Blasi

Summary of Facts and Submissions

I. Appeals were lodged by the patent proprietor (hereinafter "appellant I") and by the opponent (hereinafter "appellant II") against the interlocutory decision of the opposition division that European patent No. 1 687 328 could be maintained in amended form. The patent was filed as an international application and published as WO 2005/042569 (hereinafter "application as filed"). The patent has the title "*Process for purifying proteins in a hydrophobic interaction chromatography flow-through fraction*".

II. In the impugned decision, the opposition division held that claims 1 and 5 of the main request (corresponding to the ones of the patent as granted) did not comprise subject-matter extending beyond the content of the application as filed and that the subject-matter of claim 1 was novel over the disclosures of documents D4, D7, D9, D12, D13 and D18, but lacked novelty over the disclosure of document D11.

Claim 1 of auxiliary request 1 comprised subject-matter which extended beyond the content of the application as filed and claims 2 or 4 of auxiliary requests 2 and 3, respectively, lacked clarity.

Auxiliary request 4 filed during the oral proceedings was considered to meet the requirements of the EPC. Concerning inventive step, the opposition division considered the disclosure of document D4 as the closest prior art - rather than document D11 as suggested by the opponent - and took the view that the claimed subject-matter involved an inventive step starting from document D4 in the light of the teaching of documents

D12 and D17 (see section V below for the documents cited).

III. Appellant I submitted with its statement of grounds of appeal arguments why the main request (patent as granted) met the requirements of the EPC, in particular why the claimed subject-matter was novel over the disclosure of document D11.

Claims 1 and 5 of the main request read:

"1. A process for separating a target protein from a mixture containing the target protein and contaminants, comprising:

a) contacting the mixture with a hydrophobic adsorbent comprising branched hydrocarbon functional groups in an aqueous salt solution at a pH of at least 5.5 under conditions that permit the contaminants to bind to the adsorbent and the target protein to pass through the adsorbent in a flow-through fraction without binding to the hydrophobic adsorbent; and

b) collecting the flow-through fraction of the mixture containing the target protein that does not bind to the hydrophobic adsorbent.

5. The process of claim 1 for separating a recombinant target protein, produced as a product of cell culture expression in a host cell, from a mixture containing the target protein and contaminants, which further comprises, prior to step (a) the steps of:

i) preparing a chromatography column having a support comprising hydrophobic branched alkyl functional groups, wherein the branched alkyl functional groups

have from 4 to 8 carbon atoms, at least one of which is a tertiary carbon atom,

ii) preparing the mixture in an aqueous solution at a pH of at least 5.5 having a salt concentration such that the contaminants bind to the column while the target protein in the mixture does not bind to the column;

wherein step (a) comprises contacting the mixture with the column under said conditions; and
wherein step (b) comprises collecting from the column the flow-through fraction of the mixture containing the target protein that does not bind to the column."

IV. Appellant II submitted in its statement of grounds of appeal and its reply to appellant I's appeal arguments why, contrary to the opposition division's finding, claims 1 and 5 of the main request comprised subject-matter extending beyond the content of the application as filed and why the subject-matter of claim 1 lacked novelty over the disclosure of documents D4, D7, D10, D11, D13 and D18 (see section below).

Furthermore it submitted with regard to inventive step of the main request and the auxiliary requests that not all functional groups falling within the ambit of the feature "*branched hydrocarbon functional groups*" referred to in step a) of claim 1 achieved an improved separation. Moreover, the subject-matter of claim 1 of the main request lacked an inventive step in view of the teaching of document D12 (see section below) as the closest prior art combined with the common general knowledge of the skilled person.

V. The following documents are cited in this decision:

D4: WO 00/59927

D7: Suck *et al.*, J. Immunol. Methods, 229(1-2), 1999, p.73-80

D10: EP 1 260 518

D11: WO 03/008447

D12: US 5,468,847

D13: WO 03/031471

D18: US 6,005,081

D20: CD Römpp Chemie Lexikon, 1995, entries on "Aliphatische Verbindungen" and "Ketten"

D21: Phenyl Sepharose CL-4B: Instructions 71-7080-00 AE; Hydrophobic interaction chromatography, GE Healthcare, Manufacturer's Instruction, 2007

VI. Oral proceedings were held before the board on 10 January 2018. In its course, appellant II *inter alia* accepted that the disclosures of documents D9 and D12 were not detrimental to the novelty of the subject-matter of the main request and withdrew its objection of lack of novelty based on document D10 (see minutes, page 3 second and fourth paragraph). At the end of the oral proceedings the chairwoman announced the board's decision.

VII. Appellant I's arguments, as far as they are relevant to the present decision, may be summarised as follows:

Main request - claims of the patent as granted

Amendments (Article 100(c) EPC) - claims 1 and 5

The feature "*contacting the mixture with a hydrophobic adsorbent [...] at a pH of at least 5.5*" referred to in step a) of claim 1 had a basis on page 14, lines 1 to 7 of the application as filed read in conjunction with the disclosure on page 6, lines 3 to 8 of the application as filed.

Furthermore the feature "*preparing the mixture in an aqueous solution at a pH of at least 5.5*" referred to in step ii) of claim 5 had a basis in claim 16 as filed in the light of the claim's preamble and the disclosure on page 14, lines 1 to 7 of the application as filed in conjunction with the disclosure on page 4, lines 11 to 13 of the application as filed.

Novelty (Articles 100(a) and 54 EPC) - claim 1

The feature "*hydrophobic adsorbent comprising branched hydrocarbon functional groups*" referred to in step a) of claim 1 was structurally characterised by a central carbon (C) atom bound to three adjacent C atoms, as derivable from document D20.

Documents D4, D7, D13 and D18 all disclosed Phenyl-Sepharose™ as hydrophobic adsorbent material which contained phenyl molecules as functional groups. A phenyl molecule was characterised by a closed aromatic ring structure, wherein each C atom was linked to two adjacent C atoms. It was covalently coupled to the

Sepharose™ matrix by an ether group, i.e. an oxygen (O) atom (see document D21, page 1), so that the C atom at the linkage site in the phenyl ring was bound to two adjacent C atoms and one O atom. Thus, the phenyl group present in Phenyl-Sepharose™, although being aromatic, had a structure different from a branched hydrocarbon. Therefore, Phenyl-Sepharose™ as hydrophobic adsorbent material did not fall within the ambit of claim 1.

The feature "*that permit the contaminants to bind to the adsorbent and the target protein to pass through the adsorbent in a flow-through fraction without binding to the hydrophobic adsorbent*" in step a) of claim 1 was not disclosed in document D11. Example 3 in this document disclosed a three-step process for the separation of lactoperoxidase (target protein) from two growth factors (contaminants) in milk relying on the use of a column filled with a hydrophobic material containing as functional groups a branched hydrocarbon. The example reported that after the sample had been loaded on the column, two wash steps were carried out, the first with a solution having a salt concentration of "*0.025M phosphate with 0.25M NaCl*" (total salt: 0.275M), followed by a second solution containing "*0.1M ammoniumacetate*" (total salt: 0.1M) (see page 16, lines 11 and 12). The example further mentioned that after the washing "*lactoperoxidase was mainly present in the void and washfractions*" (see page 16, lines 15 to 16), while the growth factors remained bound to the column until eluded during a third process step.

However, example 3 was silent with regard to the amount of lactoperoxidase present in each of the various void and wash-fractions disclosed and whether or not the fractions have been pooled. Therefore, in view of the

disclosure in example 3, two equally probable separation scenarios were possible.

Firstly, that all three proteins bound to the column material after loading the sample and during the first wash step performed under high salt concentrations. Upon lowering the salt concentration by nearly three-fold during the second wash step, specifically only lactoperoxidase was eluded and found in the void and wash fractions. In this scenario the separation of lactoperoxidase from the growth factors relied on a binding-elution process.

Secondly, lactoperoxidase did not bind to the adsorbent material on the column contrary to the growth factors, and its separation from them by two wash steps resulted in lactoperoxidase's presence in the void and wash-fractions and thus relied on a flow-through process.

Therefore, since a first binding of lactoperoxidase to the adsorbent material and its later elution could not be excluded from the disclosure of document D11, the document did not directly and unambiguously disclose the process according to claim 1.

Remittal (Article 111(1) EPC)

The case should not be remitted to the opposition division for assessing inventive step on a line of argument based on document D12 since this document was in the proceedings from the beginning. Furthermore, the document was technically not complex and the board was in a position to decide the case.

Inventive step (Articles 100(a) and 56 EPC)

Admission of a line of argument based on document D12 as closest prior art (Article 12(4) RPBA)

Appellant II's argument based on document D12 as the closest prior art should be excluded from the appeal proceedings according to Article 12(4) RPBA, since the argument was deliberately dropped during the opposition proceedings as evidenced by the fact that it was no longer pursued from a certain time point during the written phase and at the oral proceedings before the opposition division, during which appellant II only relied on document D11 as the closest prior art.

Bringing up this argument now again during the appeal proceedings resembled the situation dealt with in decision T 1067/08 wherein the board held that parties were not at liberty to shift arguments brought forward in the first instance to the second instance as it pleased, since such a "forum shopping" would have jeopardised the proper distribution of functions between the departments of first instance and the boards of appeal.

Closest prior art

The process disclosed in document D12 differed from the process according to claim 1 in at least two technical features, i.e. the pH value at which it was performed and the use of a binding-elution, and not of a flow-through, process for the separation of the bovine serum albumin (BSA) as target protein from cytochrome C (Cyt C) as the contaminant.

Therefore, rather the process disclosed in document D4 represented the closest prior art, since it differed from the claimed process only in one technical feature, i.e. the use of the non-branched hydrocarbon functional group phenyl.

Admission of a line of argument based on document D4 as closest prior art in combination with document D12 (Article 13(1) RPBA)

Appellant II's line of argument based on document D4 as the closest prior art in combination with document D12 should not be admitted into the appeal proceedings since it had not been raised before in writing in the appeal proceedings and thus represented an amendment to appellant II's case. Furthermore, its submission at the oral proceedings was very late since appellant II had ample time to present the argument earlier in view of the time elapsed between the date when the appeal had been filed and the oral proceedings. Submitting it now at the oral proceedings came as a surprise for appellant I and it would be faced with a new situation and would have to react to an objection and arguments not heard before.

In view of the opposition division's finding in relation to the auxiliary request considered allowable by it, namely that document D4 was the closest prior art for the assessment of inventive step, appellant II could have expected that the board might arrive at the same conclusion in relation to the main request.

Moreover, the subject-matter of this auxiliary request was considered by the opposition division to involve an inventive step having regard of the teaching of document D4 in combination with document D12. Also,

with regard to inventive step in relation to the main request, appellant I had, in its statement of grounds of appeal, referred to the specific passages in the annex accompanying the opposition division's summons to oral proceedings in which the subject-matter of the main request was considered to involve an inventive step over the combined teachings of documents D4 and D12. Thus, these circumstances too showed that appellant II should have submitted this line of argument at an earlier stage in the appeal proceedings.

Technical problem and solution

Phenyl groups in Phenyl-Sepharose™ were not encompassed by the feature "*branched hydrocarbon functional groups*" referred to in step a) of claim 1, for the reasons set out in relation to novelty. Accordingly, the disclosure in example 3 in the patent in suit demonstrated that an improved separation of the target protein RANK:Fc (Peak B) from its misfolded form (Peak C, see column 19, line 21) was achieved by all functional groups falling within the ambit of the claim (see paragraph [0072]).

VIII. Appellant II's arguments, as far as they are relevant to the present decision, may be summarised as follows:

Main request - claims of the patent as granted

Amendments (Article 100(c) EPC) - claims 1 and 5

The feature "*contacting the mixture with a hydrophobic adsorbent [...] in an aqueous salt solution at a pH of at least 5.5*" referred to in step a) of claim 1 had no basis in the application as filed, since the passage on page 14, line 1 to 12 disclosed only two possible

scenarios for chromatographic steps to be carried out at a pH of at least 5.5.

Firstly, the equilibration of the column (see page 14, line 12), which was carried out earlier and independent of the actual chromatographic separation.
Secondly, a chromatography relying on the use of "*Citrate buffers and salts*" (see page 14, line 6) as derivable from the phrase "*Such buffers or salts can have a pH of at least about 5.5*" (see page 14, line 7), wherein the terms "*Such buffers or salts*" indicated that the pH of 5.5 exclusively related to the citrate buffers and salts mentioned in the immediately preceding sentence. Further indications for this interpretation of the disclosure on page 14 of the application as filed were derivable from page 19, lines 15 to 18 which disclosed that chromatography not relying on citrate buffers was performed at a minimum pH of "*about 6.0*", i.e. a pH higher than pH 5.5, while when citrate was used as a buffer, the pH had a "*range of 5-7*", i.e. a pH lower than 5.5.

With regard to claim 5, the feature "*preparing the mixture in an aqueous solution of at least 5.5*" in the preparation of the loading medium, i.e. the mixture comprising the target protein and the contaminants referred to in step ii), did not have a basis in the application as filed. The passage on page 14, lines 1 to 14 disclosed process steps in relation to loading/ contacting, equilibration and performing a chromatography, while it was silent on the preparation of a loading mixture.

Furthermore, the skilled person was aware of the fact, that (i) the pH of the loading mixture could be different from the aqueous solution wherein the

hydrophobic adsorbent was suspended, in particular in situations where the target protein and the contaminants were not soluble, and (ii) that a chromatography comprised several steps either performed before or during a chromatography, which might be associated with differed pH conditions.
Furthermore, the application as filed did not disclose the features referred to in steps a) and ii) of claims 1 and 5 "*contacting the mixture with a hydrophobic adsorbent [...] in an aqueous salt solution at a pH of at least 5.5* or "*preparing the mixture in an aqueous solution at a pH of at least 5.5*" for any aqueous salt solution for the reasons set out in the context of claim 1 with regard to citrate buffers and salts.

In addition, it was commonly known that certain salts mentioned on page 14, lines 4 and 5 of the application as filed did not alter the pH value of an aqueous solution at all when they were dissolved therein.

Novelty (Articles 100(a) and 54 EPC) - claim 1

Documents D4, D7, D13 and D18 all disclosed processes for the separation of target proteins from contaminants relying on hydrophobic interaction chromatography (HIC) based on the use of Phenyl-Sepharose™ as adsorbent material. The patent in suit disclosed that the feature "*branched hydrocarbon functional group*" referred to in step a) of claim 1 encompassed "*aromatic*" groups (see paragraphs [0041] and [0043]). Since phenyl was an aromatic functional group, the disclosure of a process using Phenyl-Sepharose™ fell into the ambit of claim 1.

D11 disclosed a process for separating a mixture of lactoperoxidase (target protein) from two growth

factors which could be considered as contaminants. Example 3 in this document disclosed that the separation of the proteins was carried out at pH 6.0 relying on a hydrophobic adsorbent chromatography, wherein the adsorbent comprised tert-butyl (t-butyl) functional groups, i.e. a branched hydrocarbon group, to which the two growth factors bound.

It was further reported in the document that the column was washed with salt solutions and that subsequently the lactoperoxidase was "*mainly present in the void and washfractions*", i.e. the flow-through fractions, which meant that lactoperoxidase did not bind to the hydrophobic adsorbent (see page 16, lines 10 to 16).

The subject-matter of claim 1 was thus not novel over the disclosure of any of documents D4, D7, D11, D13 and D18.

Remittal (Article 111(1) EPC)

The case should be remitted to the opposition division, since the finding of the board that the main request was novel over the cited prior art led to the situation that for the first time in the proceedings inventive step of the subject-matter of claim 1 of the main request had to be assessed.

Inventive step (Articles 100(a) and 56 EPC)

Admission of a line of argument based on document D12 as closest prior art (Article 12(4) RPBA)

The line of argument based on document D12 as the closest prior art should not be excluded from the appeal proceedings. It had not been withdrawn during

the opposition proceedings. It was with regard to the main request merely not presented at the oral proceedings in view of the opposition division's finding concerning lack of novelty.

In addition, the alleged withdrawal of the argument happened in relation to auxiliary request 2, and not in relation to the main request, and occurred thus in a different procedural situation.

Closest prior art

Document D12 disclosed a process for the separation of a target protein from a contaminant using hydrophobic interaction binding relying on a "*Macro Prep™ t-Butyl HIC™ Support*", i.e. a hydrophobic adsorbent with branched hydrocarbon functional groups. The process was carried out in a flow-through mode at pH 5.4 (see example 4), while the process according to claim 1 was carried out at pH 5.5, i.e. a 0.1 higher pH value. This small difference in pH had a low impact on the separation selectivity of target proteins from their contaminants and was therefore not an important difference between the claimed process and the process disclosed in document D12.

The process disclosed in document D4 used phenyl or alkyl residues as functional groups in the hydrophobic adsorbent material for separating target proteins from contaminants in a flow-through mode at pH 7.0 (see page 2, lines 24 to 28, page 4, lines 14 to 17, page 9, lines 5 to 19). The document was silent on branched hydrocarbon functional groups, which were thus the sole difference to the claimed process. The patent in suit disclosed in example 3 that branched hydrocarbon functional groups were essential for the achievement of

an improved separation compared to linear hydrocarbon functional groups.

Thus, while the technical effect resulting from the difference between the process disclosed in document D12 and the claimed process was only minor, the effect resulting from the difference between the process disclosed in document D4 and the claimed process was significant. Accordingly, the process disclosed in document D4 represented the closest prior art for the subject-matter of claim 1.

Admission of a line of argument based on document D4 as closest prior art in combination with document D12 (Article 13(1) RPBA)

Although a line of argument based on document D4 as closest prior art in combination with document D12 had not been submitted in writing during the appeal proceedings, both documents were in the proceedings, since an objection of lack of novelty based on document D4 had been raised (see arguments with regard to novelty above), while an objection of lack of inventive step was based on document D12 (see above).

Furthermore, a line of argument based on these two documents would not raise complex technical issues, since its submission was solely intended to address the finding of the opposition division in the decision under appeal which read: "*However, the skilled person would not combine the disclosure of D4 with D12 to arrive at the solution proposed, since D12 does not suggest to use a higher pH value in combination with a t-butyl HIC adsorbent in order to increase the efficacy of the process*".

Technical problem and solution

The patent in suit disclosed in its four examples processes for purifying target proteins from contaminants which solely relied on the use of a hydrophobic adsorbent material carrying t-butyl functional groups. These groups constituted a specific type of branched hydrocarbon since their central C atom was linked to three adjacent C atoms, while the subject-matter of claim 1 encompassed all kinds of branched hydrocarbons.

However, the patent in suit disclosed in example 3 that phenyl groups, which were comprised by the feature "*branched hydrocarbon*" referred to in step a) of claim 1 (see arguments with regard to novelty), did not achieve an improved separation of RANK:Fc from its misfolded form compared to various linear hydrocarbon groups, for example, butyl and ether (see column 20, lines 3 to 6).

Therefore, the technical problem of providing an improved process for the separation of target proteins from their contaminants was not solved by all hydrophobic branched functional groups falling within the ambit of claim 1.

IX. Appellant I requested that the decision under appeal be set aside and that the patent be maintained as granted, or alternatively, that it be maintained on the basis of the claims of auxiliary requests 1 to 17.

Appellant II requested that the decision under appeal be set aside and that the patent be revoked.

Reasons for the Decision

Main request - claims of the patent as granted

Amendments (Article 100(c) EPC) - claims 1 and 5

1. In the following the references are to passages and claims in the application as filed.

2. Any amendment to the parts of a European patent application or of a European patent (description, claims and drawings) can only be made within the limits of what a skilled person would derive directly and unambiguously, either explicitly or implicitly, using common general knowledge, and seen objectively and relative to the date of filing, from the whole of the document as filed. An implicit disclosure in this context is what the person skilled in the art would consider as necessarily implied by the disclosure of the document as a whole (Case Law of the Boards of Appeal, 8th edition 2016 (hereinafter "CLBA"), II.E. 1.2.1 and II.E.1.2.2).

3. The issue is whether or not the subject-matter of claims 1 and 5, which is *inter alia* characterised by the features "*contacting the mixture with a hydrophobic adsorbent [...] in an aqueous salt solution at a pH of at least 5.5*" and "*preparing the mixture in an aqueous solution* at *a pH of at least 5.5*" (hereinafter the "pH feature") as referred to in steps a) and ii) in claims 1 and 5, are disclosed in the application.

4. With regard to step a) in claim 1, appellant II argued that the "pH feature" did not have a basis in the

disclosure on page 14, lines 1 to 7 of the application read in conjunction with page 19, lines 13 to 18.

4.1 The application reports on page 14, line 3 to 7 that "*chromatography (and loading of the protein to be purified) can occur in a variety of buffers or salts including sodium, potassium, ammonium, magnesium, calcium, chloride, fluoride, acetate, phosphate, and/or citrate salts and/or Tris buffer. Citrate buffers and salts are preferred by those skilled in the art for their ease of disposal. Such buffers or salts can have a pH of at least about 5.5.*" (emphasis added).

Furthermore, on page 6, lines 3 to 5 the application reads: "*Chromatography: Chromatography is the separation of chemically different molecules in a mixture from one another by contacting the mixture with an adsorbent*" (emphasis added).

4.2 In the board's view, the application thus discloses to the skilled person in the passage on page 6 indicated in point 4.1 above that chromatography in general relies on the contacting between the sample mixture to be separated and the adsorbent material and that therefore the term "*contacting*" as referred to in step a) of claim 1 is necessarily implied in performing a chromatography. The passages on page 14 of the application indicated above further disclose that the chromatography is performed generally "*in a variety of buffers or salts".* Several exemplary salts and buffers are mentioned, including *inter alia* "*citrate salts*", which are also preferred due to their "*ease of disposal*". Furthermore, since buffers and salts are generally used in performing a chromatography, the skilled person would have inferred from the phrase "*Such buffers or salts can have a pH of at least about*

5.5" in line 7 on page 14 of the application that this too related to all buffers and salts disclosed for this purpose. A further indication that the disclosure of "*contacting the mixture with a hydrophobic adsorbent [...] in an aqueous salt solution at a pH of at least pH 5.5*" is not limited to citrate buffers and salts, is the disclosure of citrate buffers and their salts in a list of several exemplary buffers and salts and, although they are preferred, this is due to their "*ease of disposal*" and apparently not for their pH value.

5. Appellant II further argued that the passage in lines 13 to 18 on page 19 of the application supported its view in interpreting the disclosure on page 14 of the application with regard to the "pH feature" that it was solely related to citrate buffers and salts in the context of chromatography, while for a chromatography performed in buffers other than citrate, pH values higher than 5.5 were used.

5.1 The board is not convinced by this argument for the following reasons. It was common ground between the parties that the passage under consideration on page 19, lines 13 to 18 of the application was directed to pH conditions applied for protein chromatography, which reads as follows: "*Conditions under which these columns are used vary with the specific columns as is known in the art. For most proteins of interest, the pH range may be between about 6.0 and about 8.6, or alternatively between about 6.5 and about 7.5. However, certain proteins are known to be resistant to pH extremes, and a broader range may be possible. Typical conditions include a pH range of 5-7 and a sodium citrate concentration range of 0 to about 0.8M (e.g. 0.5M sodium citrate, pH 6.0)*" (emphasis added).

5.2 In the board's view, the skilled person would derive from the passage in the application cited in point 5.1. above, that for the chromatographic separation of "*most proteins*" the pH is selected in the range of "*between about 6.0 and about 8.6*", while a broader range, i.e. a pH lower than 6.0 or higher than 8.6, is possible for proteins being pH resistant. Thus, in the light of this disclosure it is the pH resistance of the individual protein which determines under which pH condition the chromatography is performed and not the buffer or its salt, let alone the citrate buffer or salts thereof.

6. With regard to the feature in step ii) of claim 5 "*preparing the mixture in an aqueous solution at a pH of at least 5.5*", appellant II argued that the "pH feature" did not have a basis in the application since the preparation of the loading medium, i.e. the mixture of proteins, in an aqueous solution at this pH range was neither explicitly disclosed nor necessarily implied by the steps "*equilibration*" of the column, "*chromatography*" of the protein mixture and "*loading of the protein to be purified*" disclosed on page 14, lines 1 to 12 of the application. This was so because the latter steps were all independent from the preparation of the protein mixture, since there was no need that all steps in relation to a chromatography were performed under the same pH conditions.

6.1 Step ii) in claim 5 is - with the exception of the indication of the pH - literally based on step b) of claim 16 as filed which reads: "*preparing the mixture in an aqueous solution having a salt concentration such that the contaminants bind to the column while the target protein in the mixture does not bind to the column*". Furthermore, the preamble of claim 16 recites the feature "*a mixture containing the target protein*

and contaminants" which is taken up later in step b) of claim 16 by the term "*the mixture*".

6.2 In the board's view, the skilled person would interpret the meaning of "*preparing the mixture*" in the passages of claim 16 indicated above, to relate to the preparation of a protein mixture in an aqueous salt solution under chromatographic conditions, such that the contaminants selectively bind to the column, while the target protein does not. This is derivable from the preamble of claim 16 which is directed to "*A process for separating*", step a) of claim 16 which relates to the preparation of a "*chromatography column*" and from step c) in claim 16 which reads: "*contacting the mixture with the column*", since contacting is necessarily implied in chromatography (see point 4.2 above). Furthermore, the pH condition under which a chromatography is performed is disclosed on page 14, line 7 of the application which reads "*a pH of at least about 5.5*". It follows from this that also the mixture of proteins that is separated by chromatographic means has necessarily to be prepared under the same pH condition.

7. Appellant II further argued that since the application did not provide a basis for a chromatography performed in buffers having a pH of at least 5.5, the same applied to aqueous salt solutions. Lastly, appellant II argued that it was commonly known that certain salts disclosed on page 14, lines 4 and 5 of the application did not change the pH of an aqueous solution.

7.1 The board is not convinced by these arguments either, since the application reads in line 7 of page 14 that "*Such buffers or salts can have a pH of at least about 5.5*" (emphasis added), which discloses that also salts

and thus aqueous salt solutions in general as referred to in steps a) and ii) of claims 1 and 5, respectively, have a basis in the application for the reasons set out above (see points 4.2, 6.1 and 6.2).

7.2 Furthermore, since this disclosure refers in fact to all salts, the issue that some of the salts disclosed in lines 4 and 5 of page 14 in the application might not qualify as buffers, is considered to be irrelevant in the context of the evaluation of added subject-matter.

8. Therefore, the board concludes that the subject-matter of claims 1 and 5 of the patent in suit does not extend beyond the content of the application as filed. Accordingly, the ground for opposition according to Article 100(c) EPC does not prejudice the maintenance of the patent as granted.

Novelty (Articles 100(a) and 54 EPC)

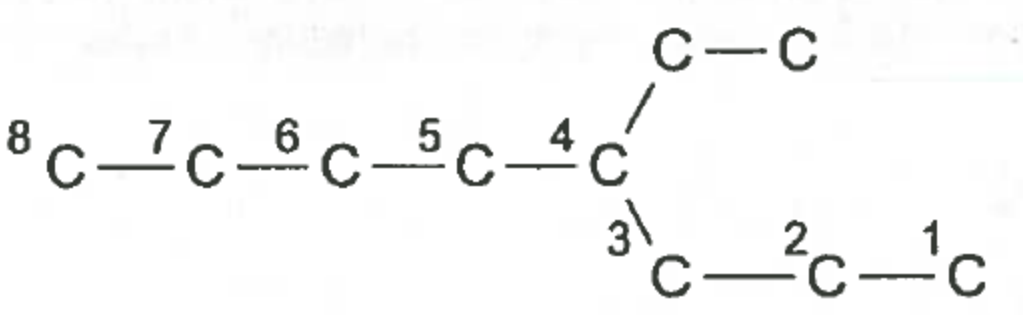
9. Claim 1 is directed to a process for separating a target protein from a mixture containing the protein and contaminants. Step a) requires that the mixture is contacted with a hydrophobic adsorbent comprising branched hydrocarbon functional groups at a pH of at least 5.5 under conditions permitting the contaminants to bind to the adsorbent, while the target protein does not bind and passes through in a flow-through fraction. Step b) requires that the target protein is then collected.

10. Appellant II argued that all of the processes for separating target proteins from contaminants disclosed in documents D4, D7, D13 and D18 were detrimental for

the novelty of the claimed process, essentially because the phenyl molecule coupled as functional group to the hydrophobic adsorbent "*Phenyl-Sepharose™*" was aromatic and hence belonged to the group of "*branched hydrocarbon*" referred to in step a) in claim 1. Evidence for this was found in the patent in suit itself disclosing that "*R is any one or more branched hydrocarbon functional group*" (see paragraph [0041]) which "*may be aromatic*" (see paragraph [0043]).

11. The issue under consideration with regard to novelty of the process according to claim 1 in the light of the disclosure of documents D4, D7, D13 and D18 is thus whether or not phenyl as a functional group in "*Phenyl-Sepharose™*" can be regarded as a branched hydrocarbon.

12. Document D20 is a chemical textbook published in 1995 which represents the understanding of the skilled person of the terms "branched carbon chains" and "ring structure" at the relevant date of the patent in suit. It discloses that branched carbon chains have the following structure:



12.1 Therefore, at the branching site (located at position "*4*" in the structure disclosed above) in a carbon chain, including hydrocarbons, the carbon (C) atom is linked to three adjacent C atoms.

12.2 The document further discloses a definition for cyclic compounds (ring structures, which includes phenyl groups), and branched forms thereof reading as follows "*Geschlossene K. liegen in cycl. Verb. (z.B. den Cycloalkanen, Catenanen u. Knoten) vor; auch diese können Verzweigungen tragen, die man Substituenten am Ring nennt*". [The following note and translation is added by the board: The term "*K.*" is the abbreviation of "Ketten", while the term "*cycl. Verb.*" stands for "cyclische Verbindungen". Translation: "Closed chains are present in cyclic compounds (e.g. cycloalkanes, catenanes and knots); also these can carry branches, designated as substitutions in a ring".]

13. It was common ground between the parties that a phenyl molecule has an aromatic, closed ring structure consisting of six C atoms. In such a ring structure, each C atom is bound to two adjacent C atoms. In "*Phenyl-Sepharose™*" as disclosed in documents D4, D7, D13 and D18, the phenyl group is covalently coupled to an agarose matrix by an ether linkage, i.e. a "C–Oxygen (O)–C" linkage (see document D21, page 1). This means that the C atom in the phenyl molecule at the coupling site is linked via one O atom to the matrix and to two C atoms in its ring structure. Furthermore, the board notes that documents D4, D7, D13 and D18 are all silent on potential substitutions of the phenyl group in the Phenyl-Sepharose™.

14. Therefore, as set out in points 12 and 12.1 above, the "*branched hydrocarbon*" functional group referred to in step a) of claim 1 is defined by a C atom at the branching point which is linked to three adjacent C atoms, while in phenyl groups contained in the "*Phenyl-Sepharose™*" disclosed in documents D4, D7, D13 and D18, the C atom at the coupling site is linked to two

adjacent C atoms and one O atom. It follows from this that the phenyl group present in "*Phenyl-Sepharose™*" is not encompassed by the term "*branched hydrocarbon*" referred to in claim 1.

15. In a further line of argument appellant II submitted that the process according to claim 1 was not novel over the disclosure in document D11. It was common ground between the parties that only the disclosure in example 3 of document D11 was relevant for the assessment of novelty of the claimed process.

16. Document D11 discloses in example 3 a process for the separation of lactoperoxidase (i.e. the target protein) from the two growth factors insulin-like growth factor-1 (IGF-1) and transforming growth factor-β (TGF-β) (i.e. the contaminants) in bovine milk relying on a hydrophobic adsorbent material substituted with tert-butyl (t-butyl), i.e. a branched hydrocarbon functional group. All process steps are carried out at pH 6 (see page 14, lines 5 to 10, page 16, lines 10 to 12). The example reports that after loading the sample on the column, the column is washed twice, first with a solution containing "*0.025M phosphate with 0.25M NaCl*" (i.e. a total salt concentration of 0.275M), followed by a solution containing "*0.1M ammoniumacetate*" (i.e. a total salt concentration of 0.1M) (see page 16, lines 11 and 12). The example further reports that after the washing "*lactoperoxidase was mainly present in the void and washfractions*" (see page 16, lines 15 to 16). Furthermore, example 3 mentions that "*during the linear gradient, an IGF-1 enriched fraction and an TGF-β enriched fraction were obtained*" (see page 16, lines 16 and 17). Example 3 is silent on the amount of lactoperoxidase in the

individual void and wash fractions and whether or not the fractions are pooled.

17. In the board's view, the skilled person would derive from the passages of example 3 in document D11 indicated in point 16 above, that the separation of the three proteins of interest is achieved by performing first two wash steps with solutions containing different salt concentrations followed by an elution step, i.e. a three-step process. In view of the disclosure in example 3, two equally probable separation scenarios appear possible.

17.1 Firstly, all three proteins bind to the column material after loading the sample and during the first wash step performed under high salt concentrations. Upon lowering the solution's salt concentration by nearly three-fold during the second wash step, lactoperoxidase may be specifically eluded from the column and is found in the void and wash fractions. In this scenario the separation of lactoperoxidase from the two growth factors relies on a process based on binding and elution.

17.2 Secondly, lactoperoxidase does not bind to the adsorbent material on the column contrary to the two growth factors, and its separation from the latter two proteins by the two wash steps into the void and wash-fractions relies on a process based on flow-through.

17.3 Therefore, since a binding of lactoperoxidase to the column material cannot be excluded in the light of the disclosure of example 3 in document D11, the document does not directly and unambiguously disclose the process according to claim 1.

18. Thus, the board concludes that the subject-matter of claim 1 is novel over the disclosure of documents D4, D7, D11, D13 and D18 and that the ground for opposition of lack of novelty does not prejudice the maintenance of the patent as granted (Articles 100(a) and 54 EPC).

Remittal (Article 111(1) EPC)

19. In view of the above conclusions, the findings of the opposition division on the lack of novelty are to be overturned and its decision is to be set aside. In cases such as the present one the board may, pursuant to Article 111(1), second sentence, EPC, either exercise any power within the competence of the opposition division or remit the case to it for further prosecution.

20. The parties were in disagreement on the question of remittal. The board, having regard to the circumstances that inventive step was assessed by the opposition division in relation to the auxiliary requests and considering that the objection raised by appellant II on inventive step was based on document D12, a document that had been filed with its notice of opposition, and relied on by appellant II for arguing lack of novelty and inventive step in relation to the patent as granted (see notice of opposition, points 5.3 and 6.1.1 and letter dated 24 November 2011, point 3.2), decided not to remit the case to the opposition division for further prosecution.

Inventive step (Articles 100(a) and 56 EPC)

Admission of a line of argument based on document D12 as closest prior art (Article 12(4) RPBA)

21. A line of argument of a lack of inventive step of the subject-matter of the main request based on document D12 as the closest prior art has been submitted by appellant II with its reply to appellant I's statement of grounds of appeal (see letter dated 28 February 2014, point 4.3). According to Article 12(1) and (2) RPBA, this line of argument is part of the appeal proceedings. The board, however, has pursuant to Article 12(4) RPBA, a discretion to hold inadmissible facts, evidence or requests, which could have been presented or were not admitted into the first instance proceedings.

22. In the present case, appellant I argued that this line of argument had been abandoned by appellant II during the opposition proceedings and that it was to be disregarded, similar to the findings in decision T 1067/08 (see e.g. catchword) as it had prevented the opposition division to decide on this aspect.

23. In the board's opinion, there is no indication on file that appellant II had withdrawn the objection of a lack of inventive step based on document D12 as the closest prior art during the opposition proceedings. Neither the minutes of the oral proceedings before the opposition division nor appellant II's written submissions during the first instance proceedings contain a statement to this effect. On the contrary, these arguments have been explicitly maintained by reference to earlier submissions (see letter dated 15 May 2012, point 3.1).

Furthermore, from the mere fact that the objection had not been presented at the oral proceedings it cannot be concluded that it was withdrawn for the following reasons. Firstly, it cannot be assumed that a party gives up any position without explicitly stating it and, secondly, the submission of an objection in writing but not at the oral proceedings might have various reasons, including for example, procedural efficiency.

24. The board further notes that inventive step was not addressed at the oral proceedings before the opposition division in relation to the main request, but only in the context of an auxiliary request and, therefore, a withdrawal of an objection on inventive step in relation to an auxiliary request would not have had, without further indications, any consequence in relation to the main request.

25. The board therefore decided that the line of argument based on document D12 as closest prior art is not excluded from the appeal proceedings in accordance with Article 12(4) RPBA.

Closest prior art

26. For assessing whether or not a claimed invention meets the requirements of Article 56 EPC, the boards of appeal normally apply the "problem and solution" approach. It requires as a first step the identification of the closest prior art. This is generally a prior art document disclosing subject-matter conceived for the same purpose or aiming at the same objective as the claimed invention and having the most technical features in common, *i.e.* requiring the

minimum of structural modifications (see CLBA, I.D.3.1).

27. Appellant I considered the disclosure of document D4, appellant II that of document D12 as the closest prior art for the subject-matter of claim 1.

28. Document D4 discloses a process for the separation of proteins produced by recombinant means from the host cell-derived contaminant endotoxin, which is preferentially bound to a hydrophobic solid material, while the protein of interest flows through (see abstract, page 2, lines 29 to 31 and page 9, lines 14 to 19). Reported hydrophobic adsorbents are either linked to aromatic groups, for example, phenyl, or are alkyl groups comprising 2 to 18 carbon atoms, with Phenyl-Sepharose™ being preferred (see page 4, lines 14 to 20). Branched hydrocarbons as functional groups are not disclosed.

29. Document D12 discloses a process for the separation of bio-molecules of interest, i.e. the target proteins, by their binding to a solid filter element from which they are subsequently eluded (see abstract, column 4, lines 36 to 54, column 9, lines 14 to 18). Example 4 in document D12 discloses the separation of bovine serum albumin (BSA) and cytochrome C (Cyt C) from a mixture in an aqueous solution relying on their different isoelectric points allowing BSA's selective binding to a "*Macro-Prep™ t-Butyl HIC™*" column contrary to Cyt C (see column 15, line 10 and line 63 to column 16, line 13 and 38 to 42, Table 1). In view of document D12's teaching that bio-molecules of interest bind to a solid stationary phase, BSA disclosed in example 4 can only be construed to represent the target protein, while Cyt

C represents the contaminant (see e.g. column 9, lines 14 to 18).

30. Thus, both documents D4 and D12 disclose processes for the separation of target proteins from contaminants in a mixture and thus share the same purpose with the claimed process.

31. As regards the technical features, the board notes that the process disclosed in document D4 differs from that according to claim 1 in that non-branched functional groups are linked to the hydrophobic adsorbent material, i.e. it differs by one feature. The process disclosed in document D12 differs from claim 1 in that the target protein binds to the hydrophobic adsorbent material, i.e. it is not separated from the contaminant in a flow-through process, and that the separation is carried out at a lower pH value, i.e. it differs by two features.

32. Appellant II argued that the process disclosed in document D12 differed in only one feature from the claimed process, namely the pH value.

33. The board, for the reasons set out in points and 29 and 31 above, is not convinced by this argument.

34. Therefore, the board, in line with the criteria set up by the case law (see point 29 above), concludes that the process disclosed in document D4 represents the closest prior art for the subject-matter of claim 1.

Admission of a line of argument based on document D4 as closest prior art in combination with document D12 (Article 13(1) RPBA)

35. Pursuant to Article 13(1) RPBA any amendment to a party's case after it has filed its grounds of appeal or reply may be admitted and considered at the board's discretion. Article 12(2) RPBA provides that the statement of grounds of appeal or the reply must contain a party's complete case. They shall set out clearly and concisely the reasons why it is requested that the decision under appeal is reversed, amended or upheld, and should specify expressly all the facts, arguments and evidence relied on. From the latter provision the board derives that a party's case comprises all its various lines of argument or objections which should be considered by the board in the appeal proceedings.

36. The line of argument based on document D4 as the closest prior art in combination with document D12 submitted by appellant II during the oral proceedings before the board had neither been raised in appellant II's statement of grounds of appeal nor in its reply to appellant I's appeal. Appellant II's submission that the subject-matter of claim 1 of the main request lacked novelty in the light of the disclosure of document D4 does not comprise the substantiation of a line of argument of a lack of inventive step based on document D4 combined with document D12.

37. Therefore, the board has a discretion to admit this line of argument into the appeal proceedings.

38. In the present case, appellant II had itself confined, as from the outset of the appeal proceedings, to argue

in relation to document D4 that its disclosure was detrimental to the novelty of the subject-matter of claim 1 of the main request. Objections of lack of inventive step were, in relation to the main request and the auxiliary requests, based on other documents as starting points. No fall-back position had been put forward by appellant II for the case that the disclosure of document D4 would neither be considered by the board as detrimental to the novelty nor that the documents relied on for an objection of a lack of inventive step with regard to the main request, in particular document D12, would not be accepted by the board as the closest prior art.

39. To present such fall-back positions or counter-arguments would, however, have been appropriate in the light of the view taken by the opposition division that had considered the disclosure of document D4, rather than any of the other documents cited by appellant II, as the closest prior art in relation to the subject-matter of claim 1 of the main request (see annex to the summons to oral proceedings, page 6, point 3.2) and the auxiliary request as considered allowable by it (see decision under appeal, page 11, third paragraph).

Moreover, the opposition division had acknowledged an inventive step having regard to the disclosure of document D4 combined with that of document D12 (see decision under appeal, page 12, sixth paragraph). In reply to appellant I's appeal in which appellant I affirmed that it shared the opposition division's approach (see appellant I's statement of grounds of appeal, page 6, point III.2), there was, from a procedural perspective, a further opportunity for appellant II to present appropriate counter-arguments. Instead, the line of argument that the subject-matter

of claim 1 of the main request lacked an inventive step having regard to the disclosure of document D4 combined with document D12 was raised for the first time at the oral proceedings before the board, and no justification was provided by appellant II for its late submission.

40. The board is not convinced by appellant II's argument that the objection of a lack of inventive step based on these two documents would not be a complex issue and should therefore be admitted, since documents D4 and D12 were known. Rather, the board considers, that the presentation of this line of argument would have led to a discussion involving issues that had not been addressed before. It therefore accepts appellant I's position that, in case of admission of this line of argument into the appeal proceedings, appellant I would have been faced with a new situation as it would hear for the first time arguments why its and the opposition division's view that the claimed subject-matter was inventive over the combined teachings of documents D4 and D12 was considered by appellant II as incorrect. Thus, the admission of this line of argument was regarded by the board as being in conflict with the principle of procedural fairness.

41. Consequently, the board did not admit this line of argument into the appeal proceedings in accordance with Article 13(1) RPBA.

Technical problem and solution

42. The patent in suit discloses in example 3 (see paragraphs [0070] and [0071]) a comparison between various hydrophobic adsorbent materials selected from "*(a) Macroprep t-butyl, (b) TosoHaas Butyl 650M, (c) Butyl Sepharose FF, (d) TosoHaas Phenyl 650M, and (e)*

TosoHaas Ether 650M" and their effects on the separation specificity of "*RANK:Fc*" (Peak B, see also column 19, line 10) from its misfolded form (Peak C, see also column 19, lines 13 and 14). The tested adsorbent materials essentially differ from each other in the structure of the functional group coupled to the adsorbent material, which can be classified as branched ("*t-butyl*": "*(a)*"), linear ("*Butyl*": "*(b)*", "*(c)*" and "*Ether*": "*(e)*"), and aromatic but not branched ("*Phenyl*": "*(d)*").

The "*Macroprep t-butyl*" adsorbent material having the branched hydrocarbon t-butyl as a functional group is reported to achieve the highest selectivity in separating the two forms of RANK:Fc from each other compared to the four other hydrophobic adsorbent materials tested (see page 12, column 19, line 53 to column 20, line 6). Furthermore, improved separation properties of the branched hydrocarbon functional group "*t-butyl*" *vis-à-vis* a linear butyl hydrocarbon having the same hydrophobicity is reported in example 4 in the patent in suit (see paragraphs [0075] to [0077]).

43. Therefore, the use of branched hydrocarbons as functional groups in the process according to claim 1 results in an improved separation of target proteins from their contaminants in a mixture.

44. Appellant II argued that the patent in suit exclusively relied on using branched t-butyl groups as functional groups in the hydrophobic absorbent material (see examples 1 to 4), while step (a) in claim 1 was directed to all types of branched hydrocarbon functional groups. Since phenyl groups were likewise comprised by the feature "*branched hydrocarbon*" referred to in step a) of claim 1 which failed to

achieve an improved separation compared to linear hydrocarbon groups as disclosed in example 3 in the patent in suit, the improved separation was not achieved by all functional groups falling within the ambit of claim 1.

45. The board is not convinced by this argument because a phenyl group, for the reasons set out above in points 13 and 14, is not to be considered as a "*branched hydrocarbon*" referred to in step a) in claim 1. Therefore, a failure of a hydrophobic material carrying phenyl groups in effectively separating a target protein from its contaminant as disclosed in example 3 of the patent in suit, cannot cast doubts on the issue that all functional groups falling within the ambit of claim 1 exhibit improved separation properties compared to non-branched hydrocarbon functional groups. Alternative arguments including experimental evidence that not all branched hydrocarbon functional groups falling within the ambit of step a) in claim 1 achieve an improved separation have not been submitted by appellant II.

46. Thus the board concludes that the technical problem to be solved by the subject-matter of claim 1 is the provision of an improved process for the separation of target proteins from contaminants in a mixture.

47. The board in view of the disclosure in examples 3 and 4 of the patent in suit is satisfied that the subject-matter of claim 1 solves this problem.

Obviousness

48. The board notes that the patent in suit in amended form on the basis of auxiliary request 4 was considered by

the opposition division to comply with the requirements of the EPC. Claim 1 of this request essentially differs from claim 1 of the present main request in that the target protein and the contaminants in the mixture are limited to recombinant proteins produced in CHO cells, while the proteins and contaminants in claim 1 of the main request can be derived from any biological source.

49. The opposition division had taken the view that the subject-matter of claim 1 of the main request lacked novelty (see section II above), a finding not shared by the board for the reasons set out in points 11 to 18 above. Furthermore, the board cannot derive any indications from the decision under appeal that the opposition division considered that claim 1 of the main request lacked an inventive step.

50. Therefore, the burden of arguing a lack of inventive step of the subject-matter of claim 1 of the main request has continued to rest with appellant II. Absent any further arguments to be considered in these appeal proceedings as to why the claimed subject-matter of the main request was obvious having regard to the disclosure of document D4 or why the opposition division's finding in the decision under appeal with regard to inventive step of auxiliary request 4 might be incorrect, the board sees no reason to deviate from the first instance position on inventive step.

51. The board therefore concludes that the ground for opposition of lack of inventive step pursuant to Articles 100(a) and 56 EPC does not prejudice the maintenance of the patent as granted.

Order

For these reasons it is decided that:

1. Appellant II's appeal is dismissed.

2. The decision under appeal is set aside.

3. The patent is maintained as granted.

The Registrar: The Chairwoman:

P. Cremona G. Alt

Decision electronically authenticated