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Datasheet for the decision of 11 October 2018

Case Number: T 2431/12 - 3.3.04

Application Number: 04715906.6

Publication Number: 1601697

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Language of the proceedings: EN

Title of invention:
Antibody purification by Protein A and ion exchange chromatography

Patent Proprietor:
Lonza Biologics plc.

Opponents:
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(02): Symphogen A/S / Biovitrum AB (opposition withdrawn)
(03): Laboratoire Français du Fractionnement et des Biotechnologies
(04): Wyeth LLC
(05): GE Healthcare Bio-Sciences AB
(06): Hexal Biotech GmbH
(07): Eli Lilly and Company

Headword:
Antibody purification/LONZA BIOLOGICALS

Relevant legal provisions:
EPC Art. 54, 56, 123(2), 123(3)
RPBA Art. 12(4)

Keyword:
Main request and auxiliary requests I to III, VII and IX - extended scope of protection (yes)
Auxiliary request IV - clarity (no)
Auxiliary request V - inventive step (no)
Auxiliary requests VI and X - unallowable amendment (yes)

Decisions cited:

Catchword:
-

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Case Number: T 2431/12 - 3.3.04

D E C I S I O N
of Technical Board of Appeal 3.3.04
of 11 October 2018

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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted on 24 September 2012 revoking European patent No. 1601697 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chair M. Blasi
Members: B. Claes
R. Morawetz

Summary of Facts and Submissions

I. The appeal was lodged by the patent proprietor (hereinafter "appellant") against the decision of the opposition division to revoke European patent No. 1 601 697 having the title "*Antibody purification by Protein A and ion exchange chromatography*", granted on European patent application 04715906.6, the latter having been filed as an international application under the PCT and published as WO 2004/076485.

II. The two independent claims 1 and 14 of the patent as granted read:

"1. Method of purifying an antibody comprising the steps of:

a. purifying an antibody by means of protein A affinity chromatography,

b. loading the purified antibody comprising a protein A-contaminant, wherein said protein A-contaminant is obtained upon eluting bound antibody from said protein A affinity chromatography column, on an anion exchange material under conditions which allow for binding of the protein A and which conditions result in retaining the antibody in the flow-through whilst the protein A-contaminant is bound and thus removed from the antibody, and

c. collecting at least 70% of the amount of antibody loaded onto the anion exchange material in the flow-through of the ion exchanger whilst the contaminant protein A is bound to the ion exchange material.

14. Method of purifying an antibody comprising the steps of:

a. Purifying an antibody by means of protein A affinity chromatography,

b. loading the purified antibody comprising a protein A-contaminant, wherein said protein A-contaminant is obtained upon eluting bound antibody from said protein A affinity chromatography column, on a first anion exchange material under conditions which allow for binding of the protein A and which conditions result in retaining the antibody in the flow-through whilst the protein A-contaminant is bound and thus removed from the antibody,

c. Collecting the antibody loaded onto the anion exchange material in the flow-through of the ion exchanger whilst the contaminant protein A is bound to the ion exchange material,

d. And further purifying the antibody by loading on, binding to and eluting it from a second ion exchanger."

Dependent claims 15 to 19 read:

"15. Method according to claim 14, characterized in that at least 70% of the amount of antibody loaded onto the first anion exchange material are recovered in the flow through.

16. Method according to one claim 15, characterized in that the antibody has a µI [*sic*] of at least 7.5 or above.

17. Method according to claim 15, characterized in that the second ion exchanger is a cation exchanger.

18. Method according to claims 14 or 17, characterized in that the purified antibody is monomeric antibody and that the second ion exchange step allows of removal of aggregated antibody.

19. Method according to one of the preceding claims, wherein the antibody is an IgG antibody."

III. Seven opponents opposed the patent on the grounds in Article 100(a) EPC, in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC), and in Article 100(b) and Article 100(c) EPC.

IV. In the decision under appeal, the opposition division held, *inter alia*, that claim 1 of the main request pending before it infringed the requirements of Article 123(2) and (3) EPC, claim 1 of auxiliary requests I to III did not comply with the requirements of Article 123(3) EPC, claim 1 of auxiliary request IV infringed the requirements of Article 123(2) EPC and claim 1 of auxiliary request V failed to meet the requirements of Article 56 EPC. The opposition division further decided not to admit auxiliary request VI into the proceedings.

Claim 1 of the **main request** read:

"1. Method of purifying an antibody comprising the steps of:

a) harvesting the antibody from a cell culture,

b) purifying the antibody harvested in step a) by means of a high affinity binding protein A affinity chromatography, wherein the protein A is a native protein A or a functional derivative thereof,

c) loading the purified antibody comprising a protein A-contaminant, wherein said protein A-contaminant is obtained upon eluting bound antibody from said protein A affinity chromatography column, on a first ion exchange material which is an anion exchanger resin under conditions which allow for binding of the protein A and which conditions result in retaining the antibody in the flow-through whilst the protein A-contaminant is bound and thus removed from the antibody,

d) collecting the antibody loaded onto the anion exchange material in the flow-through of the ion exchanger whilst the contaminant protein A is bound to the ion exchange material,

e) and further purifying the antibody by loading on, binding to and eluting it from a second ion exchanger, which is a cation exchanger."
(emphasis added by the board concerning certain amendments over claim 14 as granted)

As compared to claim 1 of the main request, in part b) of **auxiliary request I**, the wording "a high affinity binding" was deleted, and the wording "whilst the protein A-contaminant is bound and thus removed from the antibody" in part c) was amended to read "whilst the protein A-contaminant is bound to the anion exchanger resin and thus removed from the antibody" (emphasis added).

Claim 1 of **auxiliary request II** read:

"1. Method of purifying an antibody comprising the steps of:

a) harvesting the antibody from a cell culture,

b) purifying the antibody harvested in step a) by means of protein A affinity chromatography, wherein the protein A is a native protein A or a functional derivative thereof, wherein the functional derivative has a binding constant of at least $K=10^{-8}$ M for the Fc portion of mouse IgG2a or human IgG1,

c) loading the purified antibody comprising a protein A-contaminant, wherein said protein A-contaminant is a functional, IgG binding offspring of the protein A or the functional derivative thereof and is obtained upon eluting bound antibody from said protein A affinity chromatography column, on a first ion exchange material which is an anion exchanger resin under conditions which allow for binding of the protein A or its derivative and which conditions of pH and ionic strength for loading the anion exchanger resin result in retaining the antibody in the flow-through whilst the protein A-contaminant is bound to the anion exchanger resin and thus removed from the antibody.

d) collecting the antibody loaded onto the anion exchange material in the flow-through of the ion exchanger whilst the contaminant protein A is bound to the ion exchange material,

e) and further purifying the antibody by loading on, binding to and eluting it from a second ion exchanger, which is a cation exchanger." (emphasis added by the board)

As compared to claim 1 of auxiliary request II, part b) of claim 1 of **auxiliary request III** was identical but for the insertion of the wording "wherein the functional derivative comprises at least part of a functional IgG binding domain of wild-type protein A which domain is selected from the natural domains E,D,A,B,C or engineered muteins thereof which have retained IgG binding functionality" at the end.

Claim 1 of **auxiliary request IV** read:

"1. Method of purifying an antibody comprising the steps of:

a) harvesting the antibody from a cell culture,

b) purifying the antibody harvested in step a) by means of a protein A affinity chromatography,

c) loading the purified antibody comprising a protein A-contaminant, wherein said protein A contaminant is obtained upon eluting bound antibody from said protein A affinity chromatography column, on a first ion exchange material which is an anion exchanger resin under conditions which allow for binding of the protein A and which conditions of pH and ionic strength for loading the anion exchanger resin result in retaining the antibody in the flow-through whilst the protein A-contaminant is bound to the anion exchanger resin and thus removed from the antibody.

d) collecting the antibody loaded onto the anion exchange material in the flow-through of the ion exchanger whilst the contaminant protein A is bound to the ion exchange material,

e) and further purifying the antibody by loading on, binding to and eluting it from a second ion exchanger, which is a cation exchanger." (emphasis added by the board)

Claim 1 of **auxiliary request V** read:

"1. Method of purifying an antibody comprising the steps of:

a) purifying the antibody by means of a protein A affinity chromatography,

b) loading the purified antibody comprising a protein A-contaminant, wherein said protein A contaminant is obtained upon eluting bound antibody from said protein A affinity chromatography column, on a first ion exchange material which is an anion exchanger resin under conditions which allow for binding of the protein A and which conditions of pH and ionic strength for loading the anion exchanger resin result in retaining the antibody in the flow-through whilst the protein A-contaminant is bound to the anion exchanger resin and thus removed from the antibody, wherein at least 70% of the amount of antibody loaded onto the first anion exchange material are recovered in the flow-through.

c) collecting the antibody loaded onto the anion exchange material in the flow-through of the ion

exchanger whilst the contaminant protein A is bound to the ion exchange material,

d) and further purifying the antibody by loading on, binding to and eluting it from a second ion exchanger, which is a cation exchanger."

As compared to claim 1 of auxiliary request V, amended part a) of **auxiliary request VI** read "a) purifying the antibody by means of a protein A affinity chromatography, characterized in that the protein A is a recombinant protein A comprising a cysteine in the last 30 amino acids of the C-terminus of the amino acid sequence of said recombinant protein A and is coupled to the chromatographic support material through the sulphur atom of said cysteine residue via a thioether bond as the sole point of attachement [*sic*]," (emphasis added).

V. With the statement of grounds of appeal, the appellant re-submitted the main request and auxiliary requests I to VI considered by the opposition division in the decision under appeal (see section IV). The appellant submitted in addition auxiliary requests VII to X. Moreover, the appellant re-submitted a document (experimental data) already filed in the opposition proceedings that had not been considered by the opposition division.

As compared to claim 1 of auxiliary request V, amended part a) of **auxiliary request VII** read "a) purifying the antibody by means of a protein A affinity chromatography, wherein the protein A is a recombinant protein A that is engineered such as to allow of single-point attachment to a column material," (emphasis added by the board)

Claim 1 of **auxiliary request IX** read:

"1. Method of purifying an IgG antibody comprising the steps of:

a) purifying the IgG antibody by means of a protein A affinity chromatography, wherein the protein A is a recombinant protein A that is engineered such as to allow of single-point attachment to a column material,

b) loading the purified IgG antibody comprising a protein A-contaminant, wherein said protein A contaminant is obtained upon eluting bound antibody from said protein A affinity chromatography column, on a first ion exchange material which is an anion exchanger resin under conditions which allow for binding of the protein A and which conditions of pH and ionic strength for loading the anion exchanger resin result in retaining the antibody in the flow-through whilst the protein A contaminant is bound to the anion exchanger resin and thus removed from the antibody, wherein at least 70% of the amount of antibody loaded onto the first anion exchange material are recovered in the flow through and wherein the contaminant protein A is reduced to a concentration of less than 4 ng/mg antibody in the flow through of the first ion-exchanger,

c) collecting the IgG antibody loaded onto the anion exchange material in the flow-through of the ion exchanger whilst the contaminant protein A is bound to the ion exchange material,

d) and further purifying the IgG antibody by loading on, binding to and eluting it from a second ion exchanger, which is a cation exchanger."

As compared to claim 1 of auxiliary request IX, claim 1 of **auxiliary request X** specified in the preamble that it concerned a "Method of purifying an IgG antibody, wherein the IgG antibody has a pI of at least 7.5 or above" and had the wording "wherein the purified antibody is monomeric antibody and that the second ion exchange step allows of removal of aggregate antibody." inserted at the end.

VI. Joint opponents 02 withdrew their opposition with a letter dated 16 May 2013 and ceased to be a party to the appeal proceedings.

VII. Opponents 01, 04 and 06 (hereinafter for ease of reference referred to as "respondent I", "respondent IV" and "respondent VI", respectively) replied in writing to the appeal.

VIII. The board appointed oral proceedings as requested by the parties and issued a communication pursuant to Article 15(1) RPBA in annex to the summons to oral proceedings.

IX. At the oral proceedings the appellant and respondents I, IV and VI were represented. Respondents III, V and VII were not present nor represented as communicated to the board's registrar on 30 August 2018, 14 September 2018 and 18 September 2018, respectively. The appellant withdrew auxiliary request VIII filed with the statement of grounds of appeal. At the end of the oral proceedings the chair announced the board's decision.

X. The following documents are referred to in this decision:

D6: Racher *et al.* (1999), In "Manufacture of Therapeutic Antibodies", pages 246 to 274.

D8: Fahrner *et al.* (2001), Biotechnology and Genetic Engineering Reviews, Vol. 18, pages 301 to 327.

D41: WO 92/18629

D47: Iyer *et al.* (2002), BioPharm, Vol. 15, pages 14 to 20 and page 53.

XI. The appellant's arguments can be summarised as follows:

Main request and auxiliary requests I to III - claim 1 - protection conferred (Article 123(3) EPC)

Claims 1 and 14 as granted referred to "protein A chromatography". Since paragraph [0008] of the patent provided that "protein A" included both native protein A and functional derivatives thereof, the wording of claim 1 fulfilled the requirements of Article 123(3) EPC. Furthermore, paragraph [0013] provided that "a functional derivative of protein A" according to the invention was characterised by a particular binding constant for the Fc portion of mouse IgG2a or human IgG1.

A functional derivative of protein A would not have been understood as a non-protein A substance such as an anti-IgG antibody. Step c) referred to a "protein A-contaminant" and as such could only be present if

protein A was used in the chromatography and not a different substance which also could bind antibodies.

Auxiliary request IV - claim 1 - clarity (Article 84 EPC)

While the step "a) harvesting the antibody from a cell culture", which was inserted by amendment in the claimed method of purifying an antibody, could have been understood to encompass crude purification steps, the skilled person would not have understood the step to also encompass a step of chromatography. Whereas the claimed method was admittedly defined as "comprising" steps a) to d), the wording of step b) clearly excluded chromatography steps intermediate to steps a) and b) as step b) referred to "the antibody harvested in step a)".

Also, the disclosure in document D6 did not provide that the harvesting referred to in step a) possibly included chromatography.

Auxiliary request V - claim 1 - Inventive step (Article 56 EPC)

The process disclosed in example 4 of document D41 and the process disclosed in document D47 were designed for the purification of the particular and specific antibodies exemplified in these documents. There was no teaching in them that the methods would give a 70% recovery over the anion exchange step in the flow-through for the particular antibodies, and thus there was also no such teaching for different antibodies. The methods were not disclosed to be applicable to the purification of antibodies in general.

In the method disclosed in document D8, the anion exchange step followed a cation exchange step and Table 12.1 only referred to this kind of three-step antibody recovery process. The skilled person would have deduced from this table that a recovery rate of over 95% was obtained only when the anion exchange step was performed after the cation exchange step. The skilled person would further have concluded from this table that an anion step was not suitable to purify the antibody from a protein A contaminant, as the protein A concentration before and after the anion exchange step was in the same range, i.e. < 2 ng/mg antibody.

Accordingly, the claimed subject-matter would not have been obvious to the skilled person having regard to the state of the art.

Auxiliary requests VI, VII, IX and X
Admission into the appeal proceedings

The requests were filed with the statement of grounds of appeal and should not be disregarded by the board.

Auxiliary request VI - claim 1 - added subject-matter (Article 123(2) EPC)

Basis for the amendment in step a) was clearly given in the description as filed on page 4, last paragraph to page 5, first paragraph. The claim therefore did not relate to added subject-matter and complied with the requirements of Article 123(2) EPC.

Auxiliary request VII and IX - claim 1 - Article 123(3) EPC

The claims complied with the requirements of Article 123(3) EPC. No further arguments were submitted in this respect, and reference was made to earlier submitted arguments in relation to higher ranking requests.

Auxiliary request X - claim 1 - Article 123(2) EPC

This claim complied with the requirements of Article 123(2) EPC. No further arguments were submitted in this respect, and reference was made to earlier submitted arguments in relation to higher ranking requests.

XII. Respondents III, V and VII have not submitted any relevant substantive arguments. The arguments of respondents I, IV and VI can be summarised as follows:

Main request and auxiliary requests I to III - claim 1 - protection conferred (Article 123(3) EPC)

The term "protein A" would not have been understood by the skilled person to - by definition - include functional derivatives of protein A.

Paragraph [0008] of the patent did not provide an encompassing definition of the term "protein A".

Paragraph [0013] defined "functional derivatives of protein A" in accordance with a particular binding constant, and such a compound was stated to only *preferably* comprise a part of a functional part of a IgG binding domain *of wild-type protein A*. The term

"functional derivative" as defined in the patent was therefore not limited to any particular structure.

Auxiliary request IV - claim 1 - clarity (Article 84 EPC)

Although from page 7, lines 6 to 8, of the application as filed the skilled person could have understood that the harvesting step was supposed to take place prior to the purification step, the step was now part of the claimed "purification method", and this step was therefore not excluded to also encompass pre-purification steps, even chromatographic steps.

It was not clear whether the material to be loaded on the protein A column (step b) had to be taken *directly* from the newly introduced harvesting step or might also be taken directly from pre-purification materials other than ion-exchange materials and/or may be taken directly from any pre-purification materials (also including ion-exchange materials).

The disclosures on page 19 of the application as filed and on pages 262 and 263 of document D6 demonstrated that the term "harvesting the antibody from cell culture" in step a) encompassed more than just the removal of the cells by centrifugation, such as crude purification steps including chromatography.

Auxiliary request V - claim 1 - Inventive step (Article 56 EPC)

The closest prior art for the claimed invention was represented either by the disclosure in document D41, in particular example 4, or document D47.

Compared to the disclosure in example 4 of document D41, the claimed subject-matter differed solely by the explicit requirement that at least 70% of the amount of antibody loaded onto the first anion exchange material was recovered in the flow-through. Hence, starting from the disclosure in document D41, the technical problem to be solved was to ascertain that a 70% recovery was obtained.

It was reasonable to assume that - under the conditions mentioned in document D41 - a recovery rate of more than 70% of the antibody loaded onto the first anion exchange material would be obtained in the flow-through. Such rates were entirely conventional for anion exchange columns operating in flow-through mode.

If a 70% recovery rate was not achieved, it would have been within the skilled person's common general knowledge to assure a 70% recovery by modifying the purification conditions, for example by adapting the pH of the solution commensurate with the pI of the antibody to be purified ensuring that the antibody was sufficiently positively charged and not binding to the anion exchanger resin. Examples of such conditions ensuring more than 90% recovery could be found in the legend of Figure 12.3 in document D8, i.e. a pH of 8 leading to a >95% yield in the anion step (see page 308, lines 11 to 12).

The claim was not restricted to a method of purification applicable to a particular antibody. The methods of purification of the particular antibodies as disclosed in documents D41 and D47 were thus appropriate starting points for the assessment of inventive step and would, hence, not have been dismissed by the skilled person as such.

Auxiliary requests VI, VII, IX and X
Admission into the appeal proceedings

Auxiliary request VI had rightly not been admitted into the proceedings by the opposition division and should not be allowed to be reintroduced at the appeal stage. The remaining auxiliary requests should have been filed during first-instance proceedings.

Auxiliary request VI - claim 1 - added subject-matter (Article 123(2) EPC)

None of the paragraphs and claims in the application as filed which the appellant had referred to as allegedly providing a basis for the amendment to the claim, actually provided a basis. In particular the feature that the coupling as the sole point of attachment of the recombinant protein A was at least 50% through the sulphur atom of said cysteine residue was missing. The amendment therefore constituted an incomplete reflection of the disclosure of the application as filed and accordingly failed to meet the requirements of Article 123(2) EPC.

Auxiliary requests VII and IX - claim 1 -
Article 123(3) EPC

The protein A molecule as defined in the claim was a functional derivative of protein A which differed from protein A as referred to in claim 1 as granted. The arguments under Article 123(3) EPC, relevant to claim 1 of the main request and auxiliary requests I to III, thus also applied to this claim.

Auxiliary request X - claim 1 - Article 123(2) EPC

In this claim, the antibody to be purified was further characterised in that it was "an IgG antibody, wherein the IgG antibody has a pI of at least 7,5 or above". However, the application as filed failed to disclose the claimed method for an IgG antibody having the indicated pI which is not a monoclonal antibody.

Neither the description as filed, nor any of the possible combinations of the claims as filed, in particular a combination of claims 9, 10, 11 and 14, discloses the feature in absence of the monoclonal qualification.

The claim was therefore directed to added subject-matter and infringed Article 123(2) EPC.

XIII. Respondents III, V and VII have not submitted any relevant requests. The other parties' final requests were as follows.

The appellant requested that the decision under appeal be set aside and that the patent be maintained on the basis of the claims of the main request or, alternatively, on the basis of one of the sets of claims of auxiliary requests I to VI, all re-submitted with the statement of grounds of appeal or, further auxiliary, that the patent be maintained on the basis of the claims of auxiliary requests VII, IX or X filed with the statement of grounds of appeal.

Respondents I, IV and VI requested that the appeal be dismissed.

Reasons for the Decision

1. The appeal is admissible.

2. At the oral proceedings before the board respondents III, V and VII, although duly summoned, were not present or represented. In accordance with Rule 115(2) EPC and Article 15(3) RPBA, the board decided to continue the proceedings and to hold oral proceedings in their absence.

Main request and auxiliary requests I to III
Claim 1 - protection conferred (Article 123(3) EPC)

3. Whereas claims 1 and 14 as granted (see section I) and claim 1 of each of these requests is for a method of purifying an antibody by means of protein A affinity chromatography, the latter were amended to stipulate that "the protein A is a native protein A or a functional derivative thereof" (emphasis added by the board, see section IV).

4. Paragraph [0008] of the patent reads: "*According to the present invention, a method of purifying an antibody is devised which method comprises the steps of: First, purifying an antibody by means of protein A affinity chromatography wherein the protein A is a native protein A or a functional derivative thereof*" (emphasis added by the board). Paragraph [0013] of the patent reads further: "*A functional derivative of protein A is characterized by a binding constant of at least $K=10^{-8}$ M, preferably $K=10^{-9}$ M for the Fc portion of mouse IgG2a or human IgG1. An interaction compliant with such value for the binding constant is termed 'high affinity binding' in the present context.*

Preferably, such functional derivative of protein A comprises at least part of a functional IgG binding domain of wild-type protein A which domain is selected from the natural domains E,D,A,B, C or engineered muteins thereof which have retained IgG binding functionality. An example of such is (...)" (emphasis added by the board).

5. The patent thus provides that a "functional derivative of protein A" is characterised by a particular binding constant for the Fc portion of mouse IgG2a or human IgG1. However, at the same time it provides that such a "functional derivative of protein A" not necessarily (but only preferably) comprises at least part of a functional IgG binding domain of wild-type protein A which has retained IgG binding functionality.

6. The skilled person would not have considered the term "protein A" in the wording "by means of protein A affinity chromatography" of the purification step a. of claims 1 and 14 as granted, to also refer to compounds which lack any structural part of a functional IgG binding domain of wild-type protein A. However, as the patent in paragraph [0013] provides that the wording "functional derivatives of protein A" also encompasses such compounds (see point 5), the amendments to the claim by introducing this wording, and thus the reference to such compounds, extend the protection conferred by the patent.

7. This reasoning cannot be challenged by the appellant's argument that a skilled person would not have understood a "functional derivative of protein A" to be a non-protein A substance such as an anti-IgG antibody given that step c) referred to a "protein A-contaminant" and as such could only be present if

protein A was used in the chromatography and not a different substance which could also bind antibodies. The patent itself, as noted in points 4 and 5, above, instructs the skilled person that the term "functional derivative", for the purpose of determining the claimed subject-matter, is to be understood to include a different substance which also could bind antibodies.

8. Therefore, the amended claims do not comply with the requirements of Article 123(3) EPC.

Auxiliary request IV - claim 1 - clarity (Article 84 EPC)

9. Compared to the method of purifying an antibody of claim 1 of the patent as granted, the method of purifying an antibody of this claim comprises the additional step of "a) harvesting the antibody from a cell culture" and the provision in the following step b) of "purifying the antibody harvested in step a) by means of a protein A affinity chromatography (emphasis added by the board).

10. The appellant and respondents were in dispute about whether the step "a) harvesting the antibody from a cell culture" preceding step b) could be understood to encompass crude or pre-purification steps and if so whether such steps could also encompass a step of, for example, chromatography.

11. From page 7, lines 6 to 8, of the application as filed, which states that "*Preferably, according to the present invention the antibody sought to be purified is harvested from a cell culture prior to purifying the antibody be* [sic] *means of protein A affinity chromatography*" the skilled person could have inferred that in this context the harvesting step was referred

to as to take place prior to the purification step. However, step a) in the claim is part of a (claimed) "Method of purifying an antibody" and thus figures in the claim in a different context as in the passage referred to in the description. Therefore, the skilled person may, in the context of the claim, have inferred in the context of the claimed method a different technical meaning of the harvesting step, i.e. a broader meaning.

12. The latter appears to find support in the disclosure on page 19, lines 6 to 7, of the application as filed where it is stated that: "*Cell culture supernatant from a NSO myeloma cell culture was crudely purifed* [sic] *by centrifugation and in depth filtration and concentrated by ultrafiltration",* thus also implying, besides centrifugation, filtration steps, for instance, prior to the protein A affinity chromatography purification step.

13. The parties agreed that document D6, a textbook publication in the field of antibody production and purification, represented the common general knowledge of the skilled person in this technical field. Also from this publication no precise definition of the harvesting step can be taken. Document D6 discloses in the paragraph bridging pages 262 and 263 that: "*Primary recovery involves the harvesting of the cell containing media for use in the purification process. It can be considered as part of downstream processing or as part of the harvest procedure but conventionally it is considered as one or more unit operations that need to integrate well with both the preceding and subsequent steps in the manufacturing process. The main activity of primary recovery is usually to remove cells (if present) and debris from the harvest stream. The*

harvest will often require some form of polishing or preparation before the first purification step. This may include concentration of the bulk product to reduce the volumes to be handled and filtration to remove particulates. Filtration can also be used to produce a low bio-burden intermediate that may be stored within a defined expiry date to enable more convenient scheduling of purification activities". On page 263, left-hand column, lines 31 to 43, it is further stated that: "*An alternative method of product recovery has recently been introduced that promises a much simpler overall process (THOMMES et al., 1995). Expanded bed chromatography allows crude feedstocks which may contain cells and cell debris, to be applied to a chromatography column without pretreatment. Filtration or centrifugation are therefore not required, and if used in combination with an appropriate affinity ligand, expanded bed chromatography allows for a very simple and elegant combined recovery and purification process*."

14. The above-mentioned passages suggest that for a skilled person the wording "harvesting the antibody from cell culture" would have had no unambiguous conventional technical meaning which *a priori* would exclude step a) of the claim from encompassing more than just the removal of the cells by centrifugation, such as crude purification steps, including chromatography as submitted by the appellants.

15. Therefore the amendment renders the claim unclear, and the requirements of Article 84 EPC are not met.

Auxiliary request V - claim 1 - Inventive step (Article 56 EPC)
Closest prior art

16. It was not contested by the appellant that the disclosure in example 4 of document D41 could be considered to represent the closest prior art for the assessment of inventive step of the claimed subject-matter.

17. In example 4 (pages 21 to 22) document D41 discloses the 3-step antibody purification method as claimed (i.e. protein A, anion exchange chromatography, cation exchange chromatography) exemplified on the basis of recombinant murine 3F8-Type antibodies. The method is specified to comprise, like the claimed method, Sepharose-Protein A affinity chromatography, followed by Q-Sepharose chromatography operated in flow-through mode and a subsequent purification step with S-Sepharose.

The objective technical problem

18. The distinguishing feature of the claimed subject-matter over the disclosure in the closest prior art is that at least a 70% antibody recovery rate is obtained in the anion exchange step for the antibody to be purified.

19. Accordingly, the technical problem to be solved is to provide particular conditions for the anion exchange step to ascertain that 70% recovery of the antibody to be purified would be obtained.

20. The respondents have submitted that such rates are entirely conventional for anion exchange columns operated in flow-through mode to purify antibodies. In

the experiment disclosed on page 308, document D8 confirms that the conditions applied ensure more than 95% of recovery of the antibody in the anion step (see page 308, line 11 to 12).

21. The appellant has argued that it was not derivable from the prior art that a >70% antibody recovery rate could be achieved for *each and every* antibody to be purified by the disclosed purification method.

22. However, for the assessment of inventive step the whole area claimed, and thus every embodiment falling within the ambit of a claim, needs to be inventive for a claim to meet the requirements of Article 56 EPC. If an embodiment falling within the scope of a claim is obvious, the whole claim lacks an inventive step. Whether the claim also covers non-obvious embodiments, as was argued by the appellant, is not relevant.

23. The appellant has not argued that a >70% antibody recovery rate can never be achieved and, accordingly, in view of the above considerations, the board decided that the subject-matter of claim 1 fails to meet the requirements of Article 56 EPC.

Auxiliary requests VI, VII, IX and X
Admission into the appeal proceedings

24. The board decided to admit these claim requests which had been filed by the appellant with the statement of grounds of appeal into the appeal proceedings. However, since they are not allowable in substance as set out in the following, the board does not provide a reasoning in writing for its decision to admit these claim requests.

Auxiliary request VI - claim 1 - added subject-matter (Article 123(2) EPC)

25. The appellant has referred, *inter alia*, to the paragraph bridging pages 4 and 5 of the application as filed as providing support for the amendment in step a) of the claimed method.

26. The application discloses on page 4, line 23 to page 5, line 7: "*Alone or in combination with a protein A or a functional protein A-fragment or derivative as defined in the preceding sections, further preferred are protein A fragments that are engineered to allow of single-point attachement* [sic]*. (...). More preferably, such recombinant protein A or functional fragment thereof comprises a cysteine in its amino acid sequence. Most preferably, the cysteine is comprised in a segment that consists of the last 30 amino acids of the C-terminus of the amino acid sequence of the recombinant protein A or functional fragment thereof. In a further preferred embodiment of such type, the recombinant protein A or functional fragment thereof is attached by at least 50% via a thioether sulphur bond to the chromatographic support or matrix material of the protein A-affinity chromatography medium. An example of such an embodiment is described e.g. in US 6399750 from Pharmacia and is commercially available under the brandnames of Streamline™ or MabSelect™ from Amersham-Biosciences, depending on the nature of the support matrix used. (...).*" The board notes further, for the sake of completeness, that the application as filed specifies, in addition, on page 5, lines 21 to 25, that: "*In a particularly preferred embodiment, the protein A or functional protein A derivative according to the present invention is the recombinant protein A disclosed in US 6399750 which comprises a*

juxtaterminal, engineered cysteine residue and is, preferably by at least 50%, more preferably by at least 70%, coupled to the chromatographic support material through the sulphur atom of said cysteine residue as the sole point of attachment." (all above emphases added by the board)

27. The board agrees with the respondents that the amendment introduced in the claim is an incomplete reflection of the disclosure in the passages in the description referred to above. In both cited passages the feature of the attachment or coupling as the sole point of attachment is qualified to be at least 50% via a thioether sulphur bond or through the sulphur atom of said cysteine residue, respectively. However, the latter features are absent from the wording of the claim.

28. The appellant has further referred to claims 2 to 5 of the application as filed, of which the most relevant claim 5 read: "Method according to claim 3, characterized in that **the recombinant protein A** is attached by at least 50% via a thioether sulphur bond to the chromatographic support material of the protein A affinity chromatography." (emphasis added by the board)

29. This wording is not of a more general nature than the wording in the paragraph bridging pages 4 and 5 of the application as filed.

30. Accordingly, the amendments to the claim relate to added subject-matter and contravene Article 123(2) EPC as they constitute undisclosed generalisations of disclosed subject-matter.

Auxiliary request VII and IX - claim 1 - Article 123(3) EPC

31. Part a) of these claims stipulates that the protein A affinity chromatography step in the method is performed with "a recombinant protein A that is engineered such as to allow of single-point attachment to a column material" (see section V).

32. The board refers to points 3 to 6 above, which are in the context of claim 1 of the main request and auxiliary requests I to III. The present claim, by referring to a recombinant engineered protein A, also relates to a "functional derivatives of protein A" which were held by the board to not be part of the extent of protection provided by granted claims 1 and 14 given that paragraph [0014] of the patent refers to such engineered forms of protein A in their context.

33. For similar reasons therefore as claim 1 of the main request and auxiliary requests I to III, this claim infringes the requirements of Article 123(3) EPC.

Auxiliary request X - claim 1 - Article 123(2) EPC

34. The claim specifies, *inter alia*, in the preamble that it concerns a "Method of purifying an IgG antibody, wherein the IgG antibody has a pI of at least 7.5 or above".

35. The sole part in the application as filed which discloses an IgG antibody having a pI of at least 7.5 or above are the claims as filed, in particular claims 9 to 11 and 14. These claims read:

"9. Method of purifying an antibody comprising the steps of:

• Purifying an antibody by means of protein A affinity chromatography wherein the protein A is a native protein A or a functional derivative thereof,

• Loading the purified antibody on a first ion exchange material under conditions which allow for binding of the protein A or its derivative,

• Collecting the antibody, preferably collecting at least 70%, more preferably collecting at least 80%, most preferably collecting at least 90% of the amount of antibody loaded onto the ion exchange material in the flow-through of the ion exchanger whilst a contaminant protein A is bound to the ion exchange material,

• And further purifying the antibody by loading on, binding to and eluting it from a second ion exchanger.

10. Method according to claim 9, characterized in that the first ion exchanger is an anion exchanger.

11. Method according to one [sic] claim 10, characterized in that the antibody has a pI of at least 7.5 or above."

"14. Method according to one of the preceding claims, characterized in that the antibody is a **monoclonal antibody**, preferably an IgG antibody wherein the IgG antibody may be chimeric or CDR-grafted IgG antibody." (emphasis added by the board)

36. A combination of the subject-matter of claims 9, 10, 11 and 14 as filed entails a method for the purification of IgG antibody having a pI of at least 7.5, but this

combination stipulates however the IgG antibody is a monoclonal antibody.

37. However, the claim under consideration lacks the requirement that the antibody to be purified is a monoclonal antibody. The claimed subject-matter is therefore not disclosed in the application as filed.

38. Accordingly, the claim is directed to subject-matter which extends beyond the content of the application as filed and thus fails to comply with the requirements of Article 123(2) EPC.

Conclusion

39. As none of the claim requests submitted by the appellant meet the requirements of the EPC, the appeal must be dismissed.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

S. Lichtenvort M. Blasi

Decision electronically authenticated