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# Datasheet for the decision of 2 October 2023

**Case Number:** T 0782/21 - 3.3.04

**Application Number:** 11798094.6

**Publication Number:** 2583973

**IPC:** C07K1/16, C07K1/18, C07K1/20, C07K1/22, C07K16/00

**Language of the proceedings:** EN

**Title of invention:**
Method for purifying protein using amino acid

**Patent Proprietor:**
Kyowa Kirin Co., Ltd.

**Opponent:**
Michalski Hüttermann & Partner
Patentanwälte mbB

**Headword:**
Chromatography/KYOWA

**Relevant legal provisions:**
EPC Art. 123(2)

**Keyword:**
Amendments - added subject-matter (yes)

**Decisions cited:**
G 0002/10

Europäisches Patentamt
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**Case Number: T 0782/21 - 3.3.04**

# D E C I S I O N
# of Technical Board of Appeal 3.3.04
# of 2 October 2023

| | |
|---|---|
| **Appellant I:**<br>(Patent Proprietor) | Kyowa Kirin Co., Ltd.<br>1-9-2, Otemachi,<br>Chiyoda-ku,<br>Tokyo (JP) |
| **Representative:** | Hoffmann Eitle<br>Patent- und Rechtsanwälte PartmbB<br>Arabellastraße 30<br>81925 München (DE) |
| **Appellant II:**<br>(Opponent) | Michalski Hüttermann & Partner<br>Patentanwälte mbB<br>Speditionstraße 21<br>40221 Düsseldorf (DE) |
| **Representative:** | Michalski Hüttermann & Partner<br>Patentanwälte mbB<br>Kaistraße 16A<br>40221 Düsseldorf (DE) |
| **Decision under appeal:** | **Interlocutory decision of the Opposition Division of the European Patent Office posted on 6 April 2021 concerning maintenance of the European Patent No. 2583973 in amended form.** |

**Composition of the Board:**

| | |
|---|---|
| **Chairwoman** | M. Pregetter |
| **Members:** | D. Luis Alves |
| | R. Romandini |

## Summary of Facts and Submissions

I. European patent No. 2 583 973, entitled "*Method for purifying protein using amino acid*", was granted on European patent application No. 11 798 094.6, filed as an international application published in Japanese as WO 2011/162210. In this decision, reference to the "application as filed" means the A1 publication of the European patent application.

II. The patent was opposed on the grounds of lack of novelty (Article 54 EPC) and lack of inventive step (Article 56 EPC), under Article 100(a) EPC, and on the grounds under Article 100(b) and (c) EPC.

III. The opposition division decided that, account being taken of the amendments in the form of auxiliary request 5, the patent and the invention to which it related met the requirements of the EPC.

With respect to the main request and auxiliary requests 1 to 4, the opposition division held that claim 1 related to subject-matter which extended beyond the content of the application as filed (Article 123(2) EPC).

IV. Both the patent proprietor (appellant I) and the opponent (appellant II) filed appeals against this decision.

V. With the statement setting out the grounds of appeal, appellant I filed sets of claims of a main request, auxiliary request MRb and auxiliary requests 1 to 55. The main request is identical to the main request

considered by the opposition division, and auxiliary request 15 is identical to the request held allowable by the opposition division. They submitted arguments to the effect that, *inter alia,* claim 1 of each request met the requirements of Article 123(2) EPC.

VI. With the statement setting out the grounds of appeal, appellant II submitted arguments addressing, *inter alia,* the requirements of Article 123(2) EPC.

VII. Appellant I and appellant II filed replies to the statements of grounds of appeal.

VIII. The board appointed oral proceedings and in a communication pursuant to Article 15(1) RPBA informed the parties of its preliminary opinion that, *inter alia*, all requests related to subject-matter extending beyond the content of the application as filed.

IX. Appellant I subsequently submitted claim sets of auxiliary requests 15a, 51a, 52a and 55a.

X. The oral proceedings took place as scheduled. At the end of the proceedings, the Chair announced the board's decision.

XI. Claim 1 of the **main request** reads:

"1. A method for purifying an antibody to reduce the amount of polymers and host cell proteins, comprising one or more chromatographic processes, wherein the one or more chromatographic processes comprises a Protein A affinity chromatographic process and an anion exchange chromatographic process, and wherein glycine is included as the ingredient itself of the elution buffer used in the Protein A affinity chromatographic process,

the content of the glycine in the elution buffer being 100 mM, and the pH of the elution buffer being from 3.2-3.4, and wherein
(i) the ratio of the polymer in the purification product purified by the purification method is less than 4%, and/or
(ii) the content of the host cell-derived protein in the purification product purified by the purification method of the present invention is less than 100 ng/mg."

Claim 1 of **auxiliary request 15**, identical to auxiliary request 5 held allowable in the decision under appeal, reads (differences to claim 1 of the main request are highlighted by the board):

"1. A method for purifying a monoclonal antibody to reduce the amount of polymers and host cell proteins, comprising one or more chromatographic processes, wherein the one or more chromatographic processes comprises a Protein A affinity chromatographic process, ~~and~~ an anion exchange chromatographic process, and a cation exchange chromatographic process, and wherein glycine is included as the ingredient itself of the elution buffer used in the Protein A affinity chromatographic process, the content of the glycine in the elution buffer being 100 mM, and the pH of the elution buffer being from 3.2-3.4, wherein
(a) the protein A chromatographic process is carried out, followed by carrying out the cation exchange chromatographic process, preferably, further followed by carrying out the anion exchange chromatographic process, or
(b) the protein A chromatographic process and the anion exchange chromatographic process are carried out,

followed by carrying out the cation exchange chromatographic process,
and wherein
(i) the ratio of the polymer in the purification product purified by the purification method is less than 1%, and/or
(ii) the content of the host cell-derived protein in the purification product purified by the purification method of the present invention is less than 10 ng/mg."

Claim 1 of **auxiliary request 15a** reads (differences to claim 1 of the main request are highlighted by the board):

"1. A method for purifying a monoclonal antibody to reduce the amount of polymers and host cell proteins, comprising one or more chromatographic processes, wherein the one or more chromatographic processes comprises a Protein A affinity chromatographic process, ~~and~~ an anion exchange chromatographic process, and a cation exchange chromatographic process, and wherein glycine is included as the ingredient itself of the elution buffer used in the Protein A affinity chromatographic process, the content of the glycine in the elution buffer being 100 mM, and the pH of the elution buffer being from 3.2-3.4, wherein
the protein A chromatographic process and the anion exchange chromatographic process are carried out,
followed by carrying out the cation exchange chromatographic process,
and wherein
(i) the ratio of the polymer in the purification product purified by the purification method is less than 1%, and/or

(ii) the content of the host cell-derived protein in the purification product purified by the purification method of the present invention is less than 10 ng/mg."

For the text of the claims of the other auxiliary requests, the patent registry may be consulted. In general, claim 1 of those requests compares to claim 1 of the main request or auxiliary requests 15, as applicable, as follows. Claim 1 of auxiliary request MRb reads as claim 1 of auxiliary request 15 up to the pH range, i.e. it contains neither the order of steps defined in features (a) and (b) nor the purity requirements defined in features (i) and (ii). In auxiliary requests 1 to 4, claim 1 additionally includes one or more purity requirements (i) and (ii). In auxiliary requests 5 to 8, claim 1 further includes, in addition to the purity requirements (i) and (ii), a definition of the order of the chromatographic processes, defined in features (a) and (b). Auxiliary requests 9 to 17, 18 to 26, 27 to 35, 36 to 44 and 45 to 53 are based on the main request and auxiliary requests 1 to 8, respectively. Auxiliary requests 54 and 55 are based on the main request and auxiliary request 15, respectively, defining only one of the two purity requirements. Auxiliary requests 51a, 52a and 55a are based on auxiliary requests 51, 52 and 55, respectively, defining only one of the two features (a) and (b).

In addition to the amendments just described, in some auxiliary requests, claim 1 further differs from claim 1 of the main request by referring to "monoclonal antibody", by the level of purity required in features (i) and (ii), by allowing for one of those purity requirements to be optional, by defining the pH of the buffers used in the anion and cation chromatographic

processes, and/or by the deletion of "one or more" in the expression "one or more chromatographic steps". However, these amendments are immaterial to the issues decided in this appeal. For this reason, the full text of claim 1 of each request need not be reproduced here.

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

D7: T. Arakawa *et al.*, Protein Expression and Purification 36, 2004, pp. 244-8

D24: S. Ghose *et al.*, Biotechnology and Bioengineering 92(6), 2005, pp. 665-73

D31: A. Shukla *et al.*, Journal of Chromatography B 848, 2007, pp. 28-39

XIII. Appellant I's arguments relevant to this decision may be summarised as follows.

*Amendments - extension beyond the content of the application as filed (Article 123(2) EPC)*

*Main request*

The subject-matter of claim 1 was disclosed in the application as filed.

The feature "the pH of the elution buffer being from 3.2-3.4" was disclosed in the application as filed. Paragraph [0049] disclosed a pH range 2.5 to 4.5 for the elution buffer used in the Protein A affinity chromatography (PAC) process. The narrower range 3.2 to

3.4 was derivable from the examples (see pH 3.2 in Table 1 and pH 3.4 in Table 2).

Under established case law, a value of a parameter from an example could be used in a claim provided that this parameter was not inextricably linked to other parameters in the example. Therefore, the amendment did not contravene the requirements of Article 123(2) EPC merely because the parameter had an impact, even if a considerable one, on other parameters of the example (see decisions T 201/83, Reasons 6; T 876/06, Reasons 3.5 and 3.6.2; and T 500/11, Reasons 3.3).

In the case at hand, the pH value could be varied independently of the other parameters in the examples, such as the buffer and the chromatographic material. In general, experimental details were not necessarily inextricably linked merely because they were mentioned in the examples.

As regards the material of the chromatographic column, the material used in the examples was not inextricably linked to the pH of the elution buffer for a number of reasons. First, the application as filed listed several materials as suitable for PAC (see paragraph [0043] of the application as filed). Second, all Protein A columns achieved separation according to the same principle, namely the binding of the Fc portion of the antibody. Third, the examples showed that the same pH value was suitable for separation with the two materials used (see Example 1, using pH 3.2 with MabSelect and Example 2, using pH 3.2 as well, with MabSelect SuRe). As regards the disclosure in document D31, Figure 5 did not relate to experiments with a glycine buffer. As regards the disclosure in document D24, it was contested that it represented

common general knowledge. This notwithstanding, this document disclosed different elution pH values for different column materials, but it did not relate to experiments with glycine buffer, and the experiments used a pH gradient. In contrast, claim 1 was restricted to a very specific buffer: 100 mM glycine. Therefore, document D24 could not call into question the examples in the patent, which showed elution at pH 3.2 for two different column materials: MabSelect and MabSelect SuRe.

As regards the characteristics of the elution buffer, all details provided in the examples were included in the claim, namely the pH value and the glycine concentration of 100 mM.

The nature of the antibody was not relevant because it was the Fc region of the antibody that interacted with the Protein A column.

The pH of the elution buffer in the PAC step was not inextricably linked to the order of the chromatographic steps as carried out in the examples either. The pH of the PAC step had no impact on how the subsequent steps were carried out. Moreover, it was implicit for the skilled person that PAC was the first step in the claimed method.

*Auxiliary requests*

No arguments were put forward specific to the feature in question for claim 1 of any auxiliary request.

XIV. Appellant II's arguments relevant to this decision may be summarised as follows.

*Amendments - extension beyond the content of the application as filed (Article 123(2) EPC)*

*Main request*

The standard of proof to be applied to the basis for amendments was "beyond reasonable doubt". Furthermore, an amendment was only allowable if the subject-matter of the claim was directly and unambiguously derivable from the application as filed. The cases underlying the decisions cited by appellant I differed from the case at hand.

The examples in the application did not provide the basis for the pH range 3.2 to 3.4 for the following reasons.

The examples did not provide much information on the impact of the different parameters. On the other hand, it could be taken from any of documents D7, D24 and D31 that the pH was a critical parameter because a low pH caused product aggregation.

The review document D31 showed that it belonged to the common general knowledge at the relevant date that both the specific antibody to be purified and the column material used for PAC had a significant impact on the pH used in the elution. Figure 5 showed that for a given column material, the elution pH differed with the antibody. Furthermore, Figure 6 showed (e.g. in the MabSelect column) that the elution pH ranged from pH 4.0 to 3.0, depending on the antibody. Figure 6 also showed that the pH of the elution buffer differed with the column material. With the MabSelect column, the pH ranged from 3.0 to 4.0, depending on the antibody to be

purified. However, for the MabSelect SuRe, it ranged from 3.5 to 4.0. Thus, the antibody, column material and pH were interlinked. In fact, factorial experimental design of the chromatographic step was necessary (see page 37 and Figure 10 of document D31). In conclusion, the pH was taken out of context when it was added to claim 1.

The subject-matter of claim 1 contravened the requirements of Article 123(2) EPC.

*Auxiliary requests*

The objection raised for the main request applied to the auxiliary requests.

XV. Appellant I requested that the decision under appeal be set aside and that the patent be maintained in amended form on the basis of the set of claims according to the main request or, alternatively, on the basis of one of the sets of claims according to auxiliary request MRb and auxiliary requests 1 to 55, all filed with the statement setting out the grounds of appeal, and auxiliary requests 15a, 51a, 52a and 55a, all filed with the reply to the board's communication pursuant to Article 15(1) RPBA; that auxiliary requests 15a, 51a, 52a and 55a be admitted into the appeal proceedings; and that the case be remitted to the opposition division for consideration of the remaining grounds for opposition should the board reverse the opposition division's decision on Article 123(2) EPC on any of the claim requests held by the opposition division to contravene the requirements of Article 123(2) EPC.

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

## Reasons for the Decision

*Main request*

*Amendments - extension beyond the content of the application as filed (Article 123(2) EPC)*

1. Claim 1 is directed to a method of purifying an antibody involving Protein A affinity chromatography (PAC) and anion exchange chromatography (AEX). The pH and glycine concentration of the elution buffer used in the PAC step are defined in the claim. The objections raised by appellant II under Article 123(2) EPC were against, *inter alia,* the feature "*the pH of the elution buffer being from 3.2-3.4*".

2. As a basis for this feature, appellant I indicated paragraph [0049] and Examples 1 to 3 of the application as filed.

3. It is undisputed that the application as filed does not explicitly disclose a pH range 3.2 to 3.4. Instead, it discloses the broader pH range 2.5 to 4.5 (paragraph [0049]). Appellant I's argument is that the pH range 3.2 to 3.4 can be taken from Examples 1 to 3, which describe PAC carried out at the end points of this range: elution buffer pH 3.2, in Examples 1 and 2, and elution buffer pH 3.4, in Example 3.

4. The standard applied by the boards for assessing compliance with the requirements of Article 123(2) EPC is to determine whether an amendment was made within the limits of what a skilled person would derive directly and unambiguously, using common general knowledge, and seen objectively and relative to the date of filing, from the whole of the description, claims and drawings as filed (see G 2/10, Reasons 4.3).

5. In the board's view the skilled person would have read the pH value in the examples as linked to the specific experimental conditions. As argued by appellant II, it was common general knowledge at the relevant date that the pH of the elution buffer depended on the column material as well as on the antibody to be purified. This is evidenced by document D31, a review publication entitled "Downstream processing of monoclonal antibodies - Application of platform approaches". In the chapter on PAC, the elution pH for 14 antibodies is shown as a function of the antibody (see Figure 5). Figure 6 shows that the elution pH is a function of both the antibody and the chromatographic material. For the MabSelect column, the elution is carried out with a buffer pH ranging from approximately 3.0 to 4.0, depending on the antibody. For the MabSelect SuRe, the buffer pH also depends on the antibody, but the range is approximately 3.6 to 4.0. The skilled person would thus have no reason to understand that the pH values in the examples in the application relate to PAC in general.

6. Appellant I pointed out that document D31 did not concern the use of a glycine buffer in PAC. The board notes that while document D31 does not refer to glycine in the elution buffer, its teaching is not restricted to a specific buffer composition either. Hence, there

was no reason for the skilled person to discard the information in document D31 and conclude that this common general knowledge did not apply to a buffer having glycine. Accordingly, the skilled person would not directly derive from the application that the pH values used in the examples were applicable generally to any PAC step, due to the presence of glycine, or its concentration, irrespective of the chromatographic material or antibody to be purified, in spite of the common general knowledge. Even taking into account that the examples show two different chromatographic materials using the same pH value in the elution buffer (pH 3.2 in Examples 1 and 2), it is not directly derivable that the pH values used in the examples were intended to be generally applicable to all materials. In fact, the application lists a number of additional chromatographic materials for PAC (see paragraph [0043]).

7. Additionally, while the examples in the application as filed relate to methods in which PAC is followed by AEX (in turn followed by cation exchange chromatography (CEX)), claim 1 does not specify this order of chromatographic steps. In the method as defined in claim 1, PAC is not necessarily the first chromatographic step. In this case, the elution conditions, including the buffer pH, that result in separation from the other components or contaminants, depend on the preceding chromatographic steps and might or might not be the same as for a method involving PAC as the first purification step.

8. It follows from the above considerations that the requirements of Article 123(2) EPC are not fulfilled.

9. Appellant I argued that the case law of the boards distinguishes between when a parameter has an impact, possibly even considerable, on the other conditions in the examples and when the parameter is inextricably linked to the other conditions in the examples. Only in the latter were the amendments not allowable. This was illustrated by decisions T 201/83, T 876/06 and T 500/11.

10. However, in line with the case law of the boards (see point 4. above), the only relevant question is whether the claimed subject-matter is directly and unambiguously derivable, for a skilled person using common general knowledge, from the application as filed. Therefore, assessing whether a feature in the claims was only disclosed in the application as filed as inextricably linked to other features cannot have any other purpose than answering that question and cannot lead to any different conclusion than the one arrived at by the board in points 1. to 8. above.

11. For completeness, whether a parameter is inextricably linked to other conditions in an example is necessarily case specific. Hence, for the case at hand, no insight can be gained from analysing the cases underlying the decisions cited by appellant I.

*Auxiliary requests MRb, 1-55, 15a, 51a, 52a and 55a*

*Amendments - extension beyond the content of the application as filed (Article 123(2) EPC)*

12. Claim 1 of each request is directed to a method including the feature "the pH of the elution buffer

being from 3.2-3.4", analysed above for the main request.

13. In some of these requests, claim 1 includes additional features, such as the presence of a cation chromatographic process, or definitions for the sequence of the three chromatographic steps. However, these features do not overcome the lack of a direct and unambiguous disclosure set out in the reasons above for the main request. At issue is that the skilled person would not have derived directly and unambiguously from the application, and from the examples in particular, that the pH values 3.2 and 3.4 were more generally applicable than in the specific conditions of the examples. This issue remains for a claim to a method including a CEX step. For the same reasons, the deletion of some features, as is the case in claim 1 of auxiliary request MRb, does not change the reasoning.

14. In conclusion, claim 1 of each request includes subject-matter which extends beyond the content of the application as filed (Article 123(2) EPC). There is therefore no request on the basis of which the patent can be maintained.

## Order

## For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairwoman:

I. Aperribay M. Pregetter

Decision electronically authenticated