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Datasheet for the decision of 20 May 2011

Case Number: T 0305/07 - 3.3.04

Application Number: 99933442.8

Publication Number: 1095055

IPC: C07K 1/113

Language of the proceedings: EN

Title of invention:
Method for the production of recombinant peptides with a low amount of trisulfides

Patentee:
Pfizer Health AB

Opponent:
Novo Nordisk A/S

Headword:
Trisulfides/PFIZER

Relevant legal provisions:
EPC Art. 54, 56, 83, 123(2)(3), 114(2)
RPBA Art. 12(4), second half sentence

Keyword:
"Admissibility of experimental evidence (no); main request, auxiliary requests 1 and 2 - added subject-matter (yes); auxiliary request 3 - added subject-matter, extension of scope (no); sufficiency of disclosure, novelty, inventive step (yes)"

Decisions cited:
G 0002/88, T 0939/92, T 0411/02, T 0936/02, T 0190/04, T 1335/05

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0305/07 - 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 20 May 2011

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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 2 January 2007 revoking European patent No. 1095055 pursuant to Article 102(1) EPC.

Composition of the Board:

Chairman: C. Rennie-Smith
Members: G. Alt
B. Claes

Summary of facts and submissions

I. This is an appeal by the patent proprietor against the decision of the opposition division to revoke its European patent.

II. The following documents are cited in the present decision:

D1 WO 96/02570

D3 Enzyme Microb. Technol., 1992, vol. 14, April, Chen, H.-C., et al.

III. The patent at issue has the title "Method for the production of recombinant peptides with a low amount of trisulfides". It had been granted on European application No. 99 933 442.8 which originated from an international application No. WO 00/02900 (referred to in the present decision as the "application as filed").

IV. The granted patent contained one independent claim and seven claims dependent on it.

Claims 1 to 8 read:

"1. Use of an alkali metal or alkali earth metal salt in the production of recombinant peptides during or after the fermentation step for the reduction of the amount of trisulfides in the recombinant product.

2. Use according to claim 1 characterized by the addition of the salt during or after the fermentation step.

3. Use according to claim 2 in which the addition is performed directly after fermentation.

4. Use according to any of claims 1 to 3 in which [sic] pH is equal or lower than pH7.

5. Use according to any of claims 1 to 4 in which the metal preferably is potassium or sodium.

6. Use according to claim 5 in which the salt preferably is potassium- or sodium phosphate or acetate.

7. Use according to any of claims 1 to 6 in which the peptide is growth hormone.

8. Use according to claim 7 in which the peptide is human growth hormone."

V. Revocation of the patent was requested on the grounds that the claimed subject-matter was not novel, did not involve an inventive step (Article 100(a) EPC in conjunction with Articles 54 and 56 EPC) and that the patent did not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art (Article 100(b) EPC).

VI. The opposition division revoked the patent. It held that the subject-matter of claim 1 of the amended main request lacked novelty over documents D1 and D3 and that of claim 1 of auxiliary request 1 over document D3. The subject-matter of the claims of auxiliary request 2

was considered to contravene the requirements of Article 123(2) EPC.

Claim 1 of the main request read:

"1. Use of a buffer including an alkali metal or alkali earth metal salt in the production of recombinant peptides during or after the fermentation step for the reduction of the amount of trisulfides in the recombinant product; wherein the pH achieved by the use of said buffer is equal or lower than pH 7."

Claim 1 of auxiliary request 1 (denoted as "new auxiliary request 1") differed from claim 1 of the main request in that the embodiment "during" fermentation was deleted.

Claim 1 of auxiliary request 2 (denoted as "amended new auxiliary request 2") differed from claim 1 of auxiliary request 1 in that the salt in the buffer was defined as "sodium or potassium phosphate or acetate, and which does not include a sulfite or mercapto compound" and in that at the end of the claim the expression "and wherein said reduction is not achieved by conversion of the formed trisulfides back into the native form" was present.

VII. The opposition division decided moreover not to admit into the proceedings experimental data filed with the proprietor's submissions of 26 June 2006 and 21 July 2006 because their late filing constituted an abuse of the procedure and because they were not prima facie relevant. The following reasons were given therefor in point 2.4 of the decision under appeal:

"The experimental data submitted with fax of 26.06.06 was known to the Patentee since 05.12.01 that means, for more than 4 years before the Oral Proceedings. The fact that Pharmacia Aktiebolag merged [sic] *Pfizer does not seem to justify such a delay as in T 901/95, since both companies merged at least two years before the oral proceedings as seen from the provided "Certificate of formation of Pharmacia & Upjohn company LLC" of 13.08.04. A period of time of at least two years appears to this Opposition Division enough to learn about the experiments carried out in both companies, moreover, when an Opposition procedure was open. Moreover, none of the data provided with the faxes of 26.06.06 and 21.07.06 seems to be prima facie relevant. The data provided with fax of 26.06.06 seems to be similar, and therefore not prima facie relevant to that of Fig. 3[sic] of the Patent, wherein H2S-induced trisulfides are reduced in presence of sodium-phosphate at pH 6 to 7.8. The data provided with fax of 21.07.06 seems to corroborate basic chemistry knowledge relating to trisulfide formation and disruption. Thus, the Opposition Division regards as not prima facie relevant the experiments showing that hydrogen sulfide leads to formation of trisulfide impurities (also known from D1 and the Patent) and that sodium sulfide but not sodium phosphate converts trisulfide to native rhGH (as known from D1 in combination with basic sulfide chemistry knowledge)."*

VIII. With the statement of the grounds of appeal the patent proprietor (hereinafter "appellant") filed an amended main and seven auxiliary requests and it requested that the experimental data which had not been admitted by

the opposition division be admitted into the appeal proceedings.

IX. Claim 1 of the main request read:

"1. Use of an alkali metal or alkali earth metal salt, which is not a sulfite or mercapto compound, in the production of recombinant peptides during or after the fermentation step for the reduction of the amount of trisulfides in the recombinant product; wherein the pH is equal to or lower than pH 7."

Claim 1 of auxiliary request 1 differed from claim 1 of the main request in that the pH was defined as being "equal to or lower than pH 6.8."

Claim 1 of auxiliary request 2 differed from claim 1 of the main request in that the embodiment "during" fermentation was deleted.

X. Claim 1 of auxiliary request 3 differed from claim 1 of auxiliary request 2 in that at the end of the claim the expression "and wherein the addition of the salt is performed directly after fermentation" was present.

Auxiliary request 3 contained four further claims, all of them dependent on claim 1 and corresponding to claims 5 to 8 as granted (see section IV above).

XI. In its reply to the statement of the grounds of appeal the opponent (hereinafter "respondent") requested that the appeal be dismissed. It submitted that the admissibility into the appeal proceedings of the experimental data, which were not admitted by the

opposition division, was *"questionable"*. Furthermore, the letter contained arguments explaining why the subject-matter of the claims of the main request lacked novelty and inventive step and why the disclosure in the patent was not enabling with respect to the claimed subject-matter.

XII. The summons to oral proceedings enclosed a communication by the board wherein it informed the parties of its preliminary view on the admissibility of the experimental data which were not admitted by the opposition division and on the issues of novelty, inventive step and sufficiency of disclosure with regard to the main request.

XIII. By letters dated 4 April 2011 and 12 April 2011 the respondent and the appellant, respectively, informed the board that they would not attend the oral proceedings

XIV. In a letter sent via telefax on 17 May 2011 - which both parties confirmed to have received - the board informed the parties of its preliminary view that claim 1 of the main and auxiliary requests 1 and 2 contravened the requirements of Article 123(2) EPC and that auxiliary request 3 appeared to be allowable.

XV. Oral proceedings were held on 20 May 2011. None of the parties was represented. At the end of the proceedings the chairman announced the board's decision.

XVI. The appellant's arguments may be summarized as follows:

Non-admission by the opposition division of the experimental evidence filed with letters of 26 June 2006 and 21 July 2006 - Admission of this evidence into the appeal proceedings

The opposition division *"erred"* when it dismissed the experimental data at issue. The data were generated in December 2001. However, the representative only became responsible for the case in March/ April 2006. The files were revisited in March 2006 following the receipt of the summons to oral proceedings in opposition proceedings. Soon thereafter the representative became aware of the relevance of the data and decided to include them in his final submission. Thus, the belated submission was not to be considered as an abuse of the procedure. The data had been filed as early as possible.

The data were prima facie relevant. The data presented in the letter of 26 June 2006 demonstrated not only that alkali metal salts were indeed suited to reduce trisulfide variants, but also that this effect could be applied to other peptides. The data presented in the letter of 21 July 2006 concerned the comparative use of an alkali metal salt according to the present invention and an sulfite alkali metal salt according to document D1 in order to exclude the mechanism of reduction of the amount of trisulfides disclosed in document D1 as the mechanism by which the alkali metal salts of the present invention operated.

The opposition division did not admit the experimental data for the additional reason that the late filing made it impossible to fully evaluate or repeat the experiments. However, this reason had fallen away now because in the meantime the respondent - should it wish to - had had ample time both to evaluate and to repeat the experiments.

Main request and auxiliary request 2

Article 123(2) EPC

Basis for the feature "wherein the pH is equal or lower than pH 7" could be found on page 3, line 8 and claim 5 of the application as filed. The requirements of Article 123(2) EPC were fulfilled.

Auxiliary request 1

Article 123(2) EPC

Basis for the feature "wherein the pH is lower than pH 6.8" was found on page 3, lines 8 to 9 of the application as filed. The requirements of Article 123(2) EPC were fulfilled.

Auxiliary request 3

Article 123(2) EPC

The disclaimer "which is not a sulfite or mercapto compound" was disclosed on page 3, line 15 of the application as filed. The feature "wherein the pH is equal or lower than pH 7" could be found on page 3,

line 8 and in claim 5. The feature "wherein the addition of the salt is performed directly after fermentation" could be found on page 3, line 1 and in claim 3 of the application as filed. Therefore, the requirements of Article 123(2) EPC were fulfilled.

Article 123(3) EPC

The amendments were limiting. Thus, the scope of protection was not extended vis-à-vis the claims as granted.

Article 83 EPC

In toto the examples showed that the claimed measures could achieve the intended effect. Therefore, the disclosure was sufficient to enable the skilled person to carry out the invention.

Article 54 EPC

Document D1 clearly disclosed that sulfite compounds, including alkali metal or alkali earth metal sulfites - the use of which was excluded by the subject-matter of claim 1 - were used for the reduction of the amount of trisulfide variants in a preparation of human growth hormone. The use of an alkali salt was disclosed only in the context of a method for isolation of the trisulfide derivative of human growth hormone. Thus, the document did not anticipate the subject-matter of any of the claims.

Document D3 was silent about post-fermentation uses of alkali metal and alkali earth metal salts and did therefore not take away the novelty of the subject-matter of any of the claims.

Article 56 EPC

Document D1 was the closest prior art document. It disclosed the use of sulfite compounds, including alkali metal or alkali earth metal sulfites for the reduction of the amount of trisulfide variants of recombinantly produced human growth hormone.

The problem to be solved was the provision of an alternative method for reducing the amount of trisulfide derivates in a recombinant polypeptide product.

The claimed solution, i.e. the addition directly after fermentation of an alkali metal or earth alkali metal salt which was not a sulfite or mercapto compound at a pH of equal or lower than pH7 was not suggested in document D1 which explicitly taught to use sulfite compounds for this purpose. Document D3 pertained to improved fermentation methods for bacterial expression of porcine growth hormone and was completely silent about the reduction of the amount of trisulphide derivatives. Thus, the subject-matter of claim 1 and claims dependent thereon involved an inventive step.

XVII. The respondent's arguments, as far as considered relevant to the present claims, may be summarized as follows:

Non-admission by the opposition division of experimental evidence filed with letters of 26 June 2006 and 21 July 2006 - Admission of this evidence into the appeal proceedings

The opposition division was correct in not admitting the experimental data into the proceedings. It was established practice that the parties should submit their complete case as early as possible. The data presented with the letter of 26 June 2006 could have been filed earlier since they were generated in December 2001 and were available to the appellant.

Main and auxiliary requests 1 to 3

Sufficiency of disclosure

The claims related to the use directly after fermentation of any alkali metal or alkali earth metal salt for the reduction of the amount of trisulfides in the production of any recombinant peptide.

– Firstly, in view of the examples in the patent it was doubtful whether this effect could be achieved by the claimed means. This was so for the following reasons:
Example 1 did not fall within the scope of the claims. Rather, it demonstrated the influence of a change in pH on trisulfide formation. The two samples according to Example 2 differed not only

by the presence or absence of salt, but also by their pH. Since, in view of example 1, a difference in pH could itself lead to a change in trisulfide levels, one could not be sure whether the reduction was caused by the presence of the salt. Example 3 did not fall within the scope of the claims because the assay was carried out at pH 7.2. Examples 4 and 5 did not include control measurements so that it was not possible to determine whether reduction had occurred.

The results of Example 6 were difficult to interpret if only because the assay was carried out with a trisulfide human growth hormone variant that was artificially created by the addition of H_2S. Moreover, according to the appellant - see its letter dated 26 June 2006, pages 9 to 11 - there might be more than one mechanism involved in trisulfide formation during the production of recombinant peptides by fermentation. Example 6 only recreated one of them. Also for that reason it was not sure whether the results of the example actually reflected the results when the salt was used as required by the claims during a fermentation situation. If it was accepted that Example 6 demonstrated that the alkali metal/earth alkali metal salts were capable of preventing H_2S-induced production of trisulfides, the relevance of this mechanism in the claimed use depended on the extent to which this mechanism was actually involved in trisulfide formation. Thus, it was not sure, if the mechanism simulated in Example 6, was at all relevant in a fermentation situation. Even if it was and the results of Example 6 were

relevant to fermentation, the results were inconclusive. When taken in isolation, for example a comparison of the results for ammonium citrate - sodium citrate/ Tris-HCl - sodium phosphate/sodium phosphate - ammonium citrate appeared to suggest an effect of alkali metal/earth alkali metal salts. This was however not so when the results were considered as a whole because: (i) No control samples were included. Thus, the difference between alkali/earth alkali metal salts and non-alkali/non-earth alkali metal salts could arise due to an increase in the amount of trisulfide variants caused by the non-alkali/ non-earth alkali metal salts instead of by a reduction caused by alkali/earth alkali metal salts; (ii) the results showed that alkali metal salts at a pH higher than 7, i.e. a pH outside that of the claims, achieved a lower level of trisulfides than a pH according to the claims; (iii) the results as a whole suggested that it was not the cation, i.e. the alkali or earth alkali metal ion, that was important to achieve the effect, but rather the anion.

– Secondly, the examples disclosed the use of only a small number of different alkali metal or earth alkali metal salts in the production of only one peptide, i.e. recombinant human growth hormone. Moreover, the patent and the examples taught that different salts, in different concentrations and at different pHs had varied effects. The patent did not indicate how the limited teaching of the examples or of the patent as a whole could be used by the skilled reader to achieve the claimed

effect of reducing the amount of trisulfides using alkali metal or earth alkali metal salts different from the exemplified ones in the production of recombinant peptides different from human growth hormone because the patent did not provide a general teaching of how the relevant factors should be combined in order to achieve the effect.

Thus, in summary, the skilled person needed such a significant level of experimentation in order to find out the appropriate conditions for a particular protein - salt combination, that undue burden was involved when attempting to carry out the invention over the whole breadth claimed. Hence the requirements of Article 83 EPC were not fulfilled.

Novelty

Document D1 generally related to the purification of recombinant human growth hormone by the removal of trisulfide derivatives (called *"hydrophobic derivatives"* in document D1). The methods to remove such derivatives were carried out after fermentation. According to pages 5, lines 10 to 17 of document D1 suitable solvents for use in such methods were, *inter alia*, phosphate buffers at pH 7. These buffers typically included sodium and/or potassium salts, i.e. salts that were not sulfite compounds. It followed from the teaching in the patent that such a use would inherently result in a reduction of the amount of trisulfides in the recombinant peptide product. Hence, claim 1 lacked novelty over the disclosure in document D1.

Inventive step

Document D1 was the closest prior art document. The problem underlying the patent was to provide an alternative method for reducing trisulfide derivatives present in a recombinant polypeptide product.

The subject-matter of claim 1 was obvious in view of document D1. The skilled person faced with the problem of providing an alternative method for reducing the amount of trisulfide derivatives present in a recombinant polypeptide product would routinely modify the methods disclosed in document D1 and would therefore easily identify that the effect could be achieved by the means recited in claim 1.

According to decision T 939/92 the extent to which the problem was actually solved by the claimed subject-matter had to be determined. Given the variability seen in the examples and their limited scope it was not credible that the effect of reducing trisulfides in a recombinant polypeptide product could be achieved across the whole scope of claim 1. Consequently, claim 1 lacked an inventive step also for this reason.

Reasons for the Decision

Non-admission by the opposition division of experimental evidence filed with letters of 26 June 2006 and 21 July 2006

1. The appellant submits that the opposition division was wrong (i) when it refused to admit into the proceedings the experimental evidence filed with letter of 26 June

2006 for the reason that its late filing constituted an abuse of the procedure and (ii) when it refused to admit the experimental evidence filed with letters of 26 June 2006 and 21 July 2006 for its lack of prima facie relevance.

2. Thus, the question arising in view of the appellant's submission is whether or not the opposition division exercised its discretion pursuant to Article 114(2) EPC correctly when it did not admit the experimental evidence at issue.

3. As to the reason (i) above, the opposition division rejected the experimental evidence included in the letter of 26 June 2006 for the reason that its late filing - oral proceedings took place on 27 July 2006 - constituted an abuse of the procedure because, in the opposition division's view, *"[t]he fact that Pharmacia Aktiebolag merged* [sic] *Pfizer does not seem to justify such a delay as in T 901/95, since both companies merged at least two years before the oral proceedings as seen from the provided "Certificate of formation of Pharmacia & Upjohn company LLC" of 13.08.04. A period of time of at least two years appears to this Opposition Division enough to learn about the experiments carried out in both companies, moreover, when an Opposition procedure was open."*

3.1 The board notes however that the evidence referred to by the opposition division for the merger of the two companies, i.e. the *"Certificate of formation of Pharmacia & Upjohn company LLC"*, although filed by the appellant during the opposition proceedings, is - and, as was also noted by the responsible formalities

officer (see notification dated 17 December 2004) - not relevant to the present case. Indeed, this evidence merely proves *"the conversion of a Delaware Corporation under the name "Pharmacia & Upjohn Company" to a Delaware limited liability company, changing its name from "Pharmacia & Upjohn Company" to "Pharmacia & Upjohn Company LLC"."*

3.2 The pertinent evidence was however filed by the appellant with its letter dated 28 December 2004 in the form of an extract from the register of the "Patent- och Registreringsverket" in Stockholm. This shows that "Pharmacia & Upjohn Aktiebolag" changed its name to "Pharmacia Aktiebolag" on 26 September 2000 and further changed its name to "Pfizer Health AB" on 31 March 2004.

3.3 There is thus no evidence on file relating to any merger and therefore the opposition division's reason for finding that the filing of experimental data with the letter of 26 June 2006 constituted an abuse of procedure starts from a false premise. However, if the opposition division had appreciated the position correctly - namely, that one and only one company had been in possession of the experimental evidence throughout - it would probably, indeed more probably, have reached the same conclusion, i.e. that the experimental evidence submitted with the letter of 26 June 2006 could have been filed earlier. In view of the board's view below (see point 4), no decision in this matter appears necessary.

4. As to reason (ii) above, in the board's view, the opposition division has given convincing reasons why the experimental evidence filed with letters of 26 June

2006 and 21 July 2006 is not relevant (see point 2.4 of the decision under appeal; recited in section VII above, see line 13 et seq. of the cited passage). Seeing that according to established case law relevance is one of the criteria that is taken into account when deciding the admissibility of late filed evidence (see Case Law of the Board's of Appeal, 6th edition, VII.C.1.2), the board concludes thus that the opposition division exercised its discretion pursuant to Article 114(2) EPC appropriately. Hence, the board cannot come to the conclusion that the opposition division was wrong when it refused to admit into the proceedings the experimental evidence at issue.

Admissibility into the appeal proceedings of experimental evidence originally filed with letters of 26 June 2006 and 21 July 2006 and re-filed with the statement of grounds of appeal

5. The appellant filed the experimental evidence which had not been admitted by the opposition division, i.e. experimental evidence originally included in the letters dated 26 June 2006 and 21 July 2006, with its statement of the grounds of appeal and requests its admission.

6. The board is not convinced by the appellant's argument that *"the EPO and the Opponent have now had ample time to evaluate the experimental data and the Opponent has had time, should it wish to, to repeat any experiments"*. The board does not see why the respondent should react to experimental evidence which is not "in" the proceedings (even if it could be expected that it would be re-filed in appeal proceedings). The appellant's

argument overlooks the difference between filing and admissibility.

7. The admissibility of late-filed evidence during appeal proceedings is specifically regulated in Article 12(4) RPBA stipulating that, without prejudice to the power of the board to hold inadmissible evidence which was not admitted in the first instance proceedings, everything presented by the parties with the statement of the grounds of appeal shall be taken into account by the board if and to the extent it relates to the case under appeal.

8. The present claims differ from those dealt with by the opposition division and in relation to which it considered the experimental evidence as irrelevant. Thus, in the board's view, Article 12(4) RPBA, first half sentence does not apply here, but Article 12(4) RPBA, second half sentence is to be considered. In particular, the question arises whether, and if so to what extent, the experimental evidence relates to the case under appeal.

9. According to the appellant the data originally presented in the letter of 26 June 2006 demonstrate not only that alkali metal salts are indeed suited to reduce trisulfide variants in a recombinant product, but also that this effect can be applied to other peptides. However, the board considers that what the appellant aims to demonstrate is sufficiently demonstrated by the data in the patent, in particular those of Figure 2. The evidential weight of the data originally submitted with the letter of 26 June 2006 is moreover questionable, *inter alia*, since there is no

information as to when, during the recombinant production process, the salt was applied, i.e. during or after fermentation.

10. According to the appellant the experimental data as originally submitted with the letter dated 21 July 2006 was filed in order to exclude the mechanism of trisulfide reduction disclosed in document D1 as the mechanism by which the alkali metal or earth alkali metal salts of the present invention operate. However, feature or features relating to a mechanism are absent from claim 1 and therefore it is not necessary to prove that mechanism.

11. The board thus comes to the conclusion that none of the experimental evidence submitted with the statement of the grounds of appeal is relevant to the present case. Therefore, it cannot be considered to "relate" to it. Hence, in accordance with Article 12(4) RPBA, second half sentence the board decides not to admit the experimental evidence filed with the statement of the grounds of appeal.

Main request and auxiliary requests 1 and 2

Article 123(2) EPC

12. Article 100(c) EPC is not a ground of opposition in the present proceedings. Therefore, in accordance with established practice (see for example, decisions T 411/02, point 34 of the Reasons; T 936/02, point 3 of the Reasons; T 190/04, point 2.1 of the Reasons and T 1335/05, point 9 of the Reasons), only amendments

with regard to the claims as granted are considered for their compliance with regard to Article 123(2) EPC.

13. Claim 1 as granted reads (see section IV above): "Use of an alkali metal or alkali earth metal salt in the production of recombinant peptides during or after the fermentation step for the reduction of the amount of trisulfides in the recombinant product."

14. Thus, claim 1 as granted and claim 1 of the present main request and auxiliary requests 1 and 2 differ, *inter alia*, by the feature "wherein the pH is equal to or lower than pH 7" or "wherein the pH is lower than pH 6.8" (see section IX above).

14.1 In the board's view, claim 1 of those requests has to be interpreted as to encompass, *inter alia*, the use of alkali metal or earth alkali metal salts which are **inherently present** during the fermentation process - as opposed to those which are actively added. This is also the view of the appellant who observes with regard to claims which, like those at issue, do not explicitly make a distinction between *"addition"* and *"inherent presence"* (see submission dated 26 June 2006, page 7, third paragraph): *"It is also considered that the opposed claims and those of the Main Request (or indeed any of the Auxiliary Requests) do not distinguish (and do not need to distinguish) between the addition of alkali metals or alkali earth metal salts and the inherent presence of such metals and metal salts during or directly after fermentation."*

Thus, the use of "inherently present" alkali metal or earth alkali metal salts at a pH that is equal to or

lower than pH 7, or lower than 6.8, respectively, is an embodiment of claim 1 of the main request and auxiliary requests I and II, respectively.

14.2 However, a use characterized by this combination of features, i.e. the use of salts which are "anyway there" at the indicated pH range is not derivable from the application as filed. The application as filed discloses the use of alkali metal and earth alkali metal salts at the indicated pH range only in combination with the **addition** of these salts (see page 2, lines 24 to 25; page 3, lines 1 to 3, 8 to 9 and 13; the examples; and in particular claim 1 in combination with claim 5). In the board's view, the term "addition" would not be understood by the skilled person to include the meaning of "use of salts which are anyway there".

14.3 Consequently, amended claim 1 of the main request and auxiliary requests 1 and 2 contain an embodiment which is not disclosed in the application as filed. Therefore, the subject-matter of these claims extends beyond the content of the application as filed. Hence, the main request and auxiliary requests 1 and 2 do not fulfil the requirements of Article 123(2) EPC.

Auxiliary request 3

Articles 84, 123(2)(3) EPC

15. The aspect to which claim 1 relates, i.e. the use of an alkali metal or earth alkali metal salt by addition of the salt directly after fermentation and at a pH equal to or lower than pH 7 was present in the claims as

granted as a combination of claims 1 to 4. Present claims 2 to 5 are identical to claims 5 to 8 as granted (see sections IV and IX above).

16. The only difference between the claims of present auxiliary request 3 and the claims as granted is the disclaimer in claim 1 "which is not a sulfite or mercapto compound". This disclaimer is disclosed on page 3, line 15 of the application as filed. Its meaning is clear.

17. By the inclusion into claim 1 of the features "wherein the pH is equal or lower than pH 7", "wherein the addition of the salt is performed directly after fermentation" and the deletion of the feature "during" in relation to the fermentation step the scope of the present claims is limited vis-à-vis the scope of the claims as granted.

The claims of auxiliary request 3 fulfil the requirements of Articles 84, 123(2) and (3) EPC.

Sufficiency of disclosure

18. The respondent essentially pursues two lines of argument on the basis of which the sufficiency of the disclosure for the present invention in the patent should be denied: (A) in the light of the examples it is doubtful that the tridsulfide-reducing effect is achieved for the specific peptide considered in the patent, i.e. human growth hormone and (B) because the patent as a whole does not teach how relevant factors, for example type of salt or its concentration should be

combined to achieve the desired effect for a peptide different from human growth hormone and for a salt different from those exemplified in the patent, the skilled person could only with undue burden achieve the claimed use over its whole breadth.

19. In relation to the line of argument (A) above, the respondent submits with regard to Examples 1 and 3 of the patent that they do not illustrate the claimed invention. The appellant appears to agree with this view, since it states in the statement of the grounds of appeal, page 12: *"The results of Example 1 show that lowering the pH decreases the level of trisulfide variants"* and *"[h]owever it should be noted that the alkali metal treatment in Example 3 was carried out in a pH environment outside the scope of the present claims (i.e. at a pH>7)."* The board concludes that an assay which does not exemplify the invention, cannot be used as evidence that an effect, which should be achieved according to the invention, is not achieved.

20. Example 2 demonstrates a decrease in the level of trisulfide variants after the addition of water or potassium phosphate to samples at pH 7.2 and pH 6.8, respectively.

The appellant submits with regard to Example 2 that the two pH values are *"around the same"*. The respondent submits that the results of Example 2 do not demonstrate the effect to be achieved by the invention because - see Example 1 - a pH change of the size reported in Example 2 can itself lead to a change in trisulfide levels, even in the absence of salt.

The board is not convinced by the appellant's submission. The decrease from 6% to 3% in Example 2 is associated with a decrease from pH 7.2 to pH 6.8. However, according to Example 1, a comparable decrease in trisulfide levels from 5% to 3% occurred after a change in pH from pH 7.0 to pH 6.5 without addition of salt. Thus, in the board's view, Example 2 cannot be taken into account when considering the question of whether or not the examples demonstrate that the addition of alkali metal or earth alkali metal salts directly after fermentation results in the reduction of the amount of trisulfides variants in a preparation of human growth hormone.

21. The results of Examples 4 and 5, indicated as the percentage of trisulfide variants of human growth hormone are as follows (the first values are results of salt-treated samples and the second values are results of a "reference sample", i.e. a sample where the amount of trisulfides has been determined immediately after the end of fermentation):

assay E: 1.6/1.4
assay F: 3.4/3.1
assay G: 2.6/3.1

It can be seen that in assays E and F the amount of trisulfide variants in the experimental sample is higher than in the reference sample.

21.1 In the board's view, this somewhat unexpected result is convincingly explained in the patent in column 2, lines 21 to 31 and by the appellant in the statement of the grounds of appeal, namely that the mechanism of

reduction is by prevention of the formation of trisulfide derivatives and not by their back-conversion to "normal" growth hormone.

It is stated in the statement of the grounds of appeal, page 13 in the paragraph "Notes":

"This is because trisulfide variant formation is prevalent after fermentation as well as during fermentation. Thus, "reference samples" are indicative of the trisulfide levels immediately upon termination of fermentation, since they are taken for analysis just before cell harvest. In the period between the point of cell harvest and addition of the alkali metal salt, there is the possibility of post-fermentation trisulfide formation. This can cause the trisulfide level to increase. Thereafter, once the alkali metal salt is added (or not in the case of the water-treated samples), trisulfide variant formation is prevented. However, the post-fermentation trisulfide variants which were formed BEFORE addition of the alkali metal salt (or water) are not back-converted to native peptide and so remain within the experimental Extracts and contribute to the apparent "increase" in trisulfide variants compared to the "reference samples"."

21.2 The respondent does not seem to contest that this is a plausible explanation of the results of assays E and F, but submits that this explanation does not explain the results of assay G, i.e. the drop in the amount of trisulfides in the salt-treated sample to below that of the reference example. In its view the result of assay G could only be explained in two ways:

(a) Contrary to the appellant's proposed mechanism, the change in pH and/or the addition of salt reduces existing levels of trisulfide human growth hormone rather than preventing their new formation, or

(b) if the appellant's proposed mechanism applies, the difference in assay G between 2.6% and 3.1% is not significant. This level of variability makes comparisons difficult to interpret.

21.3 However, first, the mechanism of trisulfide reduction is not a feature of the claims and second, although the result of assay G may not fit with the appellant's explanations, the assay undoubtedly shows a decrease of the amount of trisulfide variants.

Moreover, the respondent's explanations could at best lead the board to the conclusion - in particular in the absence of appropriate control samples - that the experimental conditions of Examples 4 and 5 are not suited to prove an effect. This is not sufficient, however, for the board to come to the conclusion that Examples 4 and 5 provide evidence that addition of alkali metal or earth alkali metal salts directly after fermentation does not have an effect on the reduction of the amount of trisulfide variants in a preparation of human growth hormone.

21.4 According to Example 6 pure human growth hormone in water is mixed with buffers containing the salts to be tested. The samples are divided and mixed with water as a control or H_2S in three different concentrations. The samples are incubated for three hours for the

preparation of trisulfide variants, then frozen, thawed, desalted and the amount of trisulfides is analysed.

21.4.1 The respondent has questioned the relevance of Example 6, which relates to the production of recombinant peptides by fermentation, with regard to the claimed use for the following reasons. The respondent refers to the appellant's explanation in opposition proceedings that two different mechanisms are involved in trisulfide formation under fermentation conditions (see letter dated 26 June 2006, pages 9 to 11). In its view Example 6 recreates only one of these mechanisms and since it is not known to which extent the mechanism recreated according to Example 6 occurs in fact during the production of recombinant peptides by fermentation, it is not known in how far the effect obtained according to Example 6 would be the same in the fermentation situation.

21.4.2 In the absence of any statement to the contrary the board assumes that the respondent appears to accept that, firstly, two different mechanisms are involved in trisulfide formation under fermentation conditions and also that, secondly, Example 6 recreates one of them. The respondent has not provided evidence that the recreated mechanism only occurs to such a small extent in a fermentation situation that it virtually does not play a role in the reduction of trisulfides in such a situation. Thus, the board cannot come to the conclusion that Example 6 is not relevant for the present issue, i.e. the question whether or not alkali metal or earth alkali metal salts have a trisulfide reductive effect for the mechanism recreated by the conditions of Example 6.

21.4.3 The results of Example 6 as summarized in Figure 2 are interpreted in the patent in the paragraph bridging column 5 and 6 as follows:

"Ammonium citrate gave no reduction of trisulfides despite the low pH. Na-phopsphate at pH 6.0 gave the best result but also Na-phosphate at a higher pH can be used. This showed that for pure hGH the addition of a metal salt is of importance for the amount of trisulfides."

21.4.4 The appellant comments on the results of Example 6 in its statement of the grounds for appeal (see page 13) as follows:

"Samples 1-4
The results of Example 6 (sodium phosphate treatments show that addition of alkali metal salt decreases the level of trisulfide variants in combination with lower pH.

Samples 5-6
The results of Example 6 (citrate treatments) show that the presence of an alkali metal (in this case, sodium) is important for a decrease in trisulfide variants. If this was not the case, then the two citrate treatments would be comparable.

Sample 7
The results of Example 6 (no alkali treatment) show that, in the absence of an alkali metal salt, the level of trisulfide variants can be quite high. This is especially evident when the result of Sample 4 (sodium

phosphate at pH 7.8) is compared to the result of Sample 7 (Tris-HCl at pH 7.6). This comparison clearly shows that at a similar pH, the presence of an alkali metal salt is required in order to achieve a substantial decrease in trisulfide variant formation."

21.4.5 The board is convinced by the appellant's comments and considers in particular that the results of assays with ammonium citrate and sodium citrate, both carried out at pH 6.2, demonstrate the effect of alkali on the reduction of the amount of growth hormone trisulfide derivatives.

21.4.6 The respondent appears also to agree because it states on page 12 of its reply to the statement of grounds:

"These specific comparisons [note added by the board: the comparisons made by the appellant in its comments] *taken alone appear to suggest that at the stated pHs, sodium citrate or sodium phosphate led to a lower trisulfide level than ammonium citrate, and sodium phosphate leads to a lower trisulfide level than Tris-HCl."*

21.4.7 The respondent submits however that the overall results of Example 6 would call in doubt this conclusion because:

i) The results of Example 6 as summarized in Figure 2 do not include the results of an untreated control. Without a baseline the results could also be interpreted as an increase in the amount of trisulfides in the presence of non-alkali or non-earth alkali metal

salts rather than a decrease by alkali or alkali earth metal salts.

ii) The results appear to suggest that it may be the anion rather than the cation of the salt which is responsible for the effect.

iii) Sodium phosphate at a pH outside of the scope of the claims achieves a lower trisulfide level, than sodium citrate at pH 6.2, i.e. a pH inside the scope of the claims.

As to point (i) above, the respondent's interpretation of Figure 2 of the high amount of trisulfides after addition of non-alkali or non-earth alkali metal salts as showing an increase in the amount of trisulfides by the non-alkali or non-earth alkali metal salts instead of a "missing" reduction is so diametrically different from the disclosure in the patent and the submissions by the appellant, that the board would need more evidence than the mere statement that the differences highlighted by the appellant *"could"* be the result of an increase of the amount of trisulfides by non-alkali metal or non-earth alkali metal salts - for example an explanation of how, chemically, the salts used in the assay could induce an increase in the trisulfide level - in order to be convinced that the assays of Example 6 do not demonstrate a reductive effect. These observations apply also to point (ii) above. As to point (iii) above the fact that salts work at a pH outside the scope of the claims does not demonstrate that they do not work at a pH inside the scope of the claims.

21.5 The board concludes that the respondent has not made a case that in the light of the examples it is doubtful that the trisulfide-reducing effect is achieved for the specific peptide considered in the patent, i.e. human growth hormone.

22. Turning now to the respondent's second line of argument (denoted (B) in point 18 above), namely that the patent as a whole does not teach how relevant factors, such as type of salt or its concentration should be combined to achieve the effect for a peptide different from the exemplified one.

22.1 The relevant disclosure in the patent is as follows.

– The patent explains in column 2, lines 28 to 31 the mechanism by which the reduction of the amount of trisulfides is achieved: *"This reduced amount of the derivative is due to inhibition of the activity of H_2S in the medium and the prevention of the formation of the modified growth hormone comprising an extra sulphur atom."* It is emphasized in column 2, lines 25 to 27 that this mechanism differs from the known one, in that it does not rely on *"conversion of the formed trisulfides of growth hormone into the native form."*

– It is disclosed in column 2, lines 19 to 20 that the recombinant peptides can be *"e.g. both proteins and smaller peptides."*

– As to the form in which the salt is added, the patent discloses in column 2, lines 35-36 that

"[t]he addition can e.g. be done with a buffer including the salt." In fact according to the examples the salt is always added in the form of a buffer.

– As to the type of metal salt, the patent discloses in column 2, lines 42 to 50 that the metal component in the salt can be *"any metal chosen among alkali metal and earth salt"*, that the preferred metal is *"alkali, such as sodium or potassium"* and that the preferred salt is *"sodium or potassium phosphate or acetate"*. Accordingly, the salts used in the examples are sodium chloride, sodium phosphate, sodium acetate, sodium citrate and potassium phosphate.

– As to the concentration of the salt, it is disclosed that the effect of reduction of trisulfide variants is achieved *"by addition of the metal salt in molar excess"* (column 2, lines 51 to 52) or *"preferably in excess"* column 2, line 24.

– As to the point in time when the salt is added, i.e. "directly after fermentation" the patent discloses in column 2, lines 31-34: *"The addition can be done directly after fermentation, e.g. after the fermentation has been terminated and the cells are harvested and before further process steps"*. The examples disclose that the cells are harvested, concentrated and then buffer containing the salt is added (examples 2-5).

– As to the regulation of the pH, it is disclosed in column 2, lines 46 to 47 that *"pH regulation can be achieved with a selected buffer including the metal salt"*.

– Examples 4 to 6 disclose appropriate pHs, concentrations and salts.

22.2 The patent does not give explicit indications on the basis of which the skilled person could safely predict the process requirements in relation to a particular protein. However, in view of the detailed technical information in the patent as summarized above, in view of the fact that tests for trisulfide determination are known, and in the light of the successful examples, the board is not convinced by the respondent's mere statement that the skilled person would not be able to find the appropriate conditions without undue burden.

23. The requirements of Article 83 EPC are fulfilled.

Novelty

24. Claim 1 of auxiliary request 3 relates to the "[u]se of an alkali metal or alkali earth metal salt, which is not a sulfite or mercapto compound, in the production of recombinant peptides after the fermentation step for the reduction of the amount of trisulfides in the recombinant product; wherein the pH is equal to or lower than pH 7; and wherein the addition of the salt is performed directly after fermentation."

Document D1

25. It is explicitly disclosed in document D1 that the trisulfide variant of human growth hormone - this variant is referred to in document D1 as the *"hydrophobic derivative"* - is converted into its native form by treatment with sulfite compounds, in particular alkali metal sulfite or alkali earth metal sulfites (see claim 1 and page 4, lines 9-21). However, the use of sulfite compounds is excluded from claim 1.

26. Yet, according to the respondent's submission (see letter dated 28 September 2007, page 21) *"[p]ages 4 and 5 of D1 discuss treatments to be carried out on solutions that comprise growth hormone in a solvent. As explained at page 5, lines 10 to 17, a suitable solvent may be: "an aqueous buffer buffered at a pH from 3 to 11. Solutions being buffered to a pH> 6 are preferred, and more preferred are solutions buffered to about pH 7. The solvent is preferably selected from the group consisting of Tris, triethylamine, citric acid, phosphate buffer, and histidine. A preferred solution is has pH 7.0." [...] It is well-known (see for example D10) that phosphate buffers and citrate buffers will include sodium and/or potassium salts that are not sulfite or mercapto compounds."*

27. In the board's view, the skilled person would not read the disclosure on page 4 and in the first paragraph on page 5 of document D1 together with the passage cited by the respondent. But even if it is assumed that he/she would, the disclosure would not destroy the novelty of the subject-matter of claim 1 because the passages on page 4 and 5 disclose the use of **sulfite**

compounds for decreasing the amount of the hydrophobic, i.e. the trisulfide, derivative, an alternative which is disclaimed according to claim 1.

28. Rather, in the board's view, the skilled person would understand that the passage explicitly cited by the respondent, i.e. the fourth full paragraph on page 5, is to be read together with the second full paragraph on page 5. Thus, in the proper context the relevant disclosure on page 5 of document D1 would read:

"The hydrophobic derivative of growth hormone may optionally be isolated before carrying out the conversion thereof into the corresponding native growth hormone. It is preferred to treat the whole batch of growth hormone found to comprise the hydrophobic derivative of GH directly without isolating the growth hormone derivative. The solvents used to prepare the solution of derivative of the growth hormone to be treated may e.g. be an aqueous buffer [...]. The solvent is preferably selected from the group consisting of Tris, triethylamine, citric acid, phosphate buffer, and histidine."

29. The isolation of the hydrophobic derivative before the conversion treatment is disclosed in more detail on page 12 of document D1. Under the heading *"Isolation of hydrophobic Derivative of Human Growth Hormone"* it is stated that *"[s]uch isolation may be carried out by scaling up the procedure described above, or may e.g. be carried out using the method as described in Bio/Technology 5 (1987) 161-164."*

30. The cited document was not relied on by the respondent. As to the *"procedure described above"* document D1 discloses on pages 10 and 11 two different procedures, both relying on the isolation of the derivative by column chromatography, i.e. a method requiring the application of the sample comprising the growth hormone derivative onto a column.

31. Non-sulfite, alkali containing buffers are disclosed only in the context of one of the two methods, i.e. the one disclosed on page 11, and then for the elution of the fraction containing the hydrophobic growth hormone derivative from the column:

"hGH samples were analyzed on a TSK Ether 5PW (75 x 4.6 mm ID) column at ambient temperature using eluent C and D and a gradient from 40 to 50% eluent D during 30 minutes. Eluent C: 2 M $(NH_4)_2SO_4$, 20 mM Na_2HPO_4 x $2H_2O$, pH 6.0. Eluent D: 20mM Na_2HPO_4 x $2H_2O$, 0.1% PEG, pH 6.0."

32. In the board's view, the skilled person reading the above-cited disclosure on page 5 of document D1 in combination with the disclosure on page 11 of document D1 would clearly and unambiguously derive that the solvents referred to on page 5 are those used for elution of the hydrophobic derivative **after** application to the column of a sample comprising it.

33. Claim 1 recites the feature that the alkali metal or earth alkali metal salts are added "directly after fermentation" which means according to paragraph [0004], lines 31 to 34 of the patent *"after the fermentation*

has been terminated and the cells are harvested and before further process steps".

34. It follows from the observations above that according to the disclosure in document D1 the alkali-containing solution is added to the derivative-containing solution after it has been applied to a column, i.e. it is not added "directly after fermentation". Accordingly, this feature of claim 1 is not disclosed in document D1.

35. Since the disclosure in a document is considered to destroy the novelty of claimed subject-matter only if the document discloses all features of the claimed subject-matter, document D1 cannot, for the reason given in point 34 above, be considered to destroy the novelty of the subject-matter of claim 1.

Document D3

36. Document D3 discloses a process of fermentation for the production of porcine growth hormone. The fermentation medium contains a variety of alkali metal and earth alkali metal salts which are not sulphite compounds. Thus, document D3 discloses the use of the compounds at issue "during" and not "directly after" fermentation. Therefore, its disclosure does not anticipate the subject-matter of claim 1.

37. The requirements of Article 54 EPC are fulfilled.

Inventive step

38. Both parties considered document D1 as the closest prior art document. The board agrees. As noted in point 25 above it discloses the use of sulfite compounds, in particular alkali metal sulfite or alkali earth metal sulfites, for the conversion of the trisulfide derivative of human growth hormone into its native form.

The problem to be solved with regard to the closest prior art document may therefore, as suggested by the parties, be seen in the provision of an alternative method for reducing the amount of trisulfide derivates in a recombinant polypeptide product.

39. The solution according to claim 1 is the use of

a) an alkali metal or earth alkali metal salt, which is not a sulfite or mercapto compound
b) directly after fermentation
c) at a pH equal or lower than pH 7.

40. In the board's judgement this solution to the problem underlying the present invention is not suggested in any of the prior art documents available in these proceedings.

41. In contrast to the respondent's view, the board considers that it is in particular not obvious in the light of the teaching in document D1. This document teaches to use sulfite-containing compounds for the reduction of trisulfide derivatives in a preparation of human growth hormone (see also points 25 and 38 above),

a use expressly excluded from the subject-matter of claim 1.

42. The respondent's argument that the skilled person would in the light of the teaching in document D1 routinely vary methods and would thus have identified the now claimed effect of alkali metal and earth alkali metal salts does not convince the board. The respondent did not identify any teaching in either document D1 or in any other prior art document or from common general knowledge that could be taken as evidence for the motivation of the skilled person to use non-sulfite alkali metal and earth alkali metal salts for the reduction of trisulfide derivatives instead of sulfite compounds.

43. The respondent further submits that it is not credible that the effect to be achieved by the claimed subject-matter according to the problem to be solved, namely to reduce the amount of trisulphide derivatives in a recombinant polypeptide product, is exhibited across the full scope of claim 1. Therefore, in view of the reasoning in decision T 939/92 of 12 September 1995, the subject-matter of claim 1 lacks an inventive step.

44. In the case underlying decision T 939/92 the claims were directed to compounds per se without any functional restriction in the claims as to the effect to be achieved by them (see "facts and submissions", section IV of decision T 939/92).

The problem to be solved as asserted in the patent underlying decision T 939/92 was the provision of

further (alternative) chemical compounds with herbicidal activity (see the reasons, point 2.6).

The question therefore arose whether it would be credible that substantially all claimed compounds possessed this activity, because only if this question was answered in the affirmative could the technical problem formulated in the patent be taken into account.

45. The present situation differs from the one in decision T 939/92, first, because present claim 1 is directed to a use, and second, because it recites the effect which has to be achieved according to the problem to be solved, i.e. "for the reduction of the amount of trisulfides in the recombinant product".

46. In its decision G 2/88 of 11 December 1989 the Enlarged Board of Appeal deals with the interpretation of claims relating to a non-medical use, i.e. the category to which the present claims belong. It is stated in point 9 of the Reasons that *"[i]n relation to a claim whose wording clearly defines a new use of a known compound, depending upon its particular wording in the context of the remainder of the patent, the proper interpretation of the claim will normally be such that the attaining of a new technical effect which underlies the new use is a technical feature of the claimed invention."* For the present board it follows from this statement in decision G 2/88 that present claim 1 has to be interpreted as relating only to such uses where the effect stated in the claim, i.e. "for the reduction of the amount of trisulfides in the recombinant product", is actually achieved.

47. Consequently, the question which arose in the case T 939/92, namely whether or not it would be credible that substantially all claimed compounds possessed a given activity, does not arise in the present case. Hence, the respondent's argument fails.

48. Thus, the board concludes that the subject-matter of claim 1 and the claims dependent on it involves an inventive step. The requirements of Article 56 EPC are fulfilled.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the department of first instance with the order to maintain the patent based on the following documents:

   Claims 1 to 5 of auxiliary request 3 filed with the statement of the grounds of appeal dated 11 May 2007;

   Figures 1 and 2 as granted;

   and

   the description to be adapted thereto.

The Registrar: The Chairman:

P. Cremona C. Rennie-Smith