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Datasheet for the decision of 2 February 2023

Case Number: T 0412/20 - 3.3.04

Application Number: 08746526.6

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Language of the proceedings: EN

Title of invention:
Use of low temperature and/or low pH in cell culture

Patent Proprietor:
Wyeth LLC

Opponent:
Strawman Limited

Headword:
Cell culture/WYETH

Relevant legal provisions:
EPC Art. 56

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Inventive step - (no)

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Boards of Appeal

Chambres de recours

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

Case Number: T 0412/20 - 3.3.04

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

Appellant: (Patent Proprietor)	Wyeth LLC 235 East 42nd Street New York, NY 10017-5755 (US)
Representative:	Pfizer European Patent Department 23-25 avenue du Docteur Lannelongue 75668 Paris Cedex 14 (FR)
Appellant: (Opponent)	Strawman Limited Orchard Lea Horns Lane Combe, Witney Oxfordshire OX29 8NH (GB)
Representative:	Bassil, Nicholas Charles Kilburn & Strode LLP Lacon London 84 Theobalds Road London WC1X 8NL (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 18 December 2019 concerning maintenance of the European Patent No. 2139986 in amended form**

Composition of the Board:

Chairman	B. Rutz
Members:	D. Luis Alves
	L. Bühler

Summary of Facts and Submissions

I. Appeals were filed by the patent proprietor (appellant I) and the opponent (appellant II) against the interlocutory decision of the opposition division that, account being taken of the amendments in the form of auxiliary request 2, the patent and the invention to which it related met the requirements of the EPC. The patent is entitled "*Use of low temperature and/or low pH in cell culture*" and was granted on European patent application No. 08 746 526.6, which was filed as an international application published as WO 2008/131374.

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

In the decision under appeal, the opposition division held, *inter alia,* that priority was not validly claimed, that the claimed subject-matter according to the main request was not novel in view of document D1 (Article 54 EPC) and that the claimed subject-matter according to auxiliary request 1 did not involve an inventive step in view of the disclosure of document D1 (Article 56 EPC).

III. With their statement setting out the grounds of appeal, appellant I filed sets of claims of a main request and auxiliary requests 1 to 8. The main request and auxiliary requests 1, 3 to 5, 7 and 8 were identical to the main request and auxiliary requests 1 to 4, 6 and 7, respectively, considered by the opposition

division. Auxiliary request 3 corresponded to the request considered allowable by the opposition division.

Appellant I contested the opposition division's decision under Article 54 EPC (main request) and Article 56 EPC (auxiliary request 1). The decision on the validity of the claim to priority was not contested.

IV. With their statement setting out the grounds of appeal, appellant II contested the opposition division's decision under Articles 123(2), 83, 54 and 56 EPC on the claim request held allowable.

V. With the reply to appellant II's appeal, appellant I filed three documents.

VI. Appellant II submitted a reply to appellant I's appeal.

VII. The board summoned the parties to oral proceedings and, in a communication pursuant to Article 15(1) RPBA, informed them of its preliminary opinion on some of the issues in the appeal.

VIII. Oral proceedings took place as scheduled in the form of a videoconference as requested by appellant II and not objected to by appellant I. At the oral proceedings, appellant I withdrew the main request and renamed auxiliary requests 1 to 8 the main request and auxiliary requests 1 to 7, respectively.

At the end of the oral proceedings, the Chairman announced the board's decision.

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

"1. A method of producing a protein in a cell culture comprising:
(a) growing cells in the cell culture at a reduced temperature, wherein the reduced temperature is in a range of 27.0°C to less than 30.0°C; and
(b) growing cells in the cell culture at a reduced pH, wherein the reduced pH is 6.95;
to reduce production of misfolded proteins and/or aggregated proteins,
wherein the cell culture is a CHO cell culture,
wherein the protein produced is a soluble receptor
wherein said soluble receptor is TNFR-Fc."

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

"1. A method of producing a protein in a cell culture comprising:
(a) growing cells in the cell culture at a reduced temperature, wherein the reduced temperature is 29.5°C; and
(b) growing cells in the cell culture at a reduced pH, wherein the reduced pH is in a range of 6.80 to less than 7.00;
to reduce production of misfolded proteins and/or aggregated proteins,
wherein the cell culture is a CHO cell culture,
wherein the protein produced is a soluble receptor
wherein said soluble receptor is TNFR-Fc.

Claim 1 of **auxiliary request 2** corresponds to the request held allowable by the opposition division. It reads (differences to the main request highlighted by the board):

"1. A method of producing a protein in a cell culture comprising:
(a) growing cells in the cell culture at a reduced temperature, wherein the reduced temperature is 29.5°C; and
(b) growing cells in the cell culture at a reduced pH, wherein the reduced pH is 6.95;
to reduce production of misfolded proteins and/or aggregated proteins,
wherein the cell culture is a CHO cell culture,
wherein the protein produced is a soluble receptor
wherein said soluble receptor is TNFR-Fc.

Claim 1 of **auxiliary request 3** has the same wording as claim 1 of auxiliary request 2, except for step (b), which reads as follows (difference to auxiliary request 2 highlighted by the board)

"(b) growing cells in the cell culture at a reduced pH, wherein the reduced pH is a pH set point of 6.95".

Claim 1 of **auxiliary request 4** reads (differences to the main request highlighted by the board):

"1. A method of producing a protein in a large-scale cell culture comprising:
(a) growing cells in the cell culture at a reduced temperature, wherein the reduced temperature is in a range of 27.0°C to less than 30.0°C; and
(b) growing cells in the cell culture at a reduced pH, wherein the reduced pH is 6.95;
to reduce production of misfolded proteins and/or aggregated proteins,
wherein the cell culture is a CHO cell culture,

wherein the protein produced is a soluble receptor
wherein said soluble receptor is TNFR-Fc,
and wherein the volume of the cell culture is at least 10L."

Claim 1 of **auxiliary requests 5 to 7** has the same wording as claim 1 of auxiliary requests 1 to 3, respectively, except for the inclusion of the same amendments highlighted for auxiliary request 4.

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

D1: US 7,294,481 B1

D12: Declaration by Gregory Hiller dated 12 September 2019

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

Main request and auxiliary requests 1 and 2 - Claim 1 Inventive step - Article 56 EPC

Document D1 disclosed methods of producing TNFR-Fc by cell culture. It disclosed in general terms pH ranges such as 6.6 to 7.4 and 6.8 to 7.2 but did not disclose pH 6.95. It did not disclose the temperature 29.5°C either. It disclosed the temperatures 30°C and 34°C without, however, reference to a pH value or even a range of pH values. Example 2 defined a temperature range for the cell culture of cell line VA12 at pH 7.0. Example 3 described the analysis of cell culture supernatants, including of cell line VA12 culture at 30°C without, however, any direct reference to

example 2. Moreover, example 2 used present tense, whereas example 3 used the past tense. Thus, each of examples 2 and 3 had to be read on its own, and there was no disclosure of a cell culture method with combined 30°C and pH 7.0. The closest prior art was thus a method carried out at pH 7.0 at a temperature within the range 28 to 34°C.

The claimed method differed from this prior art on account of the temperature and pH values.

The experimental results in the patent showed that the temperature and pH each had a substantial effect on protein misfolding and aggregation and on sialylation. Example 1.1 described cell culture production of TNFR-Fc with a CHO cell line at different temperatures. Figure 5 showed a decrease in product misfolding and aggregation with the decrease in temperature, with a substantial decrease between 30°C and 29°C. Figure 6 showed that product sialylation was very sensitive to temperature with, however, only a slight decrease from 30°C to 29°C and an abrupt decrease only below 29°C. Thus, example 1.1 showed that the culture temperature of 29.5°C balanced the beneficial effect of glycosylation and the unfavourable effects of misfolding and aggregation. Example 1.2 described cell culture at 30°C and different pH set points, including 6.9 and 7.0. It showed an optimal pH set point for producing TNFR-Fc at 6.95.Figures 13A and 13B showed the most dramatic decrease in product misfolding to be from pH 7.0 to pH 6.9, and Figures 14A and 14B showed that the minimum pH still resulting in approximately 100% sialylation was pH 6.9. Although there were no experiments at a pH set point of 6.95, it could be seen from Figure 11A that at the set point of 6.9 the pH drifted upwards. Therefore, it could from

this data be concluded that 6.95 was a good choice of pH (see also patent, page 15, lines 28 to 32). In the experimental set up used to study the effect of pH, there was no pH control above the set point to avoid interference of CO_2 addition on the properties of the product. Nevertheless, the experiments allowed directly linking the technical effect observed to the pH (see declaration D12).

In view of these technical effects, the objective technical problem was the identification of an improved method for producing TNFR-Fc where the proportion of misfolded and/or aggregated proteins is reduced while having minimum effect on glycosylation of TNFR-Fc.

Document D1 provided no indication of the detrimental effect of temperature on sialylation. Therefore, it would not have led the skilled person to seek a balance between product sialylation and aggregation/misfolding. The skilled person would instead have used the temperature 28°C preferred in this document. There was likewise no indication in this document of an effect of pH on the product properties sialylation and aggregation/misfolding. Hence, the claimed solution was not obvious.

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

Main request and auxiliary requests 1 and 2 - Claim 1 Inventive step - Article 56 EPC

Document D1 represented the closest prior art. It disclosed a method of producing TNFR-Fc in cell culture at 30°C and pH 7.0 (see example 3). Example 1 was

essential for all the experiments described. Example 2 concerned culture conditions for each cell line disclosed in example 1. Example 3 characterised the supernatants of the cell culture in example 2, which had been carried out with the cell lines of example 1. Thus, the examples in document D1 had to be read in combination because each example built on the previous one. Example 2B disclosed experiments at pH 7.0 and a temperature range of 28 to 34°C. Example 3 indicated only the temperature, namely 30°C for VA12 samples. It referred to the experiments of example 2, and therefore the pH was 7.0. This reading was consistent with claim 32, which indicated a range of temperatures but a single pH value of 7.0.

No data were available allowing a direct comparison between a culture at 29.5°C and pH 6.95, on the one hand, and the method disclosed in document D1, i.e. 30°C and pH 7.0, on the other hand. Moreover, in the experiments in the patent, both temperature and pH were varied. Thus, from the experimental results, no conclusion could be drawn on the effect of temperature alone on product misfolding and/or aggregation and sialylation. The pH of the cell culture differed between the cultures carried out at 29°C and 30°C (see paragraph [0080] of the patent). Moreover, no difference in sialylation was observed between the cultures at 29 and 30°C (see Figures 6A and 6B and error bars in Figure 6A). Thus, no technical effect could be attributed to a cell culture at 29.5°C versus one at 30°C. According to appellant I, the product properties were very sensitive to minor pH and temperature fluctuations. Therefore, conclusions on a technical effect associated with pH 6.95 could not be drawn from data obtained with very significant fluctuations in pH. Moreover, the pH fluctuation was

more significant at pH 7.0 than at pH 6.9 (see Figure 11A). Indeed, document D12 pointed to an effect of pH control on product quality, in agreement with appellant I's argument that there were technical reasons for carrying out the experiments in the patent in the absence of pH control above the set point.

Therefore, the objective technical problem was to be formulated as the provision of an alternative method of producing TNFR-Fc.

The temperature and pH values in the claim were arbitrary choices out of the ranges disclosed in document D1. There was no reason to select pH 6.95 over 6.94 or 6.96. As regards the temperature, 29°C seemed to be best since it resulted in reduced misfolding versus 30°C, without a decrease in sialylation (see Figures 6B and 5). It was not apparent why 29.5°C had been chosen instead. Therefore, the claimed method did not involve an inventive step.

Even if the problem were formulated as the provision of a method resulting in a slightly reduced proportion of misfolded product, the solution would be obvious in view of the disclosure of document D1, which taught that reduced temperature resulted in reduced misfolding.

XIII. Appellant I requested that the decision under appeal be set aside and the patent be maintained on the basis of the set of claims of the main request or, alternatively, any of auxiliary requests 1 to 7, filed as auxiliary requests 1 to 8, respectively, with the patent proprietor's statement setting out the grounds of appeal.

Appellant II requested that the decision under appeal be set aside and the patent be revoked in its entirety. Furthermore, it requested that auxiliary requests 1 and 5 (filed as auxiliary requests 2 and 6 with the patent proprietor's statement of grounds of appeal) not be admitted into the appeal proceedings.

Reasons for the Decision

Auxiliary requests 1 and 5 - Admittance into the appeal proceedings

1. Although admittance of these sets of claims was contested by appellant II, there is no need to give reasons for their admittance since, for the reasons given below, the requests could not be allowed.

Main request and auxiliary requests 1 and 2 - Claim 1 Inventive step - Article 56 EPC

2. Claim 1 of the main request is directed to a method of producing the protein TNFR-Fc in cell culture comprising steps (a) and (b). Claim 1 of the main request and auxiliary requests 1 and 2 differ only in the temperature and pH values used in the cell culture. Claim 1 of auxiliary request 2 defines a method involving cell culture at 29.5°C and pH 6.95. This embodiment is encompassed by claim 1 of the main request and auxiliary request 1. In the following, this

is the subject-matter being analysed when the board refers to claim 1.

Closest prior art

3. It was not contested in the appeal proceedings that document D1 is comprised in the state of the art according to Article 54(2) EPC.

4. It is undisputed that document D1 represents the closest prior art for the claimed method.

5. Document D1 concerns the optimisation of mammalian cell culture for reducing misfolding of recombinant proteins, in particular TNFR-Fc (see column 3, first paragraph). It contains a general disclosure in which the production phase is carried out at a temperature within the range 28 to 34°C and a pH of about 7.0 (see claim 32). Example 1 describes the preparation of two cell lines transfected with vectors for expression of TNFR-Fc: cell line 2A5-3 and cell line VA12. Example 2 describes the culture conditions for production of TNFR-Fc using each of the two cell lines of example 1. In both cases, the temperature at the production phase is indicated as a range: 28 to 34°C. The pH is indicated to be 7.2 for cell line 2A5-3, and 7.0 for cell line VA12. Example 3 discloses the analysis of the product in the supernatant of some of the cell cultures of example 2, namely, the cell culture at 34°C for cell line 2A5-3 and at 30°C for cell line VA12. Therefore, for the cell line VA12, this document discloses a method of producing TNFR-Fc in cell culture at 30°C and pH 7.0.

6. Appellant I submitted that document D1 did not disclose cell culture at 30°C in combination with pH 7.0.

Specifically, example 3 did not refer to the experiments described in example 2, so that the temperature 30°C referred to in example 3 was not disclosed in combination with pH 7.0 of the cell culture in example 2. The examples were to be read in isolation. The fact that example 2 used the present tense whereas examples 3 and following used the past tense was an indication that each example was to be read on its own.

7. The board does not agree with this reading of document D1. Rather, the board reads the examples as describing a sequence of inter-related experiments. The board notes that example 2 describes cell culture using the cell lines described in example 1 and that example 3 describes the analysis of cell culture supernatants of those same cell lines. Example 3 reads as follows: "*Characterization of Supernatants Supernatant samples from both the 2A5-3 and VA12 cell lines in which the production culture was operated at 34° C for 2A5-3 samples and 30° C for VA12 samples were protein A purified using* [...]" (see column 19). There is no indication of the pH of the cell culture in example 3. Nevertheless, even in absence of a direct reference, in the board's view, this passage unambiguously concerns the cell cultures described in example 2. When reading this passage, the skilled person immediately understands that the temperature range in example 2 refers to different experiments at discrete temperatures within this range and that the temperature in example 3 identifies the experiment selected out of those, for each cell line, for product analysis. This is also consistent with the absence in example 3 of any indication of the pH value for the cell culture since the value for this parameter is already indicated in example 2.

Objective technical problem

8. Thus, the difference between the subject-matter of claim 1 and the disclosure in document D1 lies in the cell culture temperature of 29.5°C instead of 30°C and the pH of 6.95 instead of 7.0.

9. It is undisputed that the patent does not contain experimental results obtained with cell culture at the temperature and pH values defined in claim 1.

10. It is also undisputed that for the cell culture experiments carried out at 29°C or 30°C, the pH was not controlled above the set point (see paragraphs [0057] and [0090] of the patent), and that for the pH set points 6.9 and 7.0, the actual measured pH of the culture was in fact higher (pH drift). Figure 11A shows that for the cell culture at 30°C, with pH set point 6.9, the pH drifted up over time to between 6.9 and 7.0, whereas for the pH set point 7.0, the cell culture pH drifted up to 7.2.

The values for product aggregation/misfolding and sialylation at 29°C and 30°C, as depicted in Figures 5 and 6, were thus obtained at a different pH than the initial set point. Therefore, the experimental results which according to appellant I show an effect of lowering the temperature from 30°C to 29°C, show in fact an effect of the combined change of temperature and pH.

The patent does not describe experiments showing the product properties as a function of the temperature, in

particular 29°C versus 30°C, at a constant pH. In fact, it was precisely at these temperatures that pH drift was larger (see Figure 7). Moreover, it can be seen from Figure 11A that also the extent of pH drift changed between the initial pH set points 6.9 and 7.0. The board further notes that according to appellant I, the product properties are very sensitive to pH. Therefore, any technical effect of the temperature 30°C versus 29°C in the examples of the patent cannot be isolated from an effect of changing the pH. Even if an effect on product properties could be attributed to the temperature of 29 versus 30°C at the respective pH 7.0 to 7.2, according to the examples, it can nevertheless not be inferred what the effect would be at 29.5°C and the lower pH 6.95 as in the method claimed. The board thus concludes that based on the experimental results in the patent, no technical effect can be attributed to 29.5°C versus 30°C or to the combination of 29.5°C with pH 6.95.

11. Appellant I argued that both pH and temperature have an effect on the product properties and that 29.5°C and pH 6.95 each are optimal values for the cell culture to produce TNFR-Fc. However, for the purpose of assessing the presence of an inventive step, it is necessary to establish a technical effect attributable to the difference between the 29.5°C in the claimed method and the 30°C disclosed in D1 or between pH 6.95 in the claimed method and 7.0 disclosed in D1. Such an effect based on the differences compared to the disclosure of document D1 has not been shown in the patent. Thus, the alleged optimisation of product properties cannot be taken into account for the formulation of the objective technical problem.

12. Therefore, at best the claimed method achieves the same technical effects as provided by the method known from the closest prior art. Accordingly, the objective technical problem may be formulated as the provision of an alternative method of producing TNFR-Fc in cell culture.

Obviousness

13. For the skilled person, it would have been obvious to provide an alternative method by varying the temperature and pH within the ranges disclosed in the prior art and in particular around values already used in combination in the examples in document D1. In conclusion, the subject-matter of claim 1 of the main request and auxiliary requests 1 and 2 does not involve an inventive step.

Auxiliary requests 3 to 7 - Claim 1
Inventive step - Article 56 EPC

14. The embodiment assessed above in the context of the main request and auxiliary requests 1 and 2 is encompassed by claim 1 of auxiliary request 3. While the parties were in disagreement as to whether the pH value in claim 1 of the main request referred to a set point, there was agreement that a set point allows for fluctuations around that point. In that sense, the reasons why no technical effect could be attributed to the values in claim 1, in particular in view of the pH drift in the experiments in the patent (see points 10. and 11. above), apply equally to claim 1 of auxiliary request 3.

15. Claim 1 of auxiliary requests 4 to 7 additionally includes the feature defining a large-scale culture of at least 10 L volume. However, no reasons were put forward in writing or during the oral proceedings as to how this feature contributed to an inventive step. To the contrary, appellant I stated that this feature was intended to address novelty objections based on a different document (D2) cited in the opposition proceedings (see appellant I's statement of grounds of appeal). Therefore, the conclusions under point 13. above apply equally to claim 1 of auxiliary requests 4 to 7.

16. The subject-matter of claim 1 of auxiliary requests 3 to 7 lacks an inventive step over the disclosure of document D1 (Article 56 EPC).

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The patent is revoked.

The Registrar: The Chairman:

I. Aperribay B. Rutz

Decision electronically authenticated