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# Datasheet for the decision of 8 July 2024

**Case Number:** T 1616/22 - 3.3.04

**Application Number:** 12721821.2

**Publication Number:** 2707387

**IPC:** C07K14/755, C12N5/00

**Language of the proceedings:** EN

**Title of invention:**
A method of increasing the productivity of eucaryotic cells in the production of recombinant FVIII

**Patent Proprietor:**
Octapharma AG

**Opponent:**
WALLINGER RICKER SCHLOTTER TOSTMANN

**Headword:**
FVIII production/OCTAPHARMA

**Relevant legal provisions:**
EPC Art. 56

**Keyword:**
Inventive step - effect not made credible within the whole scope of claim - obvious alternative

**Decisions cited:**
G 0002/88, G 0006/88

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**Case Number: T 1616/22 - 3.3.04**

# D E C I S I O N
# of Technical Board of Appeal 3.3.04
# of 8 July 2024

| | |
|---|---|
| **Appellant:** (Opponent) | WALLINGER RICKER SCHLOTTER TOSTMANN<br>Patent- und Rechtsanwälte Partnerschaft mbB<br>Zweibrückenstraße 5-7<br>80331 Munich (DE) |
| **Representative:** | Zwicker Schnappauf & Partner<br>Patentanwalte PartG mbB<br>Hansastraße 32<br>80686 Munchen (DE) |
| **Respondent:** (Patent Proprietor) | Octapharma AG<br>Seidenstraße 2<br>8853 Lachen (CH) |
| **Representative:** | Ullrich & Naumann PartG mbB<br>Schneidmühlstraße 21<br>69115 Heidelberg (DE) |
| **Decision under appeal:** | **Decision of the Opposition Division of the European Patent Office posted on 19 April 2022 rejecting the opposition filed against European patent No. 2 707 387 pursuant to Article 101(2) EPC** |

**Composition of the Board:**

| | |
|---|---|
| **Chairwoman** | S. Albrecht |
| **Members:** | B. Rutz |
| | M. Blasi |

## Summary of Facts and Submissions

I. The appeal by the opponent (appellant) lies from the decision of the opposition division to reject the opposition against European patent No. 2 707 387, entitled "*A method of increasing the productivity of eucaryotic cells in the production of recombinant FVIII*".

II. The opposition proceedings were based on the grounds of Article 100(a) EPC, in relation to novelty (Article 54 EPC) and inventive step (Article 56 EPC), and Article 100(b) and (c) EPC.

III. In the decision under appeal, the opposition division concluded, *inter alia*, that the subject-matter of the claims of the patent as granted was inventive when starting from the disclosure of document D1 as the closest prior art, taken alone or in combination with common general knowledge as evidenced by document D8, or in combination with any of documents D3, D4, D5, D7 and D27.

IV. With the reply to the statement of grounds of appeal, the patent proprietor (respondent) resubmitted sets of claims of auxiliary requests 1 to 10 filed during the opposition proceedings.

V. The board summoned the parties to oral proceedings, as requested.

VI. In a communication under Article 15(1) RPBA, the board provided its preliminary view on the interpretation of claim 1 of the main request and addressed, *inter alia*, inventive step of the subject-matter of this claim. The

board furthermore indicated that it saw no reason not to admit auxiliary requests 1 to 10 into the proceedings.

VII. Claim 1 of the main request (patent as granted) reads as follows:

"1. A method of increasing the productivity, in particular cell-specific productivity, of recombinant factor VIII (rFVIII) produced in a eukaryotic cell suspension during culturing of said eukaryotic cell suspension in a culturing medium containing not more than 500 µM $CaCl_2$, at least a non-ionic detergent and other nutrient components needed for the cells to grow and produce rFVIII, **characterized in that** the said cell suspension is a suspension of human cells and is cultured under conditions inducing a shear stress by mechanical means directly to the suspension of human cells by adding a power density of 50 $W/m^3$ to 1000 $W/m^3$."

Claim 1 of auxiliary request 1 differs from claim 1 of the main request in that the term "human cells" is replaced by the term "human embryonic kidney (HEK) 293 cells".

Claim 1 of auxiliary request 2 differs from claim 1 of auxiliary request 1 in that the optional feature "in particular cell-specific productivity" has been made mandatory.

Claim 1 of auxiliary request 3 differs from claim 1 of auxiliary request 2 in that the wording "by performing a mechanical movement of the cell suspension by means of a rotating element such as a stirrer, propeller or impeller" is added at the end of the claim.

Claim 1 of auxiliary request 4 differs from claim 1 of auxiliary request 3 in that the term "recombinant factor VIII" is replaced by the term "recombinant B-domain deleted factor VIII (rBDD-FVIII)".

Claim 1 of auxiliary request 5 differs from claim 1 of auxiliary request 4 in that the power density range is restricted to a range of 113 to 610 $W/m^3$ and in that the mechanical movement of the cell suspension is performed by a rotating impeller.

Claim 1 of each of auxiliary requests 6 to 10 differs from claim 1 of each of auxiliary requests 1 to 5, respectively, in that the wording "human embryonic kidney (HEK) 293 cells" is replaced by the wording "human embryonic kidney (HEK) 293 F cells".

VIII. Oral proceedings took place before the board on 8 July 2024 in the presence of both parties. At the end of the oral proceedings, the chairwoman announced the board's decision.

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

D1 M. P. Kolind et al., "*Optimisation of the Factor VIII yield in mammalian cell cultures by reducing the membrane bound fraction*", Journal of Biotechnology 151(4), 2011, 357-62

D3 WO 2010/034428 A2

D4 WO 2011/012725 A1

D5 R. S. Senger et al., "*Effect of Shear Stress on Intrinsic CHO Culture State and Glycosylation of Recombinant Tissue-Type Plasminogen Activator Protein*", Biotechnology Progress 19(4), 2003, 1199-209

D6 Y. S. Tsao et al., "*Development and improvement of a serum-free suspension process for the production of recombinant adenoviral vectors using HEK293 cells*" Cytotechnology 37, 2001, 189-98

D7 A. W. Nienow, "*Reactor engineering in large scale animal cell culture*", Cytotechnology 50, 2006, 9-33

D8 P. Czermak et al., "*Special Engineering Aspects*", Cell and Tissue Reaction Engineering: Principles and Practice, 2009, 83-172

D12 Anonymous, "*Optimum Growth$^{TM}$ FLASKS*", Thomson Instrument Company, distributed by INFORS HT (infors-ht.com)

X. The appellant's submissions relevant to this decision can be summarised as follows.

*Main request - claim 1*
*Claim interpretation*

The cell culture medium in accordance with the claim contained $CaCl_2$ up to a limit of 500 µM. This meant that the medium could comprise no $CaCl_2$, i.e. the presence of $CaCl_2$ was optional.

"Shear stress" resulted from the mechanical force induced by the friction of fluid against the cell membrane. It was present to at least some degree in every cell culture in which there was any sort of

movement (e.g. suspension cell cultures, stirring cultures, etc.). The claimed method encompassed any non-zero amount of shear stress.

The actual level of shear stress was not indicated in the claim, and it could not be defined by the power density in $W/m^3$ alone. The range of power density did not directly correlate to the amount of shear stress.

This was apparent from Figure 4.13 in document D8, which showed that even when the power density was kept constant, there was a relative increase of shear stress when the relative vessel volume was increased. As a result, a certain amount of power density did not correlate with a certain amount of shear stress unless many other factors, including the volume of the vessel, were taken into account.

The impact of these factors was not negligible. At the left end of the curve, the shear ratio was 1. The shear stress increased 4.6 fold (this value being determined on the basis of the relationship $T \sim V^{2/9}$) when the volume was increased 1000 fold.

The range of power density specified in claim 1 could not be correlated with a numerical range of shear stress, meaning that it could not be correlated with an increase in productivity.

*Main request - claim 1*
*Inventive step (Article 100(a) in conjunction with Article 56 EPC)*

Document D1 described the use of Freestyle 293 Expression Medium™ (Invitrogen) without any defined $CaCl_2$ content for the expression of FVIII in HEK 293

suspension cell cultures. It did not disclose the power density of 50 to 1000 $W/m^3$.

No technical effect associated with the claimed $CaCl_2$ concentration in the culture medium was derivable from the patent.

The patent did not provide any explanation as to what technical effect apart from reaching an undefined amount of shear stress (see paragraph [0019] of the patent) was achieved with the specified power density.

The claim recited a method of "*increasing the productivity*" without specifying what the increase was compared to. As shown in Table 1 and Example 4 of the patent, an increase was achieved even below 50 $W/m^3$.

The indicated power density range was an arbitrary selection of a subrange of a power density required for an increase as claimed.

Although it was not specifically disclosed in D1 how much power was introduced into the suspension cell culture via the shaker, it was apparent that the shaker in D1 introduced a power density larger than zero. A power density larger than zero had an immediate effect on rFVIII productivity. Hence, shaking of the suspension culture in D1 achieved the same technical effect as the claimed method.

The objective technical problem underlying the method of claim 1 was the provision of an alternative method for producing rFVIII.

The only step remaining for the skilled person starting from the disclosure of document D1 was to identify an

(alleged) optimum of shear stress by adjusting the shaking intensity of the cell culture and thus inherently the power input. Identifying an optimum within the boundaries of given parameters did not require any inventive activity.

Document D8 reflecting common general knowledge disclosed that the reactor should be stirred to achieve a mean energy dissipation rate (i.e. the power density) of not more than 1000 $W/m^3$. Other prior-art documents also disclosed power densities for suspension cultures of mammalian cells within the claimed range (see e.g. D3, D4, D5 and D7).

The skilled person following established culturing methods would therefore have considered these teachings and arrived at the claimed method. The subject-matter thus did not involve any inventive activity.

*Auxiliary request 5 - claim 1*
*Inventive step (Article 56 EPC)*

HEK 293 cells and a B-domain deleted factor VIII had been used in document D1. Mechanical rotating means such as a stirrer, propeller or impeller were routine in the field of cell culture. The more limited range of power densities was not associated with any effect.

*Auxiliary requests 1 to 4 and 6 to 10 - claim 1*
*Inventive step (Article 56 EPC)*

The features added to the claims of auxiliary requests 1 to 4 and 6 to 10 did not contribute to an inventive step. HEK 293 cells, cell-specific productivity, B-domain deleted rFVIII and a rotating element (e.g. shaker platform) were disclosed in document D1.

Selecting a rotating impeller was obvious. The more limited range of power densities was not associated with any technical effect.

The claimed subject-matter lacked an inventive step.

XI. The respondent's submissions relevant to this decision can be summarised as follows.

*Main request - claim 1*
*Claim interpretation*

"[I]*nducing a shear stress by mechanical means directly to the suspension of human cells*" was a "qualitative feature", i.e. no specific level of shear stress was defined in the claim.

"[A]*dding a power density of 50 W/m$^3$ to 1000 W/m$^3$*" was not an unusual parameter. The numerically defined range of power densities defined in the claim and required to increase the rFVIII productivity within the suspension of human cells had to be considered separately from the qualitative requirement of a presence of a non-zero amount of shear stress.

*Main request - claim 1*
*Inventive step (Article 100(a) in conjunction with Article 56 EPC)*

Document D1 neither disclosed nor suggested (a) the addition of a power density in the claimed range nor (b) that the calcium concentration in the culturing medium should be not more than 500 µM.

The differences between the claimed invention and the method disclosed in document D1 allowed producing

rFVIII in human cells with the correct post-translational modifications and without the need to remove potentially toxic/allergenic components that might have been added during production.

The objective technical problem was the provision of an improved method to increase the productivity of rFVIII in a eukaryotic cell suspension during culturing of this suspension.

There was no pointer in document D1 to change the culturing conditions to increase rFVIII productivity. A different but disadvantageous means was used in document D1, namely adding certain ingredients prior to cell harvest to decrease the membrane-bound fraction of rFVIII. Other cited documents were either not relevant or would have led the skilled person in a different direction (D4, ADAMTS13 expression in hamster CHO cells; D5, r-tPA in CHO cells; D6, low calcium insufficient for HEK 293 expression system; D7, animal cells, e.g. BHK or insect cells; D8, unspecified hybridoma cells).

*Auxiliary request 5 - claim 1*
*Inventive step (Article 56 EPC)*

The claim was limited to a particular cell type ("human embryonic kidney (HEK) 293 cells"), a particular mechanical means ("rotating impeller"), a B-domain deleted factor VIII (rBDD-FVIII) and a restricted range of power densities ("113 $W/m^3$ to 610 $W/m^3$") corresponding to the end values tested in Example 3 of the patent. The combination of these features achieved an increased productivity of rFVIII. The prior art contained no pointer to this combination of features.

*Auxiliary requests 1 to 4 and 6 to 10 - claim 1*
*Inventive step (Article 56 EPC)*

Auxiliary requests 1 to 4 had been limited to HEK 293 cells, while auxiliary requests 6 to 10 had been limited to the HEK 293 F subtype. The arguments provided for the main request applied.

XII. The appellant requested that the decision under appeal be set aside and the patent be revoked. It further requested that:

(a) auxiliary requests 1 to 10 not be admitted into the proceedings

(b) documents D31, D33, D34, filed during opposition proceedings, and documents D35 to D40, filed in appeal, be admitted into the proceedings

XIII. The respondent requested that the appeal be dismissed and the decision to reject the opposition be upheld. Alternatively, it requested maintenance of the patent in amended form based on one of the sets of claims of auxiliary requests 1 to 10. It further requested that documents D31 to D40 not be admitted into the proceedings.

## Reasons for the Decision

*Admission of documents D31 to D40*

1. As documents D31 to D40, filed by the appellant, were not needed to reach the present decision, it was not necessary to decide on their admission.

*Main request - claim 1*
*Claim interpretation*

2. Claim 1 is directed to a method of increasing the productivity of recombinant factor VIII (rFVIII) characterised by:
(a) the composition of the cell culture:
- suspension of human cells
- in a culturing medium
- containing not more than 500 µM $CaCl_2$
- at least a non-ionic detergent
- other nutrient components needed for the cells to grow and produce rFVIII
and by:
(b) the cell culture conditions:
- inducing a shear stress by mechanical means directly to the suspension of human cells by adding a power density of 50 to 1000 $W/m^3$

3. Claim 1 further states that the purpose of the claimed method is to increase the productivity of "*recombinant factor VIII (rFVIII) produced in a eukaryotic cell suspension*". Increasing the productivity of a protein implies that the protein is produced.

4. The boards have consistently found that the criteria set out by the Enlarged Board of Appeal in G 2/88 and G 6/88 (OJ EPO 1990, 93 and 114) can be applied only to claims directed to the use of a substance for achieving an effect and cannot be extended to claims directed to a process for producing a product characterised by process steps where the purpose of carrying out the process steps is indicated in the claim (see Case Law of the Boards of Appeal of the EPO, 10th edn. 2022, I.C.8.1.3.a)).

5. "[I]*ncreasing the productivity, in particular cell-specific productivity, of recombinant factor VIII (rFVIII)*" therefore imposes no limitation on the method other than it being suitable to achieve an increase in productivity. Since there is no reference defined in the claim, any minimal increase in productivity compared to any reference method fulfils this criterion.

6. It has not been contested that "*the expression 'other nutrient components needed for the cells to grow and produce rFVIII' must be construed broadly, including even trivial ingredients such as 'water'*" (see decision under appeal, point 2.4.3.2).

7. It is also undisputed that applying a power density of 50 to 1000 $W/m^3$ (energy per volume) by mechanical means to a cell suspension in a culturing medium induces shear stress (see decision under appeal, point 2.4.6.2) as, in general, any mechanical movement in a cell culture does.

*Inventive step (Article 100(a) and Article 56 EPC)*
*Document D1 as the starting point*

8. Document D1 discloses expression of a B-domain deleted form of rFVIII (see page 358, left-hand column, last full paragraph) in human HEK 293 cells grown in suspension in Freestyle 293 Expression Medium™ (Invitrogen). Shear stress is induced by mechanical means directly to the suspension of the cells by shakers (see point 2.5 on page 359). The composition of the medium is not provided in document D1, but given the successful production of FVIII, it is undisputed that the medium contains "*nutrient components needed for the cells to grow and produce rFVIII*" as required

by the claim. It is also undisputed that Freestyle 293 Expression Medium™ comprises the non-ionic detergent Pluronic (see document D12, page 9, answer to question 7).

9. For the purpose of assessing inventive step, the board assumes, in favour of the respondent, that the feature "not more than 500 µM $CaCl_2$" is not disclosed in document D1 and thus represents a difference.

10. The parties agreed on the further difference to the disclosure of document D1 of "adding a power density of 50 $W/m^3$ to 1000 $W/m^3$".

*Effects and objective technical problem*

11. With regard to the first difference, "not more than 500 µM $CaCl_2$", the respondent argued that a culture medium with $CaCl_2$ in this concentration range prevented aggregation of the cells (see paragraph [0026] of the patent). Based on the state of the art as summarised, for example in document D6, it is credible that a reduced $CaCl_2$ concentration indeed prevents cell aggregation (see page 194, left-hand column, last paragraph: "*Reduction in calcium level was designed to minimize the HEK293 clumping which was shown to be calcium-dependent (Peshwa et al. 1993; Nadeau et al. 1996)*").

12. The respondent further argued that the reduced $CaCl_2$ concentration ("not more than 500 µM") in combination with the specified power density range resulted in increased productivity of rFVIII.

13. The board does not agree because there is no evidence to support an effect of the $CaCl_2$ concentration on this

productivity. Although productivity of rFVIII is measured in the examples of the patent (see Examples 2 and 3), the $CaCl_2$ concentration in the cell culture is not provided. The only $CaCl_2$ concentration indicated is 30 mM, which is applied for release of rFVIII from the cells and lies almost two orders of magnitude above the upper limit of the claimed range (see Examples).

14. The respondent argued that the skilled person would have recognised that the examples were carried out using a $CaCl_2$ concentration within the claimed range as this was also the only range mentioned in the description (see also decision under appeal, points 2.7.2.2 and 2.7.5.5).

15. Even assuming, in favour of the respondent, that this was the case, no comparison of different $CaCl_2$ concentrations, i.e. within and above the claimed concentration range, is provided in the patent. No effect on rFVIII productivity can therefore be derived for the $CaCl_2$ concentration range specified in the claim.

16. With regard to the second difference, "adding a power density of 50 $W/m^3$ to 1000 $W/m^3$", the respondent argued that applying this measure to a culture of rFVIII expressing human cells resulted in increased rFVIII productivity, as evidenced by the examples of the patent. Hence, it was not necessary to calculate or measure shear stress, nor to show a correlation between this shear stress and the power density added to the system.

17. The board disagrees. The only mechanistic explanation provided in the patent for an effect of increased power density on rFVIII productivity is based on increased

shear stress exerted on the cells by mechanical means. In paragraphs [0008] and [0009], prior-art studies which report a positive effect of shear stress on protein production of human cells are summarised. This sets the background for the claimed invention. Paragraph [0019] then states that: "*The shear stress is achieved by adding a power density of 50 W/m$^3$ to 1000 W/m$^3$ to the cell suspension. The conditions inducing a shear stress are events, which induce mechanical movements of the cell suspension or the cells in the suspension*". Paragraph [0038] points out that "*increased productivity can be achieved also in large-scale cultures by increasing the shear forces and energy input by increased stirring*" (see also paragraph [0039]). The relevance of "shear" or "shear forces" is further emphasised in the examples (see column 10, lines 4 to 7; column 10, lines 32 to 36) and by the wording of claim 1 (emphasis added by the board): "*inducing a shear stress <u>by</u> mechanical means <u>directly</u> to the suspension of human cells <u>by</u> adding a power density of 50 W/m$^3$ to 1000 W/m$^3$*".

18. The skilled person would therefore have taken from the patent that higher rFVIII productivity results from the increased shear stress experienced by the cells in the culture.

19. It is also undisputed that power input or density (equivalent to "*energy dissipation (rate) ε*", see paragraphs [0021] and [0030] of the patent) is not directly correlated with the amount of shear stress (see also point 2.4.6.1 of the decision under appeal).

20. It was furthermore common general knowledge at the relevant date that parameters such as the volume and form of the vessel, the type of mechanical means to

apply a power input (e.g. stirrer, shaker, propeller, impeller, centrifuge, sparger or hollow fibre unit) and the viscosity of the medium have an effect on the shear stress generated (see document D8, paragraph bridging pages 101 and 102).

21. As evidence for the influence of other parameters on shear stress, the appellant referred to document D8. Figure 4.13 on page 102 shows the correlation between shear stress (y-axis) and culture volume (x-axis) for different mechanical means at a constant power density. To allow comparison, the shear stress ratio and the volume ratio are plotted, i.e. both normalised to 1 ($T/T_0$ and $V/V_0$). This graph shows that a volume increase by a factor of 1000 for a stirred culture results in an increase of the shear stress by a factor of about 4 to 5 fold.

22. It was also common general knowledge that high shear stress results in cell damage and thus reduced productivity (see document D8, page 90, Table 4.1: "*Threshold shear stress*"). This is confirmed in the patent in paragraph [0021]: "*The power added directly to the cell suspension to introduce shear stress should not exceed a value where the cells are destroyed*". The examples of the patent also show that at higher stirring rates, the rFVIII productivity decreases (see Table 1 in Example 2).

23. In conclusion, it is not credible that at the higher end of the power density range defined in the claim an increase of the shear stress by 5 fold or more depending on the scale-up factor would be tolerated by the cells and result in a rFVIII productivity increase. It is therefore not credible that selecting a power

density in the upper end of the range gives rise to the alleged increase of rFVIII productivity.

24. On the other hand, in a larger vessel of 100 litres, a power density below the lower range defined in the claim (29 $W/m^3$) is shown to be sufficient to increase rFVIII production compared to an even lower power density (6 $W/m^3$) (see Table 2 in the patent). The lower end of the range, 50 $W/m^3$, is therefore not associated with an effect which would occur over the whole scope claimed, e.g. for any culture volume.

25. The respondent confirms that in the prior art "*the individual cellular response to power density addition must still be assessed on a case-by-case basis*" and that this depends on various parameters (see reply to the appeal, page 27). The patent, however, contains no data on the influence of any of these parameters, e.g. cell type, culture volume or mechanical means on the (cell-specific) productivity of rFVIII. Even the examples which the respondent considers to fall under the scope of the claim contain additional sources of power density, namely a sparger stone in Example 2 and a hollow fibre filter in Example 3 for which the contribution in terms of power density was not assessed.

26. During the oral proceedings, the respondent argued that it was up to the appellant to show that the effect was not achieved over the whole scope. The board agrees that it is usually up to the party that alleges a fact in opposition to provide evidence for it. However, if the patent proprietor alleges the fact that the claimed invention improves a technical effect, the burden of proof for that fact rests upon it (see also Case Law of the Boards of Appeal of the EPO, 10th edn. 2022, I.D.

4.3.1). In the case at hand, moreover, the appellant has provided, *inter alia,* document D8 representing common general knowledge to raise serious doubts that the effect can be achieved over the whole scope claimed. It is therefore up to the respondent to dispel these doubts.

27. In the decision under appeal, the opposition division considered that the claimed method was improved because document D1 required additional factors to reduce the membrane attachment of FVIII, i.e. von Willebrand Factor, Annexin V and ortho-phospho-L-serine (see sheet 16 of the decision under appeal, first and second full paragraph).

28. The board does not agree because the claim does not exclude the addition of supplementary factors to the medium ("*culturing medium containing* [...] *at least* [...]"). The claim is also not concerned with the distribution of FVIII between the soluble and the membrane fraction but only mentions "*increasing the productivity*". The absence of additional factors is therefore not a difference to the disclosure of document D1 and cannot result in an additional technical effect.

29. Summing up the above considerations, the board finds that the technical effect achieved by the claimed $CaCl_2$ concentration is the prevention of cell aggregation. However, an increase of rFVIII productivity by means of the claimed power density range in combination with the claimed $CaCl_2$ concentration cannot be accepted as being achievable over the whole scope of the claim. As a consequence, only the technical effect of preventing cell aggregation can be taken into account in the formulation of the objective technical problem posed.

30. The objective technical problem is thus the provision of a production method for rFVIII which prevents aggregation of human cells in suspension.

*Obviousness*

31. Adding a power density within the range of 50 to 1000 $W/m^3$ is disclosed in the state of the art for human cell suspension cultures (see e.g. documents D3, D4 or D8).

31.1 Document D3 discloses a power density of 56 $W\ m^{-3}$ for the production of rFVIII in HKB-11 (hybrid of kidney and B cell) cell line (page 17, line 4). The board does not agree with the respondent's argument that the cell line in document D3 was "sticky" (see page 15, lines 15 to 16: "*klebrige humane Hybrid-Zelllinie HKB-11*") and thus not representative for a suspension cell culture. The claim is not limited to any particular cell line, so any disclosure in the prior art for suspension cell culture would have been considered relevant by the skilled person.

31.2 Document D4 discloses power densities of "*about 50 $W/m^3$, about 60 $W/m^3$, about 70 $W/m^3$, about 80 $W/m^3$ or more*" (see paragraph [0031]) for the agitation of cultures of non-anchorage dependent mammalian cells, including 293 cells (see paragraph [0048]). Document D4 is not limited to the ADAMTS13 protein used in the examples, also mentioning other proteins, e.g. FVIII (see paragraph [0050]).

31.3 Document D8 discloses in a sub-chapter on "*Non-Anchorage Dependent Cells Grown in Suspension*" that "*cell damage on suspended hybridom cells grown in a*

*small stirred vessel was first observed at a stirrer speed of 300 rpm* [corresponding to a power density of 88 W/$m^3$]*; at 580 rpm* [above 400 W/$m^3$] *cell growth stopped completely*" (see Table 4.2 on page 97 and accompanying text) and concludes in general terms that "[i]*n stirred tanks the mean energy dissipation rate should be below ~ 1,000 W* $m^{-3}$" (see page 101, fifth bullet point).

32. Moreover, document D7, which relates to large-scale animal cell culture, discloses typical values of power densities for animal cell cultures of 0.01 to 0.15 W $kg^{-1}$ which corresponds to 10 to 150 W/$m^3$ (see page 12, right-hand column, first paragraph). It also states that with mean specific energy dissipation values of from 0.01 up to 0.24 W $kg^{-1}$ in baffled bioreactors with different impellers, a wide range of cells could be grown equally well (see page 13, paragraph bridging columns). The respondent argued that document D7 did not relate to human cell cultures. The board disagrees because the skilled person would consider human cells to be encompassed in the term "animal cells". Even though in document D7 only non-human mammalian cells are mentioned (e.g. CHO, BHK and mouse hybridoma), the skilled person would have considered the teaching relevant for human cells.

33. The skilled person looking for alternative production methods of rFVIII would thus have found indications of suitable power densities in the disclosure of documents D3, D4, D7 and D8. There was no reason for the skilled person to assume that these power densities which are within the claimed range could not be used in the method disclosed in document D1.

34. The effect of the indicated $CaCl_2$ concentration on cell aggregation (see point 11. above) was known from the prior art, in particular for HEK 293 cells, i.e. the cells used in the patent. Document D6 states in this regard:

> "*The basic formula of the medium used in this report (IS293-V from Irvine Scientific) contains a relatively low concentration of calcium ion (~108 µM) as opposed to ~2 mM in a typical DMEM medium. Reduction in calcium level was designed to minimize the HEK293 clumping which was shown to be calcium-dependent (Peshwa et al. 1993; Nadeau et al. 1996). In our experience, the calcium concentration can be as low as 10 µM without significant effect on cell growth or viability (data not shown).*" (page 194, left-hand column, last paragraph)
>
> And:
>
> "*Since higher concentrations of calcium cause increased levels of cell aggregation, which may at least in theory interfere with the virus infection, the time of calcium addition during subsequent development work was set at 2h post infection.*" (page 195, paragraph bridging columns)

35. The respondent argued that document D6 concerned the production of adenovirus in HEK 293 cells and would therefore not have been consulted by the skilled person concerned with rFVIII expression in human cells. The board does not agree because the state of the art summarised in document D6 provides general recommendations for the culture of HEK 293 cells and is not limited to adenovirus production (see titles of

references: Peshwa et al., 1993: "*Cultivation of mammalian cells as aggregates in bioreactors: effect of calcium concentration on spatial distribution of viability*" and Nadeau et al., 1996: "*Improvement of recombinant protein production with the human adenovirus/293S expression system using fed-batch strategies*").

36. In light of the preceding considerations, the board concludes that the subject-matter of claim 1 lacks an inventive step. The ground for opposition under Article 100(a) in conjunction with Article 56 EPC thus prejudices the maintenance of the patent as granted.

*Auxiliary requests 1 to 10*
*Admittance of auxiliary requests 1 to 10 (Article 12(4) RPBA)*

37. The sets of claims of auxiliary requests 1 to 10 are identical to those of auxiliary requests 1 to 10, respectively, submitted during the opposition proceedings but not dealt with in the decision under appeal. The board considered them to have been admissibly raised and maintained.

*Auxiliary request 5 - claim 1*
*Inventive step (Article 56 EPC)*

38. The initial argument by the respondent in oral proceedings that the features rBDD-FVIII and HEK 293 cells represented differences over the disclosure of document D1 was not maintained. Indeed, these features are disclosed in document D1 (see page 358, right-hand column, section 2.2 and page 359, left-hand column, section 2.5). The subject-matter of claim 1 thus differs from the disclosure of document D1 in the limitation of the $CaCl_2$ concentration, the use of a

rotating impeller and the specified range of power densities. The board has not been presented with any evidence that a rotating impeller achieved any technical effect compared to the shakers used in document D1. The obviousness of the limited $CaCl_2$ concentration has been addressed for the main request (see points 34. and 35. above).

39. The more restricted range of power densities defined in the claim suffers from the same problem as the broader range defined in claim 1 of the main request, namely that further parameters such as culture volume, vessel form, etc. are lacking meaning that achieving an increase in productivity over the whole claimed range is not credible (see points 16. to 25. above).

40. The objective technical problem is therefore the same as for the main request, and an obvious solution to this problem is provided in documents D8 and D7 (see points 31.3 and 32. above).

41. The subject-matter of claim 1 of auxiliary request 5 lacks an inventive step within the meaning of Article 56 EPC.

*Auxiliary requests 1 to 4 and 6 to 10 - claim 1*
*Inventive step (Article 56 EPC)*

42. In writing, the respondent referred to its submissions on inventive step of the main request (see reply, point III, pages 40 to 44). During the oral proceedings, the respondent referred to its written submissions.

43. Accordingly, the considerations for the main request or for auxiliary request 5 on inventive step equally apply to these auxiliary requests.

44. The subject-matter of claim 1 of each of auxiliary requests 1 to 4 and 6 to 10 therefore lacks an inventive step.

## Order

**For these reasons it is decided that:**

1. The decision is set aside.

2. The patent is revoked.

The Registrar: The Chairwoman:

I. Aperribay S. Albrecht

Decision electronically authenticated