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Datasheet for the decision of 11 March 2014

Case Number: T 1141/10 - 3.3.08

Application Number: 98204263.2

Publication Number: 926543

IPC: G03C1/005, G03C1/047, C07K14/78

Language of the proceedings: EN

Title of invention:
Silver halide emulsions with recombinant collagen suitable for photographic application and also the preparation thereof

Patent Proprietor:
Fuji Photo Film B.V.

Opponent:
Fibrogen Inc.

Headword:
Collagen like biopolymers Pichia pastoris/FUJI

Relevant legal provisions:
EPC Art. 83, 54, 56
RPBA Art. 12(4), 13(1)

Keyword:
Main Request:
Sufficiency of disclosure (yes), novelty and inventive step (no)
Auxiliary Requests 1-2, 6-7 and 9 - admissibility (no need to consider)
Auxiliary Requests 3-5, 8 and 10-12 - admissibility (no)

Decisions cited:
T 0455/91, T 0098/07, T 0608/07, T 0593/09

Catchword:

Europäisches Patentamt
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Case Number: T 1141/10 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 11 March 2014

Appellant: (Patent Proprietor)	Fuji Photo Film B.V. P.O. Box 80150 5000 LJ Tilburg (NL)
Representative:	Meekel, A.A.P. Nederlandsch Octrooibureau P.O. Box 29720 2502 LS The Hague (NL)
Respondent: (Opponent)	Fibrogen Inc. 225 Gateway Boulevard South San Francisco CA 94080 (US)
Representative:	Wise, D.J. Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)
Decision under appeal:	**Decision of the Opposition Division of the European Patent Office posted on 19 March 2010 revoking European patent No. 926543 pursuant to Article 101(3)(b) EPC.**

Composition of the Board:

Chairman: B. Stolz
Members: P. Julià
D. Rogers

Summary of Facts and Submissions

I. An opposition based upon Articles 100(a),(b) and (c) EPC was filed against European patent No. 0 926 543. The opposition division considered the main and sole request to contravene Article 56 EPC and, accordingly, revoked the patent.

II. An appeal was filed against this decision of the opposition division (hereinafter, "*the First Appeal*"), which led to decision T 98/07 of 28 November 2008. The Board of Appeal 3.3.06 decided to set aside the decision of the opposition division, to admit into the proceedings a new Main Request and Auxiliary Requests 1-10 filed at oral proceedings before the board, and to remit the case to the opposition division for further prosecution (Article 111 EPC).

III. In a decision dated 19 March 2010, the opposition division considered a new Main Request and Auxiliary Request 1 to contravene Article 54 EPC and Auxiliary Requests 5-6 and 8 to contravene Article 56 EPC. Auxiliary Requests 2-4, 7 and 9 were withdrawn at the oral proceedings before the opposition division. Accordingly, the patent was revoked. Except for the Main Request filed on 2 December 2009, all these requests were filed on 2 February 2010.

IV. A second appeal was lodged by the patentee (appellant) against the decision of the opposition division. This second appeal is the subject matter of the present appeal proceedings. With the statement of Grounds of Appeal, the appellant filed a new Main Request and Auxiliary Requests 1 to 12.

V. The opponent (respondent) replied to the appellant's statement of Grounds of Appeal.

VI. The board summoned the parties to oral proceedings and, in a communication pursuant to Article 15(1) of the Rules of Procedure of the Boards of Appeal (RPBA) annexed to the summons, informed the parties of its preliminary opinion on substantive issues of the case.

VII. The appellant replied to the board's communication and filed new documentary evidence (documents D35 and D36, *infra*). No substantive submissions were filed by the respondent in reply to the board's communication.

VIII. Oral proceedings took place on 11 March 2014 in the presence of both appellant and respondent.

IX. Claims 1 and 9 of the **Main Request** read as follows:

"1. A process of producing recombinant collagen like polypeptide comprising expression of a collagen like polypeptide encoding nucleic acid sequence by a methylotrophic yeast to a degree exceeding 0.95 gram/ liter, wherein the methylotrophic yeast is free of active post translational processing mechanism for processing collagen like sequences to fibrils, said nucleic acid sequence being free of procollagen and telopeptide encoding sequences and encoding a polypeptide having more than 4 different amino acid types, said recombinant collagen being free of helix structure and of hydroxyproline."

"9. A substantially pure, recombinant collagen like material being free of helix structure prepared by genetic engineering of native collagen encoding nucleic acid, said peptizer having an amino acid sequence

equivalent to that occurring in nature for collagen, wherein equivalent implies amino acid identity of at least 80% and wherein said collagen occurring in nature is collagen type I, II or III, comprising more than 4 different amino acid types, being free of hydroxyproline, having a weight on an amino acid basis of 2.5-100 kDa, and said peptizer being free of procollagen and telopeptides."

Claims 2 to 8 were directed to preferred embodiments of the process of claim 1. Claim 5 defined the methylotrophic yeast as being selected from *Hansenula*, preferably *Pichia pastoris*.

X. The claims of **Auxiliary Request 1** were identical to the claims of the Main Request, except for claim 9 which was identical to claim 9 of the Main Request but had the range of the weight on an amino acid basis of "*2.5-100 kDa*" (Main Request) replaced by "*10-100 kDa*" (Auxiliary Request 1).

XI. Claim 1 of **Auxiliary Request 2** read as follows:

"1. A process of producing recombinant collagen like polypeptide comprising expression of a collagen like polypeptide encoding nucleic acid sequence by Pichia pastoris, wherein the Pichia pastoris is free of active post translational processing mechanism for processing collagen like sequences to fibrils, to a degree exceeding 0.95 gram/liter, said nucleic acid sequence being free of procollagen and telopeptide encoding sequences and encoding a polypeptide having more than 4 different amino acid types, said recombinant collagen being free of helix structure and of hydroxyproline."

Claims 2 to 7 were directed to preferred embodiments of the process of claim 1. Claim 8 was identical to claim 9 of the Main Request.

XII. Claim 1 of **Auxiliary Request 3** was identical to claim 1 of Auxiliary Request 2 except for the additional feature "*and being free of a sequence encoding MGPR*" after the sentence "*being free of procollagen and teleopeptide encoding sequences*". Claim 8 of this Auxiliary Request was identical to claim 8 of Auxiliary Request 2, except for the presence of the same additional feature after the sentence "*being free of hydroxyproline*". Claims 2 to 7 were directed to preferred embodiments of claim 1 and were identical to claims 2 to 7 of Auxiliary Request 2.

XIII. The claims of **Auxiliary Request 4** were identical to the claims of Auxiliary Request 2 except for claim 8 which was identical to claim 9 of Auxiliary Request 1.

XIV. The claims of **Auxiliary Request 5** were identical to the claims of Auxiliary Request 3 except for claim 8 which was identical to claim 8 of Auxiliary Request 3 but had the range of the weight on an amino acid basis of "*2.5-100 kDa*" (Auxiliary Request 3) replaced by "*10-100 kDa*" (Auxiliary Request 5).

XV. **Auxiliary Requests 6, 7 and 8** were identical to the Main Request and to Auxiliary Requests 2 and 3, respectively, except for the absence of any product claim in Auxiliary Requests 6-8, i.e. deletion of claim 9 of the Main Request and claim 8 of Auxiliary Requests 2 and 3.

XVI. **Auxiliary Requests 9 to 12** contain each a single claim only. Claim 1 of Auxiliary Requests 9 and 11 were

identical to claim 9 of the Main Request and Auxiliary Request 1, respectively. Claim 1 of Auxiliary Requests 10 and 12 were identical to claim 8 of Auxiliary Requests 3 and 5, respectively.

XVII. The following documents are cited in this decision:

D1: US 5,580,712 (publication date: 3 December 1996);

D3: WO 93/07889 (publication date: 29 April 1993);

D6: WO 97/38710 (publication date: 23 October 1997);

D10: A. Vuorela et al., The EMBO J., Vol. 16, No. 22, 17 November 1997, pages 6702 to 6712;

D18: J.M. Cregg et al., Mol. Biotechnol., Vol. 16, 2000, pages 23 to 52;

D24: C.P. Hollenberg and G. Gellissen, Current Opinion in Biotechnology, Vol. 8, October 1997, pages 554 to 560;

D25: F.A. de Wolf and R.C.A. Keller, Progr. Colloid Polym. Sci., Vol. 102, 1996, pages 9 to 14;

D31: GPS. Raghava and G.J. Barton, BMC Bioinformatics, Vol. 7, 2006, pages 415 to 418;

D35: G.J. Barton and M.J.E. Sternberg, Protein Engineering, Vol. 1, No. 2, 1987, pages 89 to 94;

D36: G. Vogt et al., J. Mol. Biol., Vol. 249, 1995, pages 816 to 831.

XVIII. Appellant's submissions, insofar as they are relevant to the present case, may be summarized as follows:

Admissibility of new documentary evidence

Documents D35 and D36 were filed in reply to the board's opinion expressed in its communication under Article 15(1) RPBA. Both documents were cited in document D31 in the context of the PID2 method and they only illustrated the criteria for using this method.

Main Request
Article 83 EPC

Methods were described in the prior art and known to the skilled person for measuring both the degree of amino acid identity between two amino acid sequences and the presence or absence of helix structures in a specific amino acid sequence (document D25).

Article 54 EPC - Claim 9

Document D1 did not disclose any collagen-like material with the properties cited in claim 9, let alone the production of such a material by a recombinant method. Three types of polypeptides were identified by their specific sequence: Formulae I (SEQ ID NO: 4-5, 15), II (SEQ ID NO: 1-3, 16) and III (SEQ ID NO: 6-14). Whereas the production of trimers and multimers was contemplated for polypeptides having no more than 4 different amino acid types, i.e. Formulae I and II (column 4, lines 62-63), biopolymers with multiple peptide sequences of Formula III were not contemplated in document D1. Thus, document D1 disclosed two types of biopolymers, those of low molecular weight (up to 25 residues) with peptide sequences based on Formula III

and those of high molecular weight with peptide sequences based on Formulae I and II. Biopolymers of high molecular weight were produced by recombinant methods and biopolymers of low molecular weight by chemical or synthetic methods, as exemplified in the "Preparation Methods" 1 and 2, respectively (columns 9 and 21). The reference to multimers in column 6, lines 46-55 had to be read in the light of the disclosure in column 4, lines 62-63, and in the context of recombinantly produced sequences but not in relation to sequences prepared by chemical methods.

SEQ ID NO: 6 was a peptide sequence of Formula III and therefore, no biopolymer (trimer or multimer) of high molecular weight based on this sequence was disclosed in document D1. All polypeptides of sequence SEQ ID NO: 6 had a low molecular weight, outside the 2,5-100 kDa range of claim 9, and they were all produced by synthetic methods. These methods were complex, laborious and time-consuming, resulting always in a very low yield, not suitable for the production of trimers or biopolymers of high molecular weight. There was no basis in document D1 for the trimer of SEQ ID NO: 6 used in the sequence alignment of Annex 2 in the decision under appeal.

Moreover, this alignment showed the presence of large gaps between the residues of the trimer of SEQ ID NO: 6 when aligned to the sequence of rat alpha 1 collagen. Gap penalties were always used in all conventional methods for measuring the degree of identity between amino acid sequences. Otherwise, these methods were not technically meaningful. The degree of identity between the sequences aligned in Annex 2 of the decision under appeal, when measured by conventional methods, was much lower than the degree of identity according to claim 9

(at least 80%). As shown in documents D35 and D36, the PID2 method was appropriate only for particular conditions, such as for comparing structurally homologous sequences and sequences with conserved tertiary structures. None of these conditions were given in the present case. A trimer of SEQ ID NO: 6 did not fulfil the requirement of 80% amino acid identity with natural collagen type I, II or III.

The degree of amino acid identity in claim 9 was required to be over the full-length of the amino acid sequence of the collagen like material. The references in this claim to recombinant collagen like material being produced by genetic engineering, to native collagen encoding nucleic acid, and to the amino acid sequence being equivalent to that occurring in nature for collagen, indicated that the amino acid identity had to be over the full-length sequence of the collagen like material. Partial amino acid subsequences were excluded and not contemplated in claim 9 when determining the degree of amino acid identity.

Article 56 EPC - Claim 1

Document D1, the closest prior art, disclosed the production of biopolymers of recombinant collagen like material useful for the preparation of photographic silver halide emulsions. The production of these biopolymers using *Saccharomyces cerevisiae* as host cells was described in "Preparatory Method 1", which resulted in a very low yield (0.5 g/l) (columns 9 and 17). The objective technical problem to be solved was the provision of an improved method (in terms of yield) for the production of biopolymers of recombinant collagen like material.

As a solution, the patent proposed the use of methylotrophic yeast (*Pichia pastoris*) as host cells for expressing a specific type of collagen like material, namely the subject-matter of claim 1. An essential feature of claim 1 was the yield or degree of collagen like material obtained (0.95 g/liter). This feature limited the claim to methylotrophic yeasts capable of achieving the high yield shown in the patent, which was surprisingly higher than that reported for the method of document D1.

For the production of biopolymers with more than 4 different amino acid types (Formula III), document D1 described only methods of chemical synthesis. The sole recombinant method disclosed in document D1 was based on SEQ ID NO: 3 (Formula II), which had only 4 different amino acid types. There was no hint of the use of methylotrophic yeasts in document D1, only bacterial and yeast host cells in general were cited in this document. There was thus no reason for a skilled person to combine document D1 with other prior art documents concerned with methylotrophic yeasts or *P. pastoris*. However, even if a skilled person would have selected documents concerned with *P. pastoris* or with methylotrophic yeasts in general (documents D3, D6, D10) for the purpose of solving the technical problem (increase the yield of collagen like material), it would have introduced into these yeasts a modification system rendering them capable of expressing post-translational enzymes important to the biosynthesis of (pro)collagen (prolyl-4-hydroxylase). According to documents D3, D6 and D10, such a system was essential for achieving expression of stable molecules, helix conformation and correct folding, collagen secretion and proper collagen yields. None of these documents taught the use of methylotrophic yeasts (*P. pastoris*)

as host cells without such a modification system and technical problems would have been expected by a skilled person when using non-modified methylotrophic yeasts as host cells.

Indeed, the gist of the invention was the surprising finding that, when using methylotrophic yeast cells as host cells in the absence of a modification system, high yields of recombinant collagen like material were obtained. This was not expected by, and was completely surprising to, a skilled person. The more so since the yield of recombinant proteins expressed in *P. pastoris* showed great variability and was dependent on the protein itself (document D18). Document D24 made no mention of collagen (like material).

Admissibility of Auxiliary Requests 3-5, 8 and 10-12

These auxiliary requests were filed and submitted to the board's consideration at the earliest stage of the appeal proceedings, namely with the Grounds of Appeal. The filing at this stage of the proceedings could not be seen as a late filing. The less so since the respondent had ample time to study, prepare and react to these requests, i.e. they were not a surprise to the respondent.

XIX. Respondent's submissions, insofar as they are relevant to the present case, may be summarized as follows:

Admissibility of new documentary evidence

Documents D35 and D36 were late filed and addressed an objection that had been raised at the beginning of the proceedings. They could have been filed earlier in the proceedings.

Main Request
Article 83 EPC

The patent did not provide enough information to teach a skilled person how to determine the features "*amino acid identity of at least 80%*" (claim 9) and "*being free of helix structure*" (claims 1 and 9). As for the first feature, the scope of the claim was unclear and extended to non-disclosed variants since not all collagens occurring in nature were known. As for the second feature, it was apparent from document D25 that the assessment of helical structures depended on the experimental conditions (concentration, pH, temperature, etc.), none of them specified in the claims. The structural requirements of claims 1 and 9 were not limited to any specific sequence let alone to the sequences exemplified in the patent. In the absence of sufficient information, it was thus not possible to assess whether these features were present or absent and whether a product fell within the scope of the claims.

Article 54 EPC - Claim 9

Document D1 contemplated the production by recombinant methods of trimers and multimers (biopolymers of high molecular weight) of all peptide sequences disclosed in the document, namely sequences of Formulae I, II and III (columns 5-7). As shown by the definition of the term polypeptide which included Formula III (column 4), no distinction was made between these sequences or their methods of production. Document D1 disclosed thus a trimer of SEQ ID NO: 6 with a molecular weight (6.6 kDa) falling within the range of claim 9.

Claim 9 was not limited to any particular method for determining the required degree of sequence identity. Therefore, the method termed PID2, which was disclosed in document D31 and which did not use gap penalties, could be used for determining the degree of identity between the claimed collagen like material and native collagen. According to this method, both sequences were 100% identical. Indeed, all biopolymers disclosed in document D1 had the GXY repeat characterizing collagen and were thus structurally related to native collagen. They fulfilled the criteria defined in documents D35 and D36 for using the PID2 method. Document D36 also showed the use of a method with no gap penalty for optimizing the range of gap penalty pairs (Table 12).

In line with the case law, the sentence "having an amino acid equivalent" in claim 9 had to be broadly interpreted. Since document D1 was concerned with biopolymers mimicking native collagen, it was not necessary that these biopolymers were identical to collagen over their full-length sequence. The presence of only parts of native collagen, or subsequences identical thereto, was already sufficient. As shown in the sequence alignment of Annex 2, such subsequences were present within a trimer of SEQ ID NO: 6.

Article 56 EPC - Claim 1

Document D1, the closest prior art, disclosed the production of recombinant collagen like material by *S. cerevisiae,* yielding 500 mg/l of protein (column 17). Claim 1 did not specify under which conditions the 0.95 g/l of protein had to be produced and there was no experimental evidence on file to compare the yield obtained with the method of the patent and that of document D1. In the absence of any evidence of the

alleged improvement, the objective technical problem to be solved was the provision of a mere alternative method for producing recombinant collagen like material.

The teachings of document D1 were not limited to the use of *S. cerevisiae* as host cells since other conventional yeast cells were contemplated (column 7). Document D24, a review summarizing the common general knowledge of the skilled person, showed *P. pastoris* to be an obvious alternative since it was available as a commercial kit and its advantages were known, in particular the much higher expression levels of recombinant proteins.

There was no indication in document D24 that a modification system was necessary when using *P. pastoris* as host cells. Indeed, there was no reason for introducing such a modification system into these cells since the purpose of document D1, contrary to documents D3, D6 and D10, was not to produce collagen with helix conformation (native collagen) but only collagen like biopolymers. The modification system was not necessary when the helix conformation was not desired. Moreover, no prejudice could be derived from any of these prior art documents against the use of methylotrophic yeast without a modification system. Document D10 compared the production of recombinant collagen like material in *P. pastoris* with and without the modification system, reporting expression, secretion and production in both types of host cells (page 6707).

Although, as shown in document D18, there was great variability in the yield of recombinant proteins when using *P. pastoris* as host cells, the expectations of a skilled person were not very ambitious when looking for

a mere alternative. A discussion on the yields of the different methods was irrelevant in absence of any experimental data from comparative tests carried out in accordance with the criteria defined in the case law. Document D18 showed that *P. pastoris* was widely used in the scientific community, supporting the disclosure of document D24.

Even if, for the sake of argument, the technical problem was considered to be the provision of an improved production method, this problem was obvious from document D1 itself. Document D1 acknowledged that the expression degree (0.5 g/l) was obtained under specific conditions and was only a compromise of high expression and low background of media protein impurities (column 17). The interest of having a high yield was made evident by references to optimize the elements and features of the expression constructs so as to improve them and to increase the expression and secretion of recombinant collagen like material.

Admissibility of Auxiliary Requests 1-2, 6-7 and 9

In the light of the board's conclusions regarding the Main Request, there was no need to admit these requests into the appeal proceedings.

Admissibility of Auxiliary Requests 3-5, 8 and 10-12

The present appeal was a second appeal proceedings. The appellant had had ample time and opportunities to file requests with all desired features during earlier stages of the proceedings. And, indeed, the appellant had used these opportunities and had filed a large number of requests during these proceedings. However, none of these former requests contained the features

taken from the description and introduced in Auxiliary Requests 3-5, 8 and 10-12. No reasons had been given to explain why these requests could not have been filed earlier and why these features could not have been introduced into former requests. Thus, these auxiliary requests were late filed and should not be admitted into the present second appeal proceedings.

XX. The appellant (patentee) requested to set aside the decision under appeal and to maintain the patent upon the basis of the Main Request, or alternatively, upon the basis of one of Auxiliary Requests 1 - 12, all filed under cover of a letter dated 15 July 2010.

XXI. The respondent (opponent) requested to dismiss the appeal. In addition, the respondent requested that Auxiliary Requests 1 - 5 and 8 - 12 be not admitted into the proceedings, and that documents D35 and D36, submitted by the appellant under cover of a letter dated 11 February 2014, be not admitted into the proceedings.

Reasons for the Decision

Admissibility of new documentary evidence

1. Documents D1 to D25 and D26 to D31 were filed during the first part of the opposition proceedings and the First Appeal proceedings, respectively. Documents D32 to D34 have been filed during the second part of the opposition proceedings which resulted in the decision under appeal. The admissibility of these documents has not been contested by the parties and the board does not see any reason to do it on its own motion.

2. In reply to the board's communication pursuant to Article 15(1) RPBA, the appellant has filed documents D35 and D36 (cf. point VII *supra*). Both documents are cited in document D31 and they were filed to address comments by the board in relation to document D31. These documents do not raise any new issue in appeal proceedings but support only an argument already present in the proceedings. Under these circumstances the board decides to admit these documents into the appeal proceedings (Article 13(1) RPBA).

Main Request

3. The Main Request is identical to Auxiliary Request 1 of the decision under appeal and clearly admissible.

Article 100(c) EPC; Articles 123(2),(3) EPC

4. No substantiated objections have been raised under these articles in appeal proceedings nor were any raised at the first instance proceedings (cf. page 7, point 14.2 of the decision under appeal). The board is satisfied that the requirements of these articles are fulfilled.

Article 100(b) EPC; Article 83 EPC

5. Although Article 100(b) EPC was an original ground of opposition, there is no decision of the opposition division on this article in the decision under appeal.

5.1 In a first decision dated 12 January 2007, the opposition division decided that the main and sole request fulfilled the requirements of Article 83 EPC. In the First Appeal, the respondent/opponent submitted arguments to contest the decision of the opposition

division on Article 83 EPC. There was, however, no reasoned decision of the board on this issue in the decision T 98/07 of the First Appeal.

5.2 After the board's remittal of the case for further prosecution, both the opposition division and the patentee considered that the board had decided on Article 100(b) EPC/Article 83 EPC and that it was not an issue in the further prosecution of the case. This was contested by the opponent in opposition proceedings and in the present appeal proceedings.

5.3 In the light thereof, the board considers the ground of opposition based on Article 100(b) EPC/Article 83 EPC to be part of the present appeal proceedings and the parties' arguments on this ground are to be examined by the board.

6. The respondent's objections under this article arise essentially through the presence of ambiguity in the claims, in particular concerning the features "*amino acid identity of at least 80%*" (claim 9) and "*being free of helix structure*" (claims 1 and 9) (cf. point XIX *supra*).

6.1 According to the case law of the Boards of Appeal, care has to be taken that an insufficiency objection is not in fact an objection under Article 84 EPC. A distinction has to be made between the clarity of what has been disclosed and the clarity of what is claimed. For Article 83 EPC, it is necessary to show that the feature is so ill-defined that the skilled person is not able, on the basis of the disclosure as a whole and using its common general knowledge to identify (without undue burden) the technical measures or suitable parameters necessary to solve the problem underlying

the patent (cf. *inter alia*, T 593/09 of 20 December 2011, Catchword and point 4.1.4 of the Reasons, T 608/07 of 27 April 2009, point 2.5.2 of the Reasons).

7. As for the objected first feature, the following points are of relevance:

7.1 The objection against the first feature was raised with the respondent's letter of 23 November 2009 (page 18) for the first time in the proceedings. Although a similar feature was present in product claim 50 as granted, it was always discussed in the context of Article 84 EPC and its relevance for a broad interpretation of the claim under Article 54 EPC. In these submissions, reference was made to prior art disclosing several methods for determining the level of sequence identity and to sequence alignments with known sequences occurring in nature for collagen. No problems were encountered to argue against the novelty of the claimed subject-matter in these earlier stages of the proceedings. These arguments are also submitted by the respondent under Article 100(a) EPC/Article 54 EPC in the present appeal proceedings (*infra*).

7.2 There is obviously no information on new (non-disclosed) sequences of collagen occurring in nature and thus, the respondent's argument is based on mere assumptions, namely the existence of new collagen sequences that might be significantly different from the known collagen sequences.

8. As for the objected second feature, the following points are of relevance:

8.1 The method cited in the patent and disclosed in document D25 allows a skilled person to identify and

measure the presence of (collagen, gelatin) helix structures. Although the selection of several parameters influences the presence of these structures, these parameters are identified in document D25 (temperature, pH, ionic strength, concentration, etc.) and conditions disclosed for which the presence of these structures is most probable.

9. In view thereof, the board considers the Main Request to fulfil the requirements of Article 83 EPC.

Article 100(a) EPC; Article 54 EPC - Claim 9

10. In the light of document D1, the sole document relevant for the question of novelty, three issues were disputed by the parties: i) whether a recombinant trimer of SEQ ID NO: 6 (MW 6.6 kDa) is disclosed in document D1, ii) whether this trimer fulfils the requirement of "*at least 80% amino acid identity*" defined in claim 9, and iii) whether the requirement of degree of identity must be over the full-length of the compared sequences.

11. As for the first issue, the following points are considered to be of relevance:

11.1 Document D1 discloses the preparation of polypeptides and biopolymers which are characterized by the presence of Gly-X_{aa}-Y_{aa} repeats and which have (gelatin) collagen like properties that make them useful as (nucleation or growth) peptizers for preparing photographic silver halide emulsions (cf. *inter alia*, abstract and column 2, line 42 to column 3, line 39). These polypeptides and biopolymers are defined as "*having at least one occurrence of the ... peptide sequences identified ... as Formulae I, II and III*" (cf. *inter alia*, column 2, line 66 to column 3, line 9, column 4, lines 48 to 61).

Useful peptide sequences found in these polypeptides and biopolymers are specifically disclosed in SEQ ID NO: 1 to 16. Polypeptides and biopolymers of Formula I comprise peptide sequences SEQ ID NO: 4, 5 and 15, Formula II comprises sequences SEQ ID NO: 1 to 3 and 16, and Formula III sequences SEQ ID NO: 6 to 14 (cf. column 5, line 25 to column 6, line 40).

11.2 Document D1 defines the term "*polypeptide*" as referring to "*sequences having at least 20 amino acids, such sequences having at least one occurrence of one or more of the peptide sequences identified herein as Formulae I, II and III, or a tripeptide contained in these three peptide sequences*" without making any distinction between peptide sequences of different Formulae (cf. column 4, lines 19 to 23). In line therewith and contrary to the appellant's view that document D1 refers only to polypeptides of Formulae I and II as having multiple occurrences (preferred 3 to 20, more preferred 3 to 18) (cf. column 4, lines 62 to 63 and column 5, lines 2 and 3) but not to polypeptides of Formula III (cf. column 4, lines 64 to 65 and column 5, lines 7 to 12), the reference to "*(t)he polypeptides described herein*" in column 6, line 46, makes no distinction between the different groups of polypeptides. Accordingly, the disclosure that "*such biopolymers generally have multiple occurrences of the peptide sequences, for example, at least 3 and up to 25 occurrences*" (column 6, lines 46 to 55), applies to all specifically disclosed sequences including SEQ ID NO: 6. Thus, the board considers document D1 to disclose a trimer of SEQ ID NO: 6 and, indeed, even multimers of SEQ ID NO: 6 "*up to 25 occurrences*".

11.3 The board does not follow appellant's argument that document D1 does not disclose the recombinant

production of peptide sequences of Formula III (cf. point XVIII *supra*). On the contrary, the board is convinced that references to the preparation of the biopolymers disclosed in document D1, in particular to the use of "*conventional DNA recombinant techniques*" (cf. column 7, lines 3 to 31), apply to all the disclosed biopolymers. Therefore, document D1 discloses the preparation of a trimer of peptide sequence NO: 6 by conventional recombinant DNA techniques and, in particular, the use of *Saccharomyces cerevisiae* as the host organism, as exemplified in the "Preparatory Method 1" with SEQ ID NO: 3 and 4 (cf. column 9, lines 24 to 33). In this context, it is worth noting that the feature "*recombinant*" in claim 9 has to be interpreted in line with the case law of the Boards of Appeal concerning product-by-process claims, which *inter alia* states that process features establish novelty of a product only if they cause it to have different properties from the products previously described (cf. "Case Law of the Boards of Appeal of the EPO", 7th edition 2013, I.C.4.2.7, page 124 and II.A. 7.1, page 274).

11.4 A trimer of the peptide defined by SEQ ID NO: 6, produced by recombinant DNA techniques using *S. cerevisiae* as a host organism, is a collagen like material comprising more than 4 different amino acid types, being free of helix structure, of hydroxyproline and of procollagen and telopeptides, having a weight on an amino acid basis of 6.6 kDa, falling within the range of 2.5-100 kDa.

12. As for the second issue, the following points are considered to be of relevance:

12.1 There is no limitation in claim 9 to any particular method for measuring the degree of amino acid identity or to the particular parameters to apply when using such a method. According to the established case law, there is no need to interpret an excessively broad claim more narrowly, if it is a question not of understanding concepts that require explanation but rather of examining an excessively broad request in relation to the state of the art (cf. "Case Law", *supra*, I.C.3.8, page 114). In line with this case law, there is no reason to exclude any of the methods known in the prior art for measuring the degree of identity between amino acid sequences. Document D31 refers to several of these methods, including the method termed PID2 which considers only matched residues between the aligned sequences without taking into account gaps (no gap-penalty) (cf. page 3, right-hand column, third paragraph from the bottom and page 4, left-hand column). As shown in Annex 2 of the decision under appeal, the alignment of a trimer of the specifically disclosed sequence SEQ ID NO: 6 of document D1 with the rat type III, alpha 1 collagen chain results in a 100% identity (75 identical positions/75 aligned positions in the alignment) according to the PID2 method.

12.2 Although the PID2 method is identified in document D31 as being the least correlated and thus, the method with the lowest reliability (cf. page 3, left-hand column, last paragraph), this method is not technically meaningless. On the contrary, it might be technically relevant for certain applications. Document D35, relying on studies of five pairs of structurally homologous proteins, shows the relevance of using variable (length dependent/independent) gap-penalties depending on the properties of the aligned sequences (presence of large insertions, secondary structural

similarity, etc.). Document D36, comparing proteins of similar tertiary topology, shows the relevance of optimizing gap penalties. Several gap penalties are used in these studies and alignments with no gap penalties are also considered as shown in Table 12 (cf. page 825 of document D36). Thus, although having a low correlation and reliability, alignment methods with no gap-penalty might be technically meaningful for certain purposes. Claim 9 does not exclude any purpose or application nor is it, as stated above, limited to any method. The comparison of a collagen like material with native collagen with an alignment method with no gap penalty might well provide information of technical relevance, the more so for collagen like material of low molecular weight (2.5 kDa) (presence and/or number of different/identical residue, more/less structurally related, etc.).

13. As for the third issue, the following points are of relevance:

13.1 There is no indication in claim 9 that the required degree of amino acid sequence identity has to be over the full-length of the compared sequences. Nor does the board see such a limitation in the wording "*peptizer having an amino acid sequence equivalent to that occurring in nature for collagen*". In line with the established case law, the term "*peptizer having an amino acid sequence*" is broadly interpreted like the term "*peptizer comprising an amino acid sequence*", and includes peptizers comprising subsequences with the required degree of identity to the corresponding subsequences of the native collagen (cf. "Case Law", *supra*, II.A.3.3, page 254 and II.A.6.2, page 267). Annex 2 of the decision under appeal shows the presence of several subsequences within a trimer of the

specifically disclosed sequence SEQ ID NO: 6 of document D1 having a 100% identity to the corresponding subsequences of the rat type III, alpha 1 collagen chain.

14. It follows from the above considerations that claim 9 of the Main Request does not fulfil the requirements of Article 54 EPC.

Article 100(a) EPC; Article 56 EPC - Claim 1

15. The disclosure of the closest prior art document D1 has been summarized in points 11.1 to 11.3 *supra*. As stated therein, document D1 contemplates the preparation of collagen like polypeptides having the structural properties defined in claim 1, such as *inter alia* a trimer of peptide sequence SEQ ID NO: 6 using *S. cerevisiae* as a host organism, as exemplified in "Preparatory Method 1" for SEQ ID NO: 3 and 4 (cf. column 9, lines 24 to 33). As stated in point 11.4 *supra*, such a trimer fulfils the structural requirements defined in claim 1. With the expression construct and the conditions exemplified in this preparatory method, document D1 refers to a high production of collagen like polypeptides with "*a preferable compromise of high expression (about 500 mg/l) and low background of media protein impurities*" (cf. column 17, lines 4 to 9).

16. On the one hand, it is worth noting in this context that claim 1 does not define any particular growth conditions nor is it limited to any specific expression constructs (secretory leader peptide, promoter, etc.) for achieving the required degree of expression of 0.95 gram/liter. On the other hand, there is no evidence on file showing that the concentration of collagen like

polypeptides obtained using methylotrophic yeasts, in particular *P. pastoris*, as host organism is (always) higher than the concentration obtained using any of the the methods and conditions disclosed in document D1. As argued by the respondent (cf. point XIX *supra*), there are no experimental data on file derived from comparative tests performed as defined by the established case law (cf. "Case Law", *supra*, I.D.10.9, page 231).

17. In view thereof and starting from the closest prior art document D1, the objective technical problem to be solved is the provision of an alternative method for the production of these recombinant collagen like polypeptides. As a solution to this problem, the patent proposes the use of methylotrophic yeast, in particular *P. pastoris*, as host organism, i.e. the subject-matter of claim 1 (cf. point IX *supra*).

18. It is not contested that the proposed solution solves the technical problem.

19. As acknowledged in the case law, the skilled person always looks for alternatives in known methods when no risks are involved and no inventive skills are required (cf. "Case Law", *supra*, I.D.8.1.3, page 189; *inter alia*, T 455/91, OJ EPO 1995, page 684, point 5.1.3 of the Reasons). Indeed, document D1 itself already indicates that, for the production of collagen like biopolymers by standard DNA recombinant techniques, "*(c)onventional protein expression procedures using various bacterial or yeast host cells can be practiced*" (cf. column 7, lines 12 to 15), clearly not limiting the teachings of the document to the exemplified *S. cerevisiae* host organism. Thus, it would have been obvious for a skilled person to look for

alternative yeast strains which were known to be suitable and appropriate as host cells.

20. When looking for alternatives to *S. cerevisiae*, a skilled person would have considered as highly relevant the disclosure of document D24. According thereto, the use of methylotrophic yeasts *Hansenula polymorpha*, *Pichia pastoris* and *Candida boidinii* as production systems for recombinant proteins has favourable and most advantageous characteristics (cf. page 554, left-hand column, abstract). In particular, *P. pastoris* is "*widely distributed within the scientific community due to the availability of a commercial kit*" (cf. page 554, right-hand column, lines 28 to 31). Document D24 acknowledges that "*methylotrophs have become the preferred option among the various yeast expression systems*" since, as stated in this document, "*(i)n many instances the heterologous proteins were produced at much higher levels when compared with the respective productivities in the traditional S. cerevisiae host*" and "*(a) comprehensive catalogue of methods and components, including a commercial kit based on P. pastoris, is now available*" (cf. page 558, right-hand column, third paragraph).

21. In the light of document D24, the board is convinced that the use of methylotrophic yeast, in particular *P. pastoris*, as host cells for the production of recombinant collagen like polypeptides would have been obvious to a skilled person. All the more so, since methylotrophic yeast and *P. pastoris* had already been successfully used - with a high yield - for the production of the much more complex recombinant human collagens and procollagens (cf. documents D3, D6 and D10). For the production of these complex products, proline hydroxylation, and therefore the presence of a

post-translational (hydroxylation) modification system (cf. *inter alia*, page 1, line 21 to page 2, line 2, page 4, lines 10 to 26, page 9, lines 4 to 7, page 22, Example 9 of document D3; page 6, lines 15 to 20, page 22, lines 9 to 14, page 42, Example 9, page 70, Example 11.2 of document D6), is essential for obtaining an appropriate helical folding, secretion and self-assembly into collagen fibrils. However, for the much less complex collagen like polypeptides disclosed in document D1, there is no need, and in fact no mention in document D1 at all, of any post-translational modification. Consequently, no prejudice could be derived from any of the prior art documents D3, D6 and/or D10 against the use of methylotrophic yeast in general, i.e. without a hydroxylation modification system, as host cells.

22. The general disclosure of document D24 is supported by post-published document D18 (cited as expert opinion), a review article reporting a long list of recombinant polypeptides and proteins produced by using *P. pastoris* as host cells. The suitability of *P. pastoris* as host cells is shown by the successful expression and production of a large number of proteins and polypeptides - with very different properties and characteristics - obtained from very different sources, such as bacteria, fungi, protists, plants and vertebrates, including human. Although, as argued by the appellant (cf. point XVIII *supra*), the yields reported in document D18 show a great variability, the yield obtained for several human proteins is about or greater than 1 gram/liter.

In any case, in view of the disclosure of document D1 and in the absence of any comparative tests on file (cf. points 15 and 16 *supra*), the technical problem to

be solved has been formulated with a less ambitious goal (provision of an alternative) than that suggested by the appellant (provision of an improvement) (cf. point 17 *supra*) and, accordingly, the expectations of a skilled person were also less ambitious or challenging. In view of the reference in document D24 to the production of high level of recombinant proteins and the results from documents D3, D6 and D10, the board is convinced that a skilled person would also have had a reasonable expectation of success when using *P. pastoris* as a host cell for producing the recombinant collagen like polypeptides and biopolymers disclosed in document D1.

23. It follows from the above considerations, that the combination of documents D1 and D24 renders the process of claim 1 not inventive (Article 56 EPC).

Admissibility of Auxiliary Requests 1-2, 6-7 and 9

24. All these auxiliary request contain a process-claim or a product-claim identical to the process-claim 1 or the product-claim 9 of the Main Request (cf. points IX to XI, XV and XVI *supra*). Since none of the requests overcomes the objections raised against the Main Request, there is no need for the board to consider their admissibility into the appeal proceedings.

Admissibility of Auxiliary Requests 3-5, 8 and 10-12

25. These auxiliary requests have been amended by the introduction of features taken from the description of the patent, namely "*being free of a sequence encoding MGPR*" and/or the replacement of the range "*2.5-100 kDa*" by "*10-100 kDa*" (cf. points IX, XII to XVI *supra*). The introduction of these features raises *prima facie* new

issues under Article 123(2) EPC when considered in combination with other features present in the claims. In view of the general disclosure of document D1 (cf. points 11.1 to 11.4 *supra*), it is also *prima facie* questionable whether the introduction of these features overcomes the objections raised against the Main Request.

26. Moreover, no reasons have been provided by the appellant to explain why requests comprising these features could not have been filed at a much earlier stage of the proceedings, either during the first opposition and/or appeal proceedings and/or in the second opposition proceedings. In view of the facts of the present case (2nd appeal after 2nd opposition proceedings; cf. points I to IV *supra*), and although these auxiliary requests have been filed with the appellant's Grounds of Appeal, the filing of these requests at such a late stage of the proceedings is considered not to be justified and not to be in line with the purpose of appeal proceedings (cf. "Case Law", *supra*, IV.E.4.1, page 984 and IV.E.4.3.2.b), page 996).

27. Thus, the board, in the exercise of its discretion, decides not to admit Auxiliary Requests 3-5, 8 and 10-12 into the appeal proceedings (Article 12(4) RPBA).

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

A. Wolinski B. Stolz

Decision electronically authenticated