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Datasheet for the decision of 19 October 2018

Case Number: T 1314/13 - 3.3.08

Application Number: 98966468.5

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Language of the proceedings: EN

Title of invention:
Procedures for the extraction and isolation of bacterial capsular polysaccharides for use as vaccines or linked to proteins as conjugates vaccines

Patent Proprietor:
Pfizer Ireland Pharmaceuticals

Opponent:
Strawman Limited

Headword:
Method for purifying bacterial CPS/PFIZER

Relevant legal provisions:
EPC Art. 56, 83, 113(1), 123(2)
EPC R. 115(2)
RPBA Art. 12(4), 15(3)

Keyword:
Late-filed documents - admitted (no)
Auxiliary request C - requirements of the EPC met (yes)

Decisions cited:

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

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Case Number: T 1314/13 - 3.3.08

D E C I S I O N
of Technical Board of Appeal 3.3.08
of 19 October 2018

Respondent: (Patent Proprietor)	Pfizer Ireland Pharmaceuticals Ringaskiddy Cork (IE)
Representative:	Weinmann, Lasse Hoffmann Eitle Patent- und Rechtsanwälte PartmbB Arabellastraße 30 81925 München (DE)
Appellant: (Opponent)	Strawman Limited Orchard Lea Horns Lane Combe, Witney Oxfordshire OX29 8NH (GB)
Representative:	Wise, Daniel Joseph Carpmaels & Ransford LLP One Southampton Row London WC1B 5HA (GB)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted on 26 March 2013 concerning maintenance of the European Patent No. 1051506 in amended form.**

Composition of the Board:

Chairman B. Stolz
Members: M. Montrone
D. Rogers

Summary of Facts and Submissions

I. Appeals were lodged by the patent proprietor and the opponent against the interlocutory decision of an opposition division concerning European patent No. 1 051 506, having the title "*Procedures for the extraction and isolation of bacterial capsular polysaccharides for use as vaccines or linked to proteins as conjugates vaccines*". The patent is based on European application No. 98 966 468.5, which was filed as an international application and published as WO 99/32653 (hereinafter "the patent application").

II. In this decision, the patent proprietor will, due to the withdrawal of its appeal at the end of the oral proceedings before the board, either be referred to as "appellant I" or "respondent". The opponent will either be referred to as "appellant II" or "appellant".

III. In the decision under appeal, the opposition division held that claims 1 of the main request and the first auxiliary request contravened Article 123(2) EPC and that claim 3 of the first auxiliary request lacked clarity. It further took the view that the second auxiliary request and pages of the description adapted thereto complied with the requirements of the EPC.

IV. With its statement of grounds of appeal, appellant I submitted a main request and three auxiliary requests. The main request and auxiliary requests A and C correspond to the main, first and second auxiliary requests dealt with in the decision under appeal, while auxiliary request B is new in the appeal proceedings.

V. With its statement of grounds of appeal, appellant II submitted arguments as to why claim 1 of the second

auxiliary request as maintained by the opposition division (i.e. auxiliary request C in the appeal proceedings) comprised added subject-matter, lacked an inventive step, and was insufficiently disclosed. Appellant II filed documents D13 to D19 in support of its case on insufficiency.

VI. Both appellants replied to each other's statement of grounds of appeal. Appellant I, in support of its arguments with regard to sufficiency of disclosure, submitted a further document, while appellant II submitted *inter alia* documents D20 and D21 in reply to appellant I's arguments about inventive step.

VII. In a further letter appellant I submitted further arguments and requested that documents D20 and D21 not be admitted into the appeal proceedings.

VIII. In reply, appellant II reiterated its previously submitted arguments.

IX. The parties were summoned to oral proceedings. In a communication pursuant to Article 15(1) RPBA, the parties were informed of the board's provisional, non-binding opinion on some of the legal and substantive matters of the case. In reply thereto, appellant II announced that it would not be attending the oral proceedings, without however, submitting substantive arguments in response to the issues raised in the board's communication.

X. Oral proceedings before the board were held on 19 October 2018, in the absence of appellant II. At the oral proceedings, appellant I withdrew its appeal and thus became a respondent to the present appeal proceedings. In consequence thereof, claims 1 to 16 of

auxiliary request C, i.e. the request upon which the opposition division decided to maintain the patent, became the only remaining set of claims in the appeal proceedings.

XI. Claim 1 of the auxiliary request C reads:

"1. A method of purifying a capsular polysaccharide from cellular components of gram-negative and gram-positive bacteria, wherein the purification comprises

- providing for extraction isolated bacterial cells, concentrated bacterial supernatants from homogenized bacterial cells or conditioned medium, or pelleted cells;

- extracting the capsular polysaccharide from cellular components, wherein the cellular components include protein and nucleic acid, by contacting the isolated bacterial cells, concentrated bacterial supernatants from homogenised bacterial cells or conditioned medium, or pelleted cells with a base reagent selected from the bases NaOH, KOH, LiOH, NaH, NaOMe or KOtBu in a pH range of 12 to 14, wherein the bases are used in a range of 0.5 N to 5.0 N, and whereby bacterial DNA and RNA are degraded; and

- separating by hydrophobic-interaction chromatography (HIC) the extracted capsular polysaccharide from impurities resulting from the cellular components which comprise proteins and nucleic acids, and recovering the purified capsular polysaccharides".

Dependent claims 2 to 16 define specific embodiments of the method of claim 1.

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

D1: EP0238739 (publication date: 30.09.1987);

D2: US4413057 (publication date: 01.11.1983);

D4: M.R. Wessels *et al.*, Infection and Immunity, 1989, Vol. 57(4), pages 1089-1094;

D9: US3577527 (publication date: 04.05.1971);

D13b: D. Glick, Methods of Biochemical Analysis, John Wiley & Sons Inc., 1966, pages 133-134;

D14: A.L. Lehninger, Biochemistry, 2nd Edition, Worth Publishers Inc., 1979, pages 322-323;

D15: Wikipedia extract: Ethanol precipitation;

D19: A. Fattom *et al.*, Infection and Immunity, 1988, vol. 56(9): pages 2292-2298;

D20: O.T. Avery and W.F. Goebel, Journal of Experimental Medicine, 1933, Vol. 58(6): pages 731-755;

D21: J.F. Enders and C.-J. Wu, Journal of Experimental Medicine, 1934, Vol. 60(2): pages 127-147;

XIII. The appellant's submissions, insofar as relevant to the present decision, may be summarised as follows:

Admission of documents D20 and D21 into the appeal proceedings (Article 12(4) RPBA)

Documents D20 and D21 were filed in response to the arguments submitted by the respondent (former appellant I) in its statement of grounds of appeal, namely that the harsh conditions of the base extraction step as recited in claim 1 resulted in the deacetylation of CPS which allowed its separation from proteins and nucleic acids as impurities. Thus, both documents were not filed too late, since they could not have been filed earlier.

Furthermore, documents D20 and D21 were *prima facie* relevant for the assessment of inventive step of the claimed method because they disclosed *inter alia* that alkaline conditions caused a complete deacetylation of CPS which rendered the compound soluble and allowed its isolation (see e.g. document D20, page 731, last paragraph to page 732, first paragraph, page 736, legend of Table I). Document D21 disclosed that CPS was deacetylated in dilute alkali (see page 127, line 7).

Auxiliary request C - claim 1

Amendments (Article 123(2) EPC)

Claim 1 of auxiliary request C comprised added subject-matter because the term "hydrophobic-interaction chromatography (HIC)" encompassed any HIC-based chromatography irrespective of the stationary phase, i.e. the chromatographic material on which HIC was performed. However, the patent application solely disclosed that HIC was performed "*on phenyl sepharose*" for separating CPS from impurities comprising proteins and nucleic acids (see page 9, lines 3 to 5). Furthermore, neither the disclosure on page 9, lines 7 to 10 nor that on page 6 of the patent application provided a basis for a generalisation to any type of

HIC. Although both of these passages mentioned HIC in general, they were silent on the separation of CPS from protein and nucleic acid impurities.

Sufficiency of disclosure (Article 83 EPC)

The method of claim 1 was *inter alia* characterised by the functional requirement that bacterial DNA and RNA be degraded when extracting the capsular polysaccharide with a base reagent selected from several bases having a pH in the range of 12 to 14 and a concentration in the range of 0.5 N to 5.0 N.

However, document D13b disclosed that DNA was resistant to "*alkaline hydrolysis*" (see page 133, first paragraph).

Likewise, document D14 reported that DNA compared to RNA lacked the 2'-OH group and was therefore not hydrolysed by "*dilute alkali*" (see paragraph bridging pages 322 and 323). Thus, based on the disclosure of both documents serious doubts existed that DNA was indeed hydrolysed over the whole base concentration range recited in claim 1.

Inventive step (Article 56 EPC)

Document D9 represented the closest prior art for the method of claim 1. The document disclosed a method of purifying CPS from the bacterium *Neisseria meningitidis.* The claimed method was solely distinguished therefrom in that HIC was used for the separation of CPS from impurities resulting from cellular components.

In the decision under appeal, the opposition division erred that there was a second distinguishing feature. Document D9 already mentioned that an 1 N NaOH solution having a pH of 12 was used to extract CPS from the bacterial sample (see column 2, line 1, column 4, line 1). These conditions fell within the pH and base concentration ranges recited in claim 1, and thus must have led to the degradation of bacterial DNA and RNA.

The term "*mild alkaline hydrolysis*" reported in the abstract of document D9 did not exclude that the alkaline hydrolysis of the CPS was performed at pH 12, because "*mild*" related to the hydrolysis conditions of isolating CPS. In other words, the hydrolysis had to be sufficiently mild to ensure that the antigenic structure of CPS was not damaged. There were, however, no suggestions derivable from document D9 that "*mild*" related to the treatment of nucleic acids as impurities, i.e. that the degradation of nucleic acids should be avoided. Moreover, document D13b disclosed that RNA was degraded by an NaOH treatment at pH 11.8 (see page 133, last paragraph), which was below pH 12 defining the upper limit of the pH range disclosed in document D9 (see claim 1).

Furthermore, an EtOH precipitation in the purification of CPS as disclosed in document D9 did not indicate to the skilled person that nucleic acids were still present as impurities in the sample. This was so because document D15 mentioned that nucleic acids were precipitated in an EtOH solution (see page 1, line 1). Thus, since EtOH precipitated nucleic acids and CPS, it could not separate the compounds from each other.

The sole difference between the claimed method and that of the closest prior art was thus the use of HIC for

the purification of CPS from proteins and nucleic acids, which resulted in a further separation of CPS from impurities. The technical problem was considered as the provision of an improved method for the separation of hydrophobic and hydrophilic compounds.

The solution to this problem was obvious to the skilled person because the use of HIC for achieving this task was well established at the filing date of the patent in suit (see e.g. document D19). A separation of components using a polarity based chromatography was also known from document D4.

XIV. The respondent's arguments, insofar as relevant to the present decision, may be summarised as follows:

Admission of documents D20 and D21 into the appeal proceedings (Article 12(4) RPBA)

Documents D20 and D21 should not be admitted into the appeal proceedings since they were late filed and lacked *prima facie* relevance.

Regarding their relevance, it was *inter alia* submitted that both documents were silent on the issue whether or not the disclosed capsular polysaccharides (CPS) were purified starting from bacterial material that was base-treated and N-deacetylated as in the claimed method.

Auxiliary request C - claim 1

Amendments (Article 123(2) EPC)

Claim 1 did not comprise added subject-matter for the reasons set out in the decision under appeal.

Sufficiency of disclosure (Article 83 EPC)

The feature "and whereby bacterial DNA and RNA are degraded" in the extraction step of claim 1 was objected under sufficiency of disclosure by the appellant based on the disclosure of documents D13b and D14. It was established case law that regarding sufficiency of disclosure the opponent (appellant) bore the burden of proof.

With regard to DNA, document D13b disclosed that "*It is thus resistant to alkaline digestion*" (see page 133, first paragraph). Document D14 contained a similar statement, namely that "*DNA is not hydrolysed by dilute alkali*" (see page 322, last paragraph). However, both documents were silent on the specific alkaline conditions under which DNA was resistant to hydrolysis, and whether or not these conditions fell within the pH and base concentration ranges recited in claim 1. Furthermore, both documents provided no evidence that DNA was not degradable by alkaline hydrolysis at all. Thus, both documents did not demonstrate that any of the alkaline conditions recited in claim 1 did not degrade DNA, and hence, did not discharge the appellant of its burden of proof.

Inventive step (Article 56 EPC)

Document D9 represented the closest prior art. The claimed method differed from the closest prior art method in that the base extraction step was carried out at a final concentration of the bases in the range of 0.5 N to 5.0 N, and in that the alkaline conditions recited in the claim resulted in the degradation of DNA and RNA. In other words, document D9 failed to disclose

the harsh alkaline conditions in the extraction step of the claimed method, but rather reported "*mild alkaline*" conditions (see abstract and claim 1).

The patent in suit disclosed that the alkaline conditions recited in claim 1 had several effects. Firstly, CPS was modified and became more hydrophilic due to the deacetylation of its acetic groups (see paragraph [0023]); secondly, CPS was detached from the other cellular components to which it was bound because base-labile bonds were hydrolysed (see paragraph [0022]); thirdly, DNA and RNA as cellular impurities were degraded (see paragraph [0036]). Furthermore, the alkaline treatment of CPS caused a difference in polarity of this molecule compared to other cellular components that could be exploited for a more efficient chromatographic purification. Further, the claimed method was safer because flammable organic solvents, such as ethanol (EtOH), were not required. Moreover, since an EtOH precipitation step was likewise not required, the claimed method was simplified due to a reduced number of process steps.

Thus, the technical problem could be defined as the provision of an improved method for the purification of CPS. The experimental data in paragraph [0074] of the patent in suit demonstrated that this problem was solved by the claimed method.

The skilled person starting from the closest prior art document would not have arrived at the claimed method in an obvious manner, because it contained no pointer to use the alkaline conditions referred to in the claim for extracting CPS. On the contrary, the document suggested the use of "*mild*" alkaline conditions. Likewise, document D19 was silent on such "harsh

alkaline" conditions for the purification of CPS. The document reported alternative process steps, for example, a "*cold phenol extraction*" and a "*fractional ethanol precipitation*" for CPS's purification, which taught away from using HIC (see paragraph bridging pages 2292 and 2293).

XV. The appellant requested that the decision under appeal be set aside, and that the patent be revoked in its entirety.

XVI. The respondent requested that the appeal be dismissed. Furthermore, it requested that documents D20 and D21 not be admitted into the appeal proceedings.

Reasons for the Decision

1. The duly summoned appellant did not attend the oral proceedings, which in accordance with Rule 115(2) EPC and Article 15(3) RPBA took place in its absence.

Article 113(1) EPC

2. The board in its communication pursuant to Article 15(1) RPBA expressed a reasoned provisional opinion on the issues to be discussed at the oral proceedings, which included *inter alia* the issues of admission of documents D20 and D21 (Article 12(4) RPBA); amendments in relation to claim 1 of auxiliary request C (Article 123(2) EPC); and the presence of an inventive step of the subject-matter of claim 1 of auxiliary request C in the light of documents D9 and D19.

3. In reply to the board's communication, the appellant did not submit any substantive arguments in relation to the issues raised (see point IX above). Moreover, by not attending the oral proceedings, the appellant decided not to avail itself of another opportunity to orally address or comment on the issues raised by the board in its communication for defending its case. The present decision is therefore based on the same grounds, arguments and evidence on which the provisional opinion of the board was based.

Admission of documents D20 and D21 into the appeal proceedings (Article 12(4) RPBA)

4. In reply to the respondent's (former appellant I) statement of grounds of appeal, the appellant submitted documents D20 and D21 in support of *inter alia* its allegation that the use of alkaline conditions to deacetylate bacterial capsular polysaccharides (hereinafter "CPS") for purification purposes was well established at the filing date of the patent in suit.

5. In its communication in preparation of the oral proceedings, the board observed that the passages indicated by the appellant in documents D20 and D21 did not disclose that an alkaline hydrolysis was used for the purification of CPS, as in the method of the invention, rather an acidic hydrolysis was reported for this purpose. Accordingly, the issue of the *prima facie* relevance of documents D20 and D21 for assessing the claimed method arose and hence, the board considered it doubtful whether both documents could be admitted into the appeal proceedings.

6. The appellant neither replied to the board's communication nor did it attend the oral proceedings. In these circumstances the board exercised its discretion according to Article 12(4) RPBA and decided not to admit documents D20 and D21 into the appeal proceedings.

Auxiliary request C

Amendments (Article 123(2) EPC) - claim 1

7. The issue to be assessed is whether or not the feature "*separating by hydrophobic-interaction chromatography (HIC)*" in claim 1 can be directly and unambiguously derived by the skilled person, using common general knowledge, from the patent application as a whole.

8. The appellant argued that HIC in general did not have a basis in the passage on page 9, lines 2 to 10 of the patent application, even when read in conjunction with page 6, second and third paragraph, since a separation of CPS from protein and nucleic acid impurities was only reported for a HIC performed "*on phenyl sepharose*".

9. The cited passage on page 9 of the patent application reads:

"*More preferred is hydrophobic-interaction chromatography on phenyl sepharose which will remove most of the high-molecular-weight, uv-active contaminants from the base extract. Capsular polysaccharide will elute in the beginning of the high-pH (pH 10 to pH 8), high-salt (2 N to 1 N) elution, while the more hydrophobic protein and nucleic acids*

will be retained. Non-limiting examples of the hydrophobic-interaction chromatographic method are alkyl agarose or sepharose resins with Phenyl Sepharose HP (Pharmacia Biothech; Piscataway, NJ) being a preferred resin" (emphasis added).

10. Furthermore, page 6, first to third paragraphs read as follows:

"*This invention provides a method for obtaining capsular polysaccharides from gram-negative and gram-positive bacteria by using base hydrolysis of the base-labile bond that attaches the CPS to the cellular components. [...].*

A wide variety of conditions can be used to hydrolyze the base-labile bond in either aqueous or organic solvent according to the invention. The extent to which N-acetyl bonds of the carbohydrates are also hydrolyzed can be controlled by the reaction conditions. The hydrolysis of the N-acetyl groups is advantageous for separating the CPS from the other cellular components because the greater extent to which the N-acetyl bonds are cleaved, the more hydrophilic, relative to the rest of the cellular components, the CPS becomes. This difference in polarity can be exploited to effect an efficient chromatographic separation. The separation of two or more components of a mixture based on differences in polarity is well known to those skilled in the art.

For example, using hydrophobic-interaction chromatography, compounds of relatively greater hydrophobicity are retained longer on the column relative to those compounds that are more hydrophilic" (emphasis added).

11. As set out above, page 9, lines 2 to 10 of the patent application mentions at the end of the passage that "*Non-limiting examples of the hydrophobic-interaction chromatographic method are alkyl agarose or sepharose resins with Phenyl Sepharose HP*", in other words that various HIC chromatographic media, i.e. different stationary phases, might be used for the separation of CPS, because "*the more hydrophobic protein and nucleic acids will be retained*".

12. Thus, although HIC performed on "Phenyl Sepharose" is the preferred chromatographic material for removing the more hydrophobic proteins and nucleic acids from CPS, in the board's view, the skilled person would have derived from the passage on page 9, lines 2 to 10 of the patent application as a whole that HIC in general can be used for this task.

13. This view is also supported by the paragraphs on page 6 of the patent application (see point 10 above). The cited passages teach that in the method of the invention the base treatment detaches the CPS from the cellular components by cleaving base-labile N-acetyl groups. It further reports that an increased cleavage of these groups correlates with an increased hydrophilicity of CPS relative to the other cellular components present in the mixture. In other words, CPS as a result of the base treatment is more hydrophilic relative to the then more hydrophobic other cellular components, which may be exploited for separation purposes.

14. In this context HIC is mentioned in the immediately following paragraph on page 6 (see point 10 above), including the principle on which HIC-based separation

rests, namely that "*compounds of relatively greater hydrophobicity*", i.e. proteins and nucleic acids (see point 9 above), are "*retained longer on the column relative to those compounds that are more hydrophilic*", i.e. CPS. Thus, this principle applies to HIC in general, irrespective of the stationary phase material used.

15. In view of the considerations above, the board is not persuaded by the appellant's arguments, and hence concludes that the subject-matter of claim 1 of auxiliary request C does not extend beyond the content of the application as filed. Thus, auxiliary request C meets the requirements of Article 123(2) EPC.

Sufficiency of disclosure (Article 83 EPC) - claim 1

16. The appellant submitted that in view of the disclosure in documents D13b and D14 serious doubts existed that bases used in the concentration range of 0.5N to 5.0N, as referred to in claim 1, degraded DNA across the whole range.

17. It is established case law that an objection of lack of sufficient disclosure presupposes that there are serious doubts, substantiated by verifiable facts, and that in order to establish insufficiency, the burden of proof rests generally on the opponent (see Case Law of the Boards of Appeal, 8th edition, 2016, II.C.8).

18. Thus, the issue to be assessed is whether or not the appellant, based on the information disclosed in documents D13b and D14, has made a convincing case that bases in the concentration range of 0.5 N to 5.0 N

cannot degrade DNA over substantially the whole of this concentration range.

19. Document D13b discloses that "*In the case of RNA, on exposure to alkali a cyclic triester is formed with the hydroxyl group on the C_2 of the ribose and this spontaneously hydrolyzes to yield 2' or 3' nucleotides. In the case of DNA, the deoxypentose bears no hydroxyl group on C_2 and in consequence cannot form the essential 2',3' phosphotriester. It is thus resistant to alkaline digestion*" (see page 133, first paragraph, emphasis added).

Similarly, document D14 reports that "*DNA is not hydrolysed by dilute alkali, whereas RNA is because of the 2'-hydroxyl groups it contains*" (see page 322, last paragraph, emphasis added).

20. In other words the presence or absence of a hydroxyl group at the C2 position of a ribose sugar in DNA and RNA molecules determines whether or not these molecules are hydrolysed, i.e. degraded, under alkaline conditions. In the board's opinion, the skilled person would not construe the two statements "*It is thus resistant to alkaline digestion*" and "*DNA is not hydrolysed by dilute alkali*" in an absolute sense, i.e. to mean that DNA is not degradable under alkaline conditions at all.This has also not been argued by the appellant.

21. Furthermore, since documents D13b and D14 report that the lack of a hydroxyl group at the C2 position of DNA's deoxyribose is responsible for its resistance to alkaline degradation, the skilled person would have construed the terms "*resistant to alkaline digestion*"

and "*DNA is not hydrolysed by dilute alkali*" to relate to the same (dilute) alkaline conditions.

22. The board further observes that documents D13b and D14 are both silent on the specific (dilute) alkaline conditions under which DNA is resistant to hydrolysis, in other words the base concentration to which the terms "*dilute alkali*" and "*resistant to alkaline digestion*" refer.

23. In these circumstances, it cannot be established whether or not the resistance "*to alkaline digestion*" or the "*dilute alkali*" reported in documents D13b and D14, respectively, relate to base concentrations that fall within the concentration range of 0.5 N to 5.0 N recited in claim 1, and hence, whether or not a base concentration encompassed by this range is incapable of degrading DNA. In other words both documents fail to demonstrate that the invention cannot be performed over substantially the whole of the claimed range.

24. Consequently, the board concludes that the provisions of Article 83 EPC are met.

Inventive step (Article 56 EPC)

Closest prior art

25. It is common ground between the parties that document D9 represents the closest prior art for the method of claim 1.

26. Document D9 discloses methods for purifying various CPS antigens from several *Neisseria meningitidis* serogroups subjected to a "*mild alkaline hydrolysis*" (see

abstract, column 1, lines 45 and 46, 60 to 65, column 2, lines 7 and 8).

In a first step, isolated bacterial cells are provided as source material (see column 1, lines 66 to 72); secondly, the CPS is extracted by incubating the cells for one or two hours in a saline solution at "*pH 11.0*" or "*pH 10*" adjusted with "*1 N NaOH*" followed by a pH readjustment to either 6.5 or 7.0 (see column 1, last line to column 2, line 2, column 3, lines 4 to 8); thirdly, extracted CPS is separated from either (i) an "*insoluble residue*" (i.e. impurities) by precipitation in absolute ethanol (EtOH) followed by a re-suspension in saline solution leaving the CPS in the supernatant (see column 2, lines 3 to 7), or (ii) from a "*precipitated sediment*" in a fractional EtOH precipitation starting with 20% EtOH leaving CPS in solution followed by the addition of EtOH up to 85% that precipitates CPS, followed by a gel-chromatography based purification (see column 3, lines 8 to 20).

27. Document D9 is silent on the types of impurities that have been separated from CPS following the EtOH precipitation protocols (i) and (ii) set out above.

28. The appellant submitted that these impurities did not include nucleic acids since they precipitated, like CPS, in EtOH (see document D15, lines 1 to 2). In other words an EtOH precipitation was unsuitable to separate CPS from nucleic acid impurities.

29. The board is not convinced by this argument, since nucleic acids precipitate in 20% EtOH, while CPS requires higher EtOH concentrations (see e.g. document D2, column 4, lines 18 to 22). Moreover, document D9 reports that CPS is resuspendable in aqueous solution

after precipitating it in absolute EtOH, while an "*insoluble residue*" remains that is discarded (see column 2, lines 3 to 7). In other words, the reversibility of the precipitation in absolute EtOH is exploited to separate CPS from an impurity. Thus in the board's view, the skilled person being aware that nucleic acids have a different solubility in EtOH than CPS, would have rather derived from the teaching in document D9 that the described precipitation separates impurities that comprise nucleic acids from CPS.

30. This conclusion is in line with the use of "*mild alkaline hydrolysis*" conditions in document D9 for extracting CPS which implies, as set out in point 19 above, that DNA has not been substantially degraded, since it is resistant to hydrolysis under "*dilute alkali*" conditions. Furthermore, the presence of protein impurities in the purified CPS extract disclosed in document D9 is not excluded, because process steps that are generally performed for their removal are not reported. These include for example, a "*chloroform-butanol*" extraction (see document D1, page 4, lines 6 to 13, page 12, line 13), a precipitation by strong ionic salts or an enzymatic degradation (see document D2, column 3, lines 22 to 24, column 4, lines 8 to 35, example 3, column 16, lines 36 to 43). Moreover, a precipitation of proteins in EtOH is neither reported nor suggested in the available prior art documents

31. The appellant further submitted that DNA and RNA must have been degraded by the alkaline conditions disclosed in document D9, because the conditions mentioned in the document fell within the pH and concentration ranges recited in claim 1.

32. The board is not convinced by the appellant's argument for the following reason. Claim 1 refers to a concentration range of "0.5 N to 5 N" for various bases in a reagent used for extracting CPS, while document D9 reports that the cell suspension is "*adjusted to pH 11.0 with 1 N NaOH*" (see column 9, last line to column 2, line 1). This implies that a 1 N NaOH stock solution is used for adjusting the pH until the desired value is reached, but not that the concentration of NaOH in the cell suspension is 1 N. Consequently, at least the concentration of the base NaOH reported in document D9 is not considered to fall within the range recited in claim 1.

33. In view of the considerations set out above, the board concludes that the claimed method differs from that of the closest prior art in at least two features, namely the use of HIC and the use of alkaline conditions that result in the degradation of bacterial DNA and RNA. These differences result in CPS containing less impurities.

34. Consequently, the technical problem is defined as the provision of an improved method for the purification of CPS.

35. In the light of the experimental data disclosed in paragraph [0074] of the patent in suit, the board is satisfied that the method according to claim 1 solves this problem.

Obviousness

36. It remains to be assessed whether or not the skilled person starting from the closest prior art method and faced with the technical problem identified above would

have arrived at the claimed method in an obvious manner.

37. Document D9 discloses for the purification of CPS either an extraction from bacterial material at pH 11 or pH 10 at unknown NaOH concentrations followed by various EtOH precipitations, including a final gel-permeation chromatography (see point 26 above). Thus, the document discloses no pointers to how to possibly improve the method for purifying CPS, let alone by using the alkaline extraction conditions combined with HIC as referred to in claim 1. Thus, the claimed method cannot be considered obvious for the skilled person based on the teaching of document D9 alone.

38. Appellant II submitted that the use of HIC for separating a hydrophobic protein from hydrophilic CPS was obvious for the skilled person since HIC was used for this purpose in the prior art, for example, in documents D19 and D4.

39. Document D19 discloses the synthesis of *Streptococcus pneumoniae* type 12F (Pn12F) CPS conjugated to diphtheria toxoids (DT). It reports that the Pn12F is obtained from the company Eli Lilly, and that before being conjugated to DT, Pn12F is purified by a "*cold phenol extraction*" (which seems to remove residual protein impurities), followed by a "*fractional ethanol precipitation*" (which removes residual nucleic acids, see point 29 above). Lastly, the samples are run over a "*CL-4B Sepharose column*", i.e. a HIC column, to obtain purified Pn12F that "*contained less than 1% (wt/wt) protein or nucleic acids*" (see page 2292, column 2, last paragraph to page 2293, column 1, first paragraph).

40. Thus, although document D19 discloses HIC in a method for the purification of CPS, the purification starts from a commercial product that is pre-purified from impurities that comprise proteins and nucleic acid in a two step procedure, before as a final step HIC is used. However, pointers to omit these pre-purification steps and to perform instead an alkaline extraction of bacterial samples under the conditions recited in claim 1 are not derivable from document D19.

41. Document D4 discloses two methods for purifying antigenic bacterial CPS from group B streptococci. The first method describes the detachment of CPS mediated by the enzyme mutanolysin, an N-acetyl-muraminidase that disrupts the cell walls, followed by a DNase, RNase and pronase treatment of the samples to degrade DNA, RNA and protein impurities, respectively. In a last step, the solution is loaded on a DEAE-Sephacel™ column (i.e. an anion exchange chromatographic material) which binds to the negatively charged sialic acid residues of the CPS, while the protein and nucleic acid impurities seem to run through (see page 1090, column 1, first paragraph, page 1093, column 1, second and third paragraphs). Regarding the second method, CPS is purified directly from the culture supernatant, based on a fractionated EtOH precipitation, followed by a DNase, RNase and pronase treatment. Again the final step is performed on a DEAE-Sephacel™ column (see page 1090, column 1, second paragraph).

42. Neither the alkaline extraction conditions nor a HIC-based purification of CPS as recited in claim 1 are disclosed or suggested in document D4.

43. Therefore, the claimed method cannot be considered obvious for the skilled person in the light of the

combined teachings of documents D9 and D4 or D19 either.

44. Accordingly, the board concludes that the method of claim 1 is based on an inventive step and that, hence, auxiliary request C meets the requirements of Article 56 EPC.

Order

For these reasons it is decided that:

The appeal is dismissed

The Registrar: The Chairman:

L. Malécot-Grob B. Stolz

Decision electronically authenticated