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Datasheet for the decision of 24 April 2008

Case Number: T 1844/06 - 3.3.04

Application Number: 00108605.7

Publication Number: 1048738

IPC: C12P 13/04

Language of the proceedings: EN

Title of invention:
A process for the preparation of phosphatidylserines

Patentee:
CHEMI S.p.A.

Opponent:
Fidia Farmaceutici S.p.A.

Headword:
Phosphatidylserines/CHEMI S.p.A.

Relevant legal provisions:
EPC Art. 54, 56, 83, 115(1)

Keyword:
"Main request: novelty (yes), inventive step (yes), sufficiency of disclosure (yes)"

Decisions cited:
T 0305/87

Catchword:
-

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Case Number: T 1844/06 **-** 3.3.04

D E C I S I O N
of the Technical Board of Appeal 3.3.04
of 24 April 2008

Appellant: (Opponent)
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Decision under appeal:
Decision of the Opposition Division of the European Patent Office posted 26 July 2006 rejecting the opposition filed against European patent No. 1048738 pursuant to Article 102(2) EPC 1973.

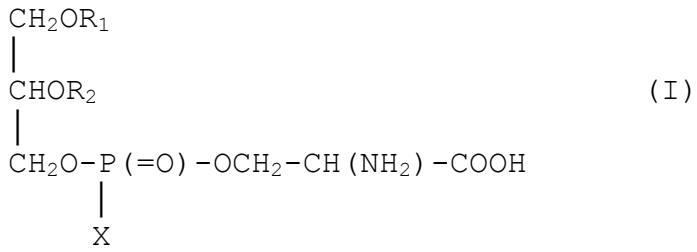
Composition of the Board:

Chair: U. Kinkeldey
Members: R. Gramaglia
R. Moufang

Summary of Facts and Submissions

I. European Patent No. 1 048 738 based on application No. 00 108 605.7 and having the title "A process for the preparation of phosphatidylserines" was granted on the basis of 22 claims, of which claim 1 read as follows:

"1. A process for the preparation of phosphatidylserines of formula (I)



(I)

in which R_1 and R_2 are independently saturated, monounsaturated or polyunsaturated acyl C_{10}-C_{30}, X = OH or OM, wherein M = alkali or alkaline-earth metal, ammonium, alkylammonium (including the inner salt), comprising the reaction of phosphatides of general formula (II)

(II)

in which R_1, R_2 and X have the meanings defined above and R_3 = CH_2-CH_2-NH_2 or CH_2-CH_2-$N^+(CH_3)_3$, with racemic or enantiomerically pure serine, preferably with (L)-

serine, in the presence of a phospholipase D (PLD), characterized in that the reaction medium is an aqueous dispersion and in that the reaction is carried out in presence of one or more surfactants in amounts lower than 0.4 g per gram of phosphatides."

Claims 2 to 22 related to specific embodiments of the process of claim 1.

II. Notice of opposition was filed by the opponent requesting the revocation of the European patent on the grounds of Articles 100 (a) and (b) EPC for lack of novelty, lack of inventive step and insufficiency of disclosure. By a decision dated 26 July 2006 the opposition division rejected the opposition.

III. The appellant (opponent) lodged an appeal against the decision of the opposition division.

IV. On 18 February 2008 a third party filed observations according to Article 115(1) EPC and cited document A1 in annex.

V. Oral proceedings were held on 24 April 2008.

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

A1 Salvador G.A. et al., Lipids, Vol. 33, No. 9, pages 853-860 (1998);

D1 Comfurius P. et al., Journal of Lipid Research, Vol. 31, pages 1719-1721 (1990)

D3 JPO5-42917

D6 Lichtenberg D., Biochim. Biophys. Acta, Vol. 821, pages 470-478 (1985);

D7 Properties of Detergents from Dr Shaun D. Black, last update June 1998;

D9 Heller M, Adv. Lip. Res., Vol. 16, pages 267-326 (1978);

D17 Wade A. and Weller P.J., Handbook of Pharmaceutical Excipients, Second Edition, pages 375-378 and 173-174 (1994);

D21 D'Arrigo P. et al., J. Chem. Soc., Perkin Trans. I, pages 2651-2656 (1996);

D30 EP-B-1 231 213.

VII. The appellant's arguments in writing and during the oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Novelty
Document D3

– This document disclosed a process for the preparation of phosphatidylserines of formula (I), comprising the reaction of phosphatides of general formula (II) with serine, in the presence of a phospholipase D (PLD), wherein the reaction medium could be water alone. However, putting an insoluble phospholipid into "water alone" would

automatically yield an aqueous dispersion. Therefore, document D3 anticipated the process of claim 1.

Document D9

– This document showed the general equation for a transphosphatidylation reaction and stated that the conditions for a transphosphatidylation reaction were similar to those described for hydrolysis. As regards hydrolysis, document D9 prescribed an aqueous solution as reaction medium and gave instructions to mix phosphatidylcholine (PC) and the detergent SDS in molar ratios ranging from 1 to 3. Hence, document D9 anticipated the process of claim 1.

Inventive step

– The reaction medium described in document D1 was an aqueous dispersion. Hence, the only difference between the process of claim 1 and that described in document D1 lay with the amount of surfactant per gram of phosphatide. In view of this difference, the problem underlying the contested patent was the provision of a process for the preparation of phosphatidylserine on an industrial scale that overcame the drawbacks of the prior art by e.g. avoiding the use of an excessive quantity of detergents and their recovery. The solution lay with using a lower amount of surfactant in comparison to document D1.

– In the case the CMC (critical micellar concentration) of a surfactant was lower than the CMC's of the surfactants used in Table 1 of document D1 (deoxycholate and octylglucoside), it would be obvious to the skilled person to reduce the amount of surfactant and arrive at the range stated in claim 1. This view was supported by Fig. 4 at page 857 of documents A1, showing that a surfactant was not a promoter of the reaction at any concentration but only within a specific range, around its CMC value.

– Document D3 taught the use of "water alone", whereas document D9 suggested an "aqueous dispersion". Therefore, the skilled person would have gone into the direction of an aqueous dispersion for solving the above problem.

– Present claim 1 covered non-inventive embodiments that did not solve the underlying technical problem:

  – The process of claim 1 failed in the case of surfactants having a high CMC such a octylglycoside, used in amounts <0.4 g/gram of phosphatides. This was shown by the comparative test (submission dated 11 June 2004, page 14) involving 0.32 g octylglycoside/gram phosphatide.

  – The process of claim 1 also failed in the case of phospholipases D other that the exemplified one (Streptomyces ATCC 55717),

or in the case of low concentrations of the enzyme. This was shown by a comparative test.

Sufficiency of disclosure

– The patent did not provide sufficient information for the skilled person to successfully carry out the process within the whole range of claim 1, covering any phospholipase D and/or any enzyme concentration.

VIII. The respondent's arguments in writing and during the oral proceedings, insofar as they are relevant to the present decision, may be summarized as follows:

Novelty
Document D3

– This document did not contain any example in which the claimed reaction was performed in water alone but it only contained examples in which the reaction was performed in a biphasic system consisting of water and diethyl ether in a 1:1 ratio.

Document D9

– This document did not disclose a process for manufacturing phosphatidylserine (PS) by transphosphatidylation in an aqueous dispersion in the presence of surfactant at a concentration lower than 0.4g/gram of phosphatide.

– The few transphosphatidylation reactions described in document D9 were carried out in ether.

Inventive step

– The differences over document D1 were the scaling up and the reaction medium being an aqueous dispersion. The technical problem was to be seen in a simpler way to recover the final product by means of a filtration and not with solvents.

– There was no suggestion in the prior art documents which would have induced the skilled man wishing to solve the above technical problem to modify the process described in document D1 as done in the patent in suit.

– Document D1 taught away from turning to an aqueous dispersion. Document D17 was not relevant to the present case. Document D3 taught that water should be kept under 10% to avoid side-reactions.

– Document A1 related to a different reaction since ethanol instead of serine was added to phosphatidylcholine, to yield phosphatidylethanol. But ethanol was much more reactive than serine (see document D21).

Sufficiency of disclosure

– The evidence on file demonstrated that the claimed reaction could be performed without surfactant or using surfactants having different properties and by using PLD from different sources. The data

provided by the appellant confirmed that the claimed reaction could be reproduced without undue burden.

IX. The appellant (opponent) requested that the decision under appeal be set aside and that the patent be revoked.

The respondent (patentee) requested that the appeal be dismissed.

Reasons for the Decision

Novelty

1. To summarize, claim 1 relates to a process for the preparation of phosphatidylserines by means of a transphosphatidylation reaction taking place in a reaction medium which is an aqueous dispersion, in the presence of one or more surfactants at a concentration lower than 0.4 g per gram of phosphatide.

2. One feature of claim 1 relates to the reaction medium, which should be an aqueous dispersion. This expression means that the phospholipids do not undergo complete solubilisation but are under the form of small particles held in water by agitation, the particles being the dispersed phase, while water, i.e., the suspending medium, is the continuous phase. Unlike the case where complete solubilisation occurs, an aqueous dispersion, such as the one described in the patent, can be both filtered (see paragraphs [0029], [0034], [0037], [0040], [0043] and [0050] of the patent) or

decanted (see paragraph [0019] and Example 8: "separatory funnel"). Therefore, interpreting the expression "aqueous dispersion" in claim 1 as meaning "complete solubilisation" would go against the fact that the reaction medium described in the patent can be filtered/decanted (see also paragraph [0030]: "...recovery can be effected by simple filtration" and paragraph [0019]: "...decanting the **suspension**"; emphasis by the board).

The appellant apparently agrees that the reaction medium referred to in claim 1 is a "suspension" since it states in the submissions dated 14 June 2006 (page 6) and 28 November 2006 (paragraph bridging pages 48 and 49) that the "EP'738 process is clearly carried out in suspension, namely with a dispersed phase of particles" (emphasis by the appellant).

3. Another feature of claim 1 is that "the reaction is carried out in presence of one or more surfactants in amounts lower than 0.4 g per gram of phosphatides". The board notes that the function of the surfactant is to promote the dispersion of the substrate and hence the reaction rate (see paragraph [0015]), not to completely solubilise the substrate, i.e., the surfactant is added in "sub-solubilising" amounts. This way to proceed is illustrated by Examples 2, 3, 9 and 10, according to which the surfactant Tween 80® or AOT is added, but "...the solid was filtered" (see paragraphs [0036], [0039], [0050] and [0052]).

Document D3

4. This document describes a process for the preparation of phosphatidylserines of formula (I), comprising the reaction of phosphatides of general formula (II) with serine, in the presence of phospholipase D (PLD). According to page 2, line 10 of this document, the reaction medium can be "water alone". The appellant argues that document D3 anticipates the process of claim 1 because putting an insoluble phospholipid into "water alone" would automatically yield an aqueous dispersion.

5. However, as regards the phospholipids, it is merely stated in document D3 that "...the phosphatidylcholine usable in this invention may be either a natural product... or a synthetic product" (see page 1, lines 4-5 from the bottom). Hence, in the absence of further information about the nature of the phospholipid, the skilled reader would take it that document D3 relates to both water-insoluble and water-soluble phospholipids (depending on the chain length of the fatty acids). Therefore, the board cannot adhere to the appellant's view that once "the insoluble phospholipids of document D3" are put into "water alone" (see page 2, line 10), they would automatically yield an aqueous dispersion. In conclusion, the expression "water alone" in document D3 does not represent a direct and unambiguous disclosure of the feature "aqueous dispersion".

6. Moreover, the board observes that the wording "water alone" is contradicted by page 2, line 19 of document D3, stating that water should be kept under 10% "for

suppression of the side-reaction", and by all the Examples, which use a two-phase water/ether system.

Document D9

7. This document is a review on phospholipase D describing the general conditions under which the hydrolysis and transphosphatidylation processes catalysed by this enzyme can occur. As regards transphosphatidylation, the general equation for such a reaction is shown on page 269, line 5. On page 275, lines 8-9, it is stated that the conditions for a transphosphatidylation reaction are similar to those described for hydrolysis.

8. The appellant maintains that document D9 anticipates the process of claim 1 because this document prescribes for hydrolysis (and hence transphosphatidylation) the use of an aqueous solution as reaction medium (see page 269, line 5 from the bottom) as well as the use (see page 274, first paragraph and lines 7-8) of the detergent SDS and phosphatidylcholine (PC) in molar ratios from 1 to 3 (corresponding to 0.12 g to 0.37 g SDS/1 g of PC), i.e., within the range stated in present claim 1.

9. However, the passage on page 275 merely states that the conditions for transphosphatidylation are similar to those for hydrolysis. This does not mean that they are identical. Further, the passage on page 269, line 5 from the bottom relied on by the appellant merely teaches that the nucleophilic acceptor, i.e. the primary alcohol (e.g. serine) should be dissolved in water. However, the skilled person is not taught that the reaction medium should be water, let alone an

aqueous dispersion. Finally, the appellant combines the above two passages from document D9 with one specific embodiment selected among the three possible reaction mediums proposed by document D9 on pages 273-274, namely the ether system (i.e., a biphasic system), the detergent system and the monolayer system.

10. Even assuming, against the rationale of decision T 305/87 (OJ EPO 1991, 429), that the above three unrelated passages from document D9 can be combined for questioning novelty, as does the appellant, there is still no direct and unambiguous disclosure in document D9 of the feature "aqueous dispersion" stated in present claim 1.

11. In view of the foregoing, the subject-matter of claim 1 and dependent claims 2 to 22 satisfies the requirements of Article 54 EPC.

Inventive step

Closest prior art and problem to be solved

12. The closest prior art is represented by document D1, disclosing a one-phase system for the enzymatic synthesis of phosphatidylserine from phosphatidylcholine catalysed by phospholipase D. There is also a teaching in document D1 to add from 0.5 to 5 g of detergent per gram of phosphatide (see Table 2 on page 1720).

13. In the appellant's opinion, the wordings in document D1 "to disperse the lipids" (see page 1719, r-h column, line 12 and page 1721, l-h column, line 10) and "increasing amounts of PC are dispersed" (see page 1720,

r-h column, line 7) imply that the reaction medium described in this document is an aqueous dispersion. Hence, the appellant maintains that the only difference between the process of claim 1 and that described in document D1 lies with the amount of surfactant per gram of phosphatide (claim 1: "lower than 0.4 g per gram of phosphatides"; document D1: "from 0.5 to 5 grams per gram of phosphatides").

14. In view of this sole difference, the appellant argues that the problem underlying the contested patent is the provision of a process for the preparation of phosphatidylserine on an industrial scale that overcomes the drawbacks of the prior art by e.g. avoiding the use of an excessive quantity of detergents and their recovery (see [0005] to [0008] of the patent). The solution, in the appellant's view, lies with using a lower amount of surfactant in comparison to document D1.

15. However, as admitted by the appellant in the submission dated 11 June 2004 (see page 11), document D1 teaches to use a quantity of detergent sufficient to completely solubilise the lipids. It is indeed expressly stated on page 1720, l-h column, third line under the heading "Results and Discussion" that the detergents act as a "solvent". On page 1720, r-h column, lines 8-9 of document D1, it is further stated that phosphatidylcholine ("PC") should be "solubilized in the form of mixed micelles". Finally, the fact that the resulting lipid mixture is recovered by extraction with solvents (see 1720, l-h column, line 9) rather than by a simple filtration (as in the examples of the patent in suit; see point 2 supra), confirms that the reaction

medium described in this document is a true solution rather than an aqueous dispersion as required by present claim 1.

16. Taking into account this further difference, the problem underlying the contested patent can be seen, in the board's view, as the provision of a process for the preparation of phosphatidylserine of good purity and in highly satisfactory yields, wherein the recovery can be effected by simple filtration without the need for solvents (see paragraphs [0009], [0030] and [0031]), and wherein interfering alcohols, if present, can be eliminated by simple decantation (see paragraphs [0018] and [0019]). The above problem is solved by carrying out the reaction in an aqueous dispersion, wherein one or more surfactants may be present in amounts lower than 0.4 g per gram of phosphatides. In view of the examples in the patent, the board is satisfied that the above problem has been solved. Examples 1 to 11 indeed show that it is possible to make suspensions comprising 45-140 g/l of PC which are converted to PS with yields ranging from 40-88%, compared to the best result (25 g/l PC; 46% yield) described in document D1, obtained with 2% w/v octylglucoside (see page 1720, Table 1 and r-h column, line 8: "25 mg/ml"). Moreover, the examples in the patent illustrate the recovery of phosphatidylserine by filtration (see paragraphs [0029], [0034], [0037], [0040], [0043] and [0050]), avoiding the need for organic solvents for extracting the product, unlike the technique described in document D1 (see 1720, l-h column, line 9). Decantation of the aqueous dispersion to remove interfering ethanol is shown in Example 8 (c.f. "separatory funnel" and "phosphatidylethanol < 0.1%").

17. The relevant question to the inventive step issue is thus whether there was any suggestion in the prior art documents which would have induced the skilled person wishing to solve the underlying technical problem to modify the process described in document D1 as done in the patent in suit. Document D1, the only document before the board disclosing a one-phase system rather than an ether-water biphasic system, prescribes that complete solubilisation of the phosphatide should be achieved (see point 15 supra). On page 1720, r-h column, lines 8-9 of this document, it is further stated that if more phosphatide is added for a given quantity of detergent (10 mg/ml octylglucoside), there is a sudden increase in light scattering (turbidity) due to the formation of bilayer structures, which should be avoided as unworkable. The term "solubilisation" in the field of lipids means the passage from a bilayer structure to a micellar (or mixed micelle) structure, yielding a transparent solution. Vice-versa turbidity is a sign of insolubility (see e.g., document D6, page 471, paragraph bridging l-h and r-h columns). Otherwise stated, sub-solubilizing amounts of surfactants (and hence the formation of an aqueous dispersion) had to be avoided. Therefore, in the board's judgement, document D1 encouraged the skilled person to increase the surfactant content rather than to reduce it. Going against this teaching established by document D1, the examples of the patent demonstrate that the reaction still works if the medium is an aqueous dispersion, either in the absence or in the presence of sub-solubilizing amounts of surfactants. Hence, it must be concluded that the process of present

claim 1 does not follow from the prior art in an obvious way.

18. The appellant relies on documents D3 or D9 for arguing that the skilled person would have gone into the direction of an aqueous dispersion for solving the above problem. It is the appellant's view that document D3 suggests the use of "water alone", whereas document D9 points to an "aqueous dispersion". However, as already emphasised in the context of novelty (see points 5 and 8 to 10 supra), the expression "water alone" in document D3 or the expression "aqueous solutions" in document D9 (see page 269, line 5 from the bottom) do not mean or suggest "aqueous dispersion". The board further observes that document D3 taught that water should be kept under 10% to avoid side-reactions (hydrolysis) while "aqueous solutions" in document D9 related to hydrolysis, not to transphosphatidylation. Under these circumstances, these documents did not point into the direction of an aqueous dispersion as the solution of the problem to be solved.

19. In a different line of argument, the appellant maintains that document D1 relates to surfactants such as DOC (deoxycholate) and octylglucoside having high CMC (critical micellar concentration), the CMC being the concentration of detergent at which mixed micelles form. The appellant argues that if the CMC value of a surfactant (e.g. Tween®, a surfactant highly recommended by document D17) is lower than the CMC's of the surfactants used in Table 1 of document D1, it would be obvious to the skilled person (and also obligatory) to reduce the amount of surfactant and arrive at the range stated in claim 1.

20. In a similar reasoning, the appellant relies on Fig. 4 (see page 857) of documents A1 for arguing that a surfactant was not a promoter of the reaction at any concentration but only within a specific range around its CMC value and that it would be obvious to the skilled person to reduce the amount of surfactant and arrive at the range stated in claim 1, in the case the CMC value of a surfactant is lower than the CMC's of the surfactants used in Table 1 of document D1.

21. Yet, in the board's view, even accepting in the appellant's favour that the skilled person would use, in the light of document D17 or document A1, a surfactant having a lower CMC value and arrive at the surfactant range stated in claim 1, he/she would nevertheless be bound by the fundamental requirement set out in document D1 that the formation of an aqueous dispersion had to be avoided (see point 17 supra). Therefore, he/she would obtain a transparent solution, not an aqueous dispersion as required by present claim 1.

22. In a further line of argument the appellant maintains that the process of claim 1 fails in the case of surfactants having a high CMC such a octylglycoside, used in amounts <0.4 g/gram of phosphatides. To buttress this view, the appellant refers to a comparative test (see submission dated 11 June 2004, page 14) involving 0.32 g octylglycoside/g phosphatide (yields = 38%) and concludes that present claim 1 covers non-inventive embodiments that do not solve the underlying technical problem.

23. The appellant's reasoning behind the above line of argument is that the CMC of a detergent reflects its "solubilising power" in the sense that if a detergent has a low CMC, less detergent is needed to form (soluble) mixed micelles, and vice-versa, when the CMC is high as in the case of octylglycoside (25 mM: see document D7)), more detergent is required to obtain the same effect. Therefore, surfactants having a high CMC such a octylglycoside, used in amounts <0.4 g/gram of phosphatides would, in the appellant's view, not succeed in solubilising the phosphatides and thus the reaction of claim 1 would fail.

24. However, as emphasised under points 2 and 3 supra, the lack of solubilisation of the substrate (not the solubilisation argued by the appellant), regardless of the presence or absence of a detergent, is the important feature of the process of claim 1. Therefore, the above appellant's arguments are neither pertinent nor convincing.

25. Moreover, the appellant views the yields of 38% of its comparative test as a proof that the process of claim 1 fails in the case of surfactants having a high CMC such a octylglycoside, used in amounts <0.4 g/gram of phosphatides. However, according to page 1720, r-h column of document D1, turbidity turns up at 10 mg octylglycoside/25 mg PC, i.e at 0.4 g octylglycoside/gram PC. Since the appellant's comparative test involves less detergent (0.32 g octylglycoside/gram phosphatide), the reaction must take place as an aqueous dispersion, in keeping with the requirement of present claim 1. And indeed, the appellant's yields of 38% are in line with the yields

of 39.1 % of Example 5 of the patent. In conclusion, the appellant's comparative test confirms rather than disproves that the claimed reaction takes place with an amount of surfactant lower than 0.4 g per gram of phosphatides.

26. The appellant also maintains that the process of claim 1 fails in the case of a phospholipases D other than the exemplified one (Streptomyces ATCC 55717), or in the case of a low concentration of the enzyme (see comparative test submitted on 29 November 2006, page 24, Table C). In the board's view, the appellant's test involving Streptomyces hachijoense shows that a phospholipases D other than Streptomyces ATCC 55717 does work. This finding is confirmed by later document D30 (see the Table on page 3), demonstrating that further phospholipases D are able to catalyse the reaction of claim 1. As for the reaction's failure in the case of a low concentration of the enzyme (1 U/g phospholipases D from Streptomyces hachijoense, compared to the 16,100 U used in the appellant's own patent (see document D30, page 4, paragraph [0025])), claim 1 is deemed to only cover "reasonable" situations and exclude instances where the skilled person would use thousand times less enzyme than usually needed.

Sufficiency of disclosure

27. The evidence before the board, including the comparative tests carried out by both the appellant (see e.g. the submission dated 11 June 2004, page 14) and the respondent (see the annex to the submission dated 17 May 2006) and later document D30, shows that yields ranging from about 39% to about 90% can be

obtained, depending on the different reaction conditions, either with or without surfactants having different properties (i.e. Tween® 20, Tween® 80, AOT, octylgucoside, Triton®-X), and using PLD from different sources. The board thus concludes that no case of insufficiency of disclosure has been made out.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chair:

P. Cremona U. Kinkeldey