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D E C I S I O N
of 8 August 1995

Case Number: T 0965/92 - 3.3.2

Application Number: 84107490.9

Publication Number: 0130577

IPC: A61K 9/50

Language of the proceedings: EN

Title of invention:
Method for producing liposomes

Patentee:
DAIICHI SEIYAKU CO., LTD.

Opponent:
Henkel Kommanditgesellschaft auf Aktien

Headword:
Liposomes/DAIICHI SEIYAKU CO. LTD.

Relevant legal provisions:
EPC Art. 54, 56, 83, 84, 123

Keyword:
"Novelty and inventive step of main request - yes - prior art must be read in the light of the common general knowledge available at its publication date"

Decisions cited:
T 0127/85; T 0229/90, T 0219/83

Catchword:
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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0965/92 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 8 August 1995

Appellant: (Opponent)
Henkel
Kommanditgesellschaft auf Aktien
TFP / Patentabteilung
D-40191 Düsseldorf (DE)

Representative:
-

Respondent: (Proprietor of the patent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office dated 28 August 1992 concerning maintenance of European patent No. 0 130 577 in amended form.**

Composition of the Board:

Chairman: F. Antony
Members: U. Oswald
R. E. Teschemacher

Summary of Facts and Submissions

I. European patent No. 0 130 577 relating to a method for producing liposomes was granted on 24 May 1989, with thirteen claims, in response to the European patent application No. 84 107 490.9 filed on 28 June 1984 and claiming priority from Japanese application JP 118006/83 filed on 29 June 1983.

II. Opposition was filed against the granted patent on 12 February 1990.

Of the numerous documents cited during the opposition, only the following remain relevant for the present decision:

(1) US-A-1 995 281,

(2) Prof. Dr. Fritz Gstirner, "Grundstoffe und Verfahren der Arzneibereitung", Ferdinand Enke Verlag Stuttgart (1960), page 365,

(6) J.A.C.S., **101** (1979), pages 5231 to 5234.

III. According to the interlocutory decision of the Opposition Division dated 28 August 1992 the patent could be maintained in amended form on the basis of twelve claims.

Claim 1 reads as follows:

"1. A method for producing liposome preparations which comprises mixing liposome forming membrane components with a water-soluble non-volatile physiologically acceptable organic solvent selected from glycerin, polyglycerin, propylene glycol, polypropylene glycol, polyethylene glycol, maltitol, glycerin esters, benzyl alcohol and mixtures thereof and dispersing the

resulting mixture in an aqueous medium at a temperature not lower than the phase transition temperature of the liposome membrane component."

Claim 12 relates to "Liposome preparations obtainable according to the method of anyone of Claims 1 to 11".

The decision under appeal took the view that the phase transition temperature of the liposome membrane component according to Claim 1 was a commonly accepted technical term in the art and thus the disclosure of the patent in suit was sufficient in terms of Article 83 EPC.

The subject-matter according to Claim 1 was novel over each of the cited documents. In particular, even if liposomes were indeed obtained (which was doubtful) by the Opponent's experiments with the process of document (2), which document was published before the discovery of liposomes, this would at least require a particular selection of the membrane forming component and of the dispersion temperature in the knowledge of the invention.

For the assessment of inventive step, document (6) represented the closest state of the art. It related to a method for producing multi- or singlewalled vesicles having monolayer membranes, using dimethylformamide, a solvent not comprised by Claim 1 of the patent in suit. In the absence of at least one bilayer as a compulsory prerequisite for liposomes, and taking into account the lack of information about a lower temperature limit for the dispersing step, there was no suggestion in document (6) for the preparation of liposomes as defined by Claim 1 of the patent in suit. Since furthermore none of the other prior art documents disclosed a process

which definitely would yield liposomes, the requirement of inventive step was met by Claim 1 and the dependent claims.

The subject-matter of the product-by-process Claim 12 was likewise novel by selection, and its inventive step was based on the uniform particle size of the liposomes obtained, proved by the Opponent's comparative examples and the relevant photos, showing that the particles according to the prior art exhibited a wide distribution in size.

IV. The Appellant (Opponent) lodged an appeal together with reasons on 14 October 1992. Oral proceedings took place on 8 August 1995.

V. In his grounds of appeal and in further submissions, the Appellant maintained his view that the term "phase transition temperature" did not relate to a defined temperature. It was well-known in the art that the phase transition of liposome forming components such as egg lecithin covered a broad temperature range. Accordingly, the patent in suit did not disclose the invention in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art.

The Appellant furthermore denied the novelty of Claim 1 in the light of document (2). Present Claim 1 did not define a particular lipid quality. Taking into account a phase transition temperature of egg lecithin below 0°C and the fact that the commercially available product Ovothin 160 fulfilled the requirements of drug quality for injection purposes, the skilled person following the procedure for the preparation of injection solutions according to document (2) using glycerin as water-soluble non-volatile solvent would inevitably obtain a liposome preparation within the scope of Claim 1. It was

irrelevant whether or not the authors of document (2) were aware of this.

Since the term "vesicle" was used by those skilled in the art in the same sense as the term "liposomes" when describing colloidal phenomena, it was clear that the claimed subject-matter lacked an inventive step with regard to document (6). The monolayer vesicles according to this prior art represented complete analoga to the well-known bilayer vesicles. It was furthermore to be noted that the subject-matter of Claim 1 did not relate to the membrane forming components as such, but to a method of forming liposomes, and that the technical problem as stated in the patent in suit was to improve the known injection method to be suitable for the industrial production of liposomes. In this respect document (6) comprised the essential features of the claimed method, namely the use of a non-volatile water-soluble solvent (DMF) which, after injection of an cationic two-headed amphiphile together with didodecyldimethylammonium bromide or with cholesterol, could form vesicles in hot water. Dimethylformamide was well-known to be used as a solvent in several drugs and could be regarded as comparable with the solvents presently claimed, e.g. benzyl alcohol, in regard to its physiological properties. Since at the priority date of the patent in suit it was well-known that the formation of liposomes would take place preferably above the phase transition temperature, it was obvious to generate the required temperature value by using hot water.

Finally, it was to be noted that according to the patent in suit, column 3, line 56 up to column 4, line 5, the particle size distribution was not controlled by the claimed preparation method as such but was only adjusted by an ultrafiltration method.

Comparative examples representing a repetition of the procedure described in document (2) showed that, in contrast to the Respondent's test results, there was no need to use highly purified membrane lipids instead of commercially available egg-lecithin when preparing liposome products. The requirement to use highly purified lecithin was also in contrast to certain statements in the patent in suit according to which the membrane component could comprise auxiliary materials such as antioxidants, and was not in conformity with the intended use of the liposomes to encapsulate drugs.

VI. The Respondent contested these arguments and filed on 19 October 1993 a set of Claims 1 to 12 corresponding to Claims 1 to 12 on which the disputed decision had been based. Furthermore, the Respondent submitted an amendment to column 3 of the description of the patent specification in addition to the amendments already made during the proceedings before the Opposition Division. The Respondent took the view that a definition of the term "phase transition temperature" could be found in the entire literature cited in this regard, and that such temperature values, in particular for lipid components could be looked up in most of the publications related thereto. If, however, a particular phase transition temperature was not known, it could be easily determined by a person skilled in the art by calorimetric methods. There was thus no valid objection under Article 100(b) EPC.

As regards novelty, not all available lipids were liposome-forming membrane components. This was demonstrated by comparative examples based on the use of lecithins with different phospholipid purities.

When assessing inventive step in the light of the disclosure of document (6), the Appellant did not

evaluate the content of this scientific paper in an objective manner. A person skilled in the art would not consider its teaching in order to solve the problem underlying the patent in suit, namely to provide an improved, industrially applicable process for preparing liposome dispersions.

In particular, it had to be emphasized that the dispersion of the amphiphilic compounds of document (6) did not give bilayers and vesicles were only obtained when adding additional compounds.

Surprisingly it had been found that a liposome suspension according to the invention was considerably more stable when compared with a liposome suspension including dimethylformamide. As regards the particle size, it was to be noted that the examples in the patent in suit showed that, without the need of any special equipment or procedures involving the use of additional components, a narrow particle size distribution of the liposomes could be achieved.

VII. The Appellant requested that the decision under appeal be set aside and that the patent be revoked.

The Respondent requested that the appeal be dismissed and that the patent be maintained on the basis of the Claims 1 to 12 as filed with the letter of 19 October 1993 (main request), or on the basis of two sets of claims submitted during oral proceedings (Claims 1 to 12 auxiliary request I, Claims 1 to 11 auxiliary request II).

Reasons for the Decision

1. The appeal is admissible.

2. Claim 1 of the main request is based on Claim 1 originally filed as well as Claim 1 as granted and finds further support on page 3, lines 7 to 16; page 5, lines 3 to 12; page 6, lines 22 to 24 of the original description (col. 1, line 64 up to col. 2, line 10; col. 2, lines 55 to 65 and col. 3, lines 40 to 42 of the patent specification as granted). Claims 2 to 12 correspond to Claims 3 to 13 as granted but renumbered. The claims are of narrower scope than the granted claims. The requirements of Article 123(2) and 123(3) EPC are accordingly satisfied.

   The amended description referring to additional cited prior art documents and adapted to the amendments of Claim 1 also fulfils the requirements of Article 123(2) EPC.

3. The worked examples of the description originally filed and correspondingly those of the patent specification unequivocally contain the required information about the phase transition temperature of the liposome forming membrane component used. Furthermore, the said phase transition temperature Tc is a well-known thermodynamic parameter which can either be taken from the literature or determined, e.g. by differential scanning calorimetry. This scanning method provides a graphic plot of the heat absorption of the test sample versus the temperature as measurable variable. The final plot shows an endothermic peak. Tmax is the temperature at which maximum heat uptake occurs and Tc is read at both ends of the endothermic peak.

Given this clear-cut situation, the Board cannot see how the mere fact that phase transition may occur within a certain temperature range could make the patent in suit objectionable under Article 100(b) (insufficiency of disclosure of the invention).

Accordingly, in the absence of counterevidence from the Appellant, showing that either the Tc values according to the worked examples of the patent in suit are incorrect or that the Tc values of membrane forming components, obtained for example from calorimetric methods, are not suitable as characterising parameters within the scope of the claimed invention, the Board is convinced that the requirements of Article 83 EPC have been satisfied.

4. Although neither contested in the proceedings, nor a ground for opposition according to Article 100 EPC, it is the Board's duty to ensure that amended Claim 1 satisfies the requirements of Article 84 EPC and indeed all other articles of the EPC.

Having regard to the functional nature of certain parameters of Claim 1, in particular "liposome preparations" and "liposome forming components", the objection made under Article 100(b) in relation to the sufficiency of the disclosure, and at least part of the objections with respect to lack of novelty in view of an alleged non-restrictive meaning of said functional definitions are inevitably linked to the question of clarity of these features within the meaning of Article 84 EPC insofar as these articles are related to the technical contribution made by the invention (cf. T 127/85, OJ EPO 1989, 271, points 2.1 to 2.3 of the reasons).

Both these parameters of Claim 1 are addressed to the skilled person, who does not rely solely on the literal meaning of the text, but is well aware that liposome preparations within the scope of the invention are aggregates (vesicles) in which an aqueous volume is entirely enclosed by a membrane composed of lipid molecules, usually phospholipids forming at least one bilayer membrane. Accordingly, the teaching of Claim 1 considered objectively can only be taken to mean that such **vesicles inevitably form part of a certain solvent system**, and therefore Claim 1 cannot reasonably be construed to relate to a method for producing individual liposomes or separated liposome particles.

Taking the same technical background into account, there is no doubt that Claim 1 is intended to relate to preparations or solvent systems in which most of the aggregates form stable liposomes as opposed to undefined dispersed particles. Any doubts that might nonetheless occur to the mind of a skilled person would be removed by the description of the patent in suit.

5. The Board cannot accept the Appellant's position that the preparation of the egg-lecithin suspension according to document (2), page 365 under the heading "Lecithinum", would destroy the novelty of the subject-matter of Claim 1. It is not denied that this prior art discloses the process steps of grinding 1 kg egg-lecithin with 1.5 kg glycerin and subsequently slowly dispersing the resulting lecithin/glycerin mixture in 7.5 kg physiological sodium chloride solution, to form a suspension containing about 10 per cent lecithin, the said suspension being further treated to be suitable as aseptic injection solution for medical purposes. However, in document (2) there is no mention of any specific temperature or even a temperature limit for dispersing the lecithin/glycerin mixture in the

physiological sodium chloride solution nor is there any information about the aggregate structure of the suspended particles.

In this respect the Appellant argued that the method of document (2) using the commercially available and for pharmaceutical applications accepted egg-lecithin Ovothin 160 at a dispersing temperature of 20°C (which is not lower than the phase transition temperature of this lecithin), would inevitably yield liposomes; the presence of such aggregates was said to be proven by photos taken from comparative examples.

The Respondent, on the other hand, contested the relevance of the Appellant's comparative examples with respect to the use of Ovothin 160. This component was not only selected with hindsight in the knowledge of the invention, but comparative examples and photos would show that the formation of liposomes depended on the purity of the lecithin product used and that Ovothin 160 could not generally be considered to be a liposome forming membrane component within the scope of the invention.

Independent from this aspect, and as will become entirely clear from point 7.2 below, the Board is unable on the strength of its own specialised knowledge to espouse the assertions of either the Appellant or the Respondent as to an **absolutely unequivocal meaning of the presented photos.** In the judgement of the Board, it is then the party whose argument rests on these alleged facts who loses thereby (cf. T 219/83, OJ EPO 1986, 211, in particular point 12 of the reasons.

In the absence of an **unequivocal proof** that the method of document (2) would **inevitably** yield liposomes, it is therefore decided that document (2) must be read in the

light of the common general knowledge available at its publication date in 1960, namely representing a disclosure of a method of producing a lecithin suspension. Common general knowledge about liposomes, which only became available in 1968, cannot be used to interpret such a document when deciding on the question of novelty within the meaning of Article 54 EPC (cf. T 229/90 of 28 October 1992, last but two paragraph in point 4 of the reasons.

The same reasoning would apply mutatis mutandis to the numerous other documents relating in general to methods for suspending or dispersing lipid components but published before the discovery of liposomes. The novelty of the subject-matter of Claim 1 can accordingly be recognized.

6. Document (6) was accepted by the Opposition Division and each of the parties as representing the closest state of the art. The Board sees no strong enough reason to deviate from this point of view.

6.1 This document is a scientific paper entitled "Formation of Stable Monolayer Membranes and Related Structures in Dilute Aqueous Solution from Two-Headed Ammonium Amphiphiles". Experimental studies were said to show that the hydrophobic portion of a particular group of bilayer-forming amphiphiles can be replaced by a single-chain unit which contains a rigid segment. Lamellar and rod-like structures are formed in water spontaneously for example from condensation products of 10-(p-formyl-phenoxy)decyltrimethylammonium bromide and 10-(p-amino-phenoxy)decyltrimethylammonium bromide, compound **1**, or 1,10-bis(p-aminophenoxy)decane and 10-(p-formyl-phenoxy)decyltrimethylammonium bromide, compound **3**. The aqueous solution is prepared by a sonication method forming solutions "very similar to that of aqueous

solutions of lecithin liposomes", or by an injection method. In the latter, 10 mg of the amphiphiles is dissolved in dimethylformamide and the solution is injected by a syringe into 10 ml of hot water and sonicated for 2 to 5 minutes. The aggregates formed in the dilute aqueous solution are described as huge and as stably dispersed in water for at least several weeks. It is then stated that "the preparative method of the sample solutions (sonication or injection) did not affect the aggregate structure" (cf. page 5231, right column; page 5232 left column; same page, right column, first paragraph, lines 1 to 4, second paragraph, lines 4 to 8, third paragraph, lines 9 to 10; page 5233, Table I; and page 5234, left column "Amphiphile 1-4").

Further experiments were said to demonstrate that the so-called rigid-looking lamellar structure of compound **1** can be transformed into multiwalled vesicles by cosonication with didodecyldimethylammonium bromide or into single-walled vesicles by cosonication with cholesterol. The vesicles are 1000 Å to 3000 Å in diameter, and their membrane thickness is at least 60 Å to 70 Å (cf. page 5233, left column and Figure 2).

6.2 In the light of the said prior art, the technical problem underlying the patent in suit can be seen in providing a simplified and industrially useful method for producing liposome preparations in which the liposomes are suitable to encapsulate a drug.

The problem is solved by the method according to Claim 1 (see paragraph III above). According to the experimental evidence in the description of the patent in suit, the Board is convinced that any special equipment or additional procedure such as cosonication with so-called second components is not required to produce liposome preparations comprising mainly vesicles spherical and

uniform in size and with an encapsulation efficiency suitable for industriel large scale production. Moreover, the Respondent submitted, with letter dated 19 October 1993, further evidence demonstrating that liposome preparations with encapsulated glucose using dimethylformamide instead of glycerin, a solvent according to the invention, are less stable. The glycerin containing preparation shows a leakage of only 8.5%, whereas one containing dimethylformamide (representing the solvent of document (6)) shows a leakage of 39.7%. Accordingly, the Board is satisfied that the problem has indeed been solved.

7. It remains to be established whether the proposed solution involves an inventive step.

7.1 Document (6) without any dispute describes an injection method in which a water-soluble non-volatile organic solvent, namely dimethylformamide, is not removed before determination of the aggregate structure of different amphiphiles. However, the discussion of the experimental results show, that the preparation method did not affect the aggregate structure, but that, using the injection method vesicles could only be obtained by carrying out further steps, i.e. cosonication with other compounds, whereas there is the possibility to directly obtain a solution similar to lecithin liposomes only using a sonication method. Under these circumstances, with the existing problem in mind, there was no reason for a person skilled in the art to expect that, under selected solvent and temperature conditions, liposomes could be obtained by the injection method without any further step. In other words, there was no incentive to modify the said injection method in a direction towards the claimed solution. The skilled person's attention was clearly drawn into the direction of sonication methods as being seemingly more promising to form liposomes, and

was led away from the idea to modify the water-soluble non-volatile organic solvent.

7.2 Document (2), as discussed under point 5 above, represents prior art published before the discovery of liposomes. It neither mentions the problem of forming vesicles, nor does it comprise any hint to encapsulate drugs into lipid containing dispersions; hence it could neither alone, nor in combination with document (6) lead the skilled person into the direction of the claimed solution.

7.3 Apart from a brief reference to document (1) during the oral proceedings as background, the Appellant has no longer relied on the other prior art cited during the previous proceedings, and such art is deemed to be of less relevance than the documents discussed above.

7.4 The subject-matter of Claim 1 and of the dependent Claims 2 to 11 according to the main request therefore involves an inventive step.

7.5 The closest state of the art with regard to the liposome preparations according to Claim 12 is again document (6).

7.6 In the light of the said prior art, the underlying technical problem can be seen in providing preparations of liposomes suitable to encapsulate a drug and obtainable on an industrial scale for pharmaceutical applications (cf. description of the patent in suit col. 2, lines 13 to 24).

This problem is to be solved by the liposome preparations obtainable according to Claim 1 and comprising a defined solvent system.

For the same reasons as set out under paragraph 6.2 above, the Board is convinced that also this problem has been solved in a plausible manner.

7.7 It follows from the discussion under points 4 and 5 above that not only the process of Claim 1, but also the **thermodynamic systems** in the sense of liposome preparations which are obtainable thereby are novel within the meaning of Article 54 EPC.

7.8 The Respondent has demonstrated to the Board's satisfaction that liposome preparations had not so far been available in comparable quality, i.e. with an encapsulation efficiency as proved by the worked examples of the patent in suit accompanied by an extraordinary narrow particle size distribution never achieved by known liposome preparations, which properties are essential for the use of liposomes in pharmaceutical applications. Although the Appellant has shown that vesicles in other systems such as known from document (6) may exist with particles having nearly the same absolute size as presently claimed, there is no evidence showing that the known liposome preparations exhibit an overall **particle size distribution** making them suitable for pharmaceutical applications. If the state of the art had been capable of producing liposome preparations with a quality like the one achieved according to the teaching of the patent in suit, this would have been reflected in the technical literature or in technical knowledge of some other form capable of being substantiated.

7.9 It is accordingly the Board's view that the subject-matter of the product-by-process claim of the main request would not have been obvious from either citation taken singly or in combination. Thus Claim 12 also satisfies the requirements of Article 56 EPC.

8. Since the Board have found the Respondent's main request to be allowable, it is not necessary to consider the auxiliary requests.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to maintain the patent in the following version:

Claims:
No. 1 to 12 filed with the letter of 19 October 1993,

Description:
Of the patent specification as amended in the proceedings before the Opposition Division, and with the further amendment in column 3 received during oral proceedings.

The Registrar: The Chairman:

P. Martorana F. Antony