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D E C I S I O N
of 17 September 1999

Case Number: T 0195/96 - 3.3.2

Application Number: 86116943.1

Publication Number: 0224934

IPC: A61K 49/00

Language of the proceedings: EN

Title of invention:
Contrast agent, process for its preparation and its use for ultrasonic imaging

Patentee:
Feinstein, Steven B.

Opponent:
Andaris Limited

Headword:
Sonicated microbubbles/FEINSTEIN

Relevant legal provisions:
EPC Art. 54, 56

Keyword:
"Novelty (yes): no explicit or implicit disclosure of the claimed subject-matter"
"Inventive step (yes): underlying technical problem: providing an "improved" contrast agent not simply an "alternative".

Decisions cited:
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Catchword:

-

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Case Number: T 0195/96 - 3.3.2

D E C I S I O N
of the Technical Board of Appeal 3.3.2
of 17 September 1999

Appellant: (Opponent)
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 15 January 1996 rejecting the opposition filed against European patent No. 0 224 934 pursuant to Article 102(2) EPC.**

Composition of the Board:

Chairman: P. A. M. Lançon
Members: C. Germinario
C. Rennie-Smith

Summary of Facts and Submissions

I. European Patent No. 0 224 934 was granted pursuant to European patent application No. 86 116 943.1 on the basis of a set of 6 claims for all the designated Contracting States except AT and an additional set of 6 claims for AT.

The text of claim 1 of the first set of claims reads:

"A stabilized ultrasonic imaging agent obtainable by preparing an aqueous solution of protein or derivatives thereof, subjecting said solution to high frequency sonication to form a dispersion of microbubbles of relatively uniform size therein, the sonication heating said solution and the dispersed bubbles to denature portions of the protein and thereby encapsulate the microbubbles."

II. Notice of opposition was filed by Delta Biotechnology, which later assigned the benefit of the right to pursue the opposition to Andaris Ltd (appellant), requesting revocation of the patent in its entirety on the grounds of lack of sufficiency of disclosure, novelty and inventive step.

The following documents were cited, *inter alia*, during the proceedings before the opposition division:

(1) WO 84/02838

(2) US Patent 4 247 406

(3) Ultrasonic Imaging, vol. 2, pages 67 to 77 (1980)

(4) Radiology, vol. 143, pages 747 to 750, June 1982

(6) US Patent 4 466 442

(7) US Patent 4 844 882 (not a prior art document)

(10) American Heart Journal, vol. 114, No. 3, September 1987, pages 570 to 575 (not a prior art document)

III. The opposition division decided that the subject-matter of the patent in suit met the requirements of sufficiency of disclosure, novelty and inventive step and therefore rejected the opposition under Article 102(2) EPC.

IV. The appellant lodged an appeal against this decision. Oral proceedings were held on 17 September 1999. The appellant, having withdrawn in writing its request for oral proceedings, was not represented.

In the statement setting out the grounds of appeal, the appellant firstly objected to the novelty of claim 1 with regard to claim 10 of document (1). While this document mainly referred to two independent embodiments, namely sonicated microbubbles of saccharide nature, and solid particles of amino acid polymer matrix, claim 10 represented, in the appellant's opinion, a further independent embodiment bringing together different features from the previous two and relating to microparticles of amino acid polymer matrix containing a gas such as air. Since the only process taught in (1) for preparing air-containing agents was the sonication method, this technique would have been used by the skilled person to implement the

teaching of claim 10, producing as a result the same microbubbles as in claim 1.

The appellant further relied on document (2), either as an independent prior art document or in the context of (1), in which it was incorporated by reference. This document disclosed, in the appellant's contention, the production by sonication of solid or semi-solid microspheres of albumin comprising large vacuoles. Since the patent in suit did not prescribe any particular sonication regime in order to encapsulate the microbubbles, it was argued that the effect of the sonication in (2) was to obtain microcapsules identical to the microbubbles of claim 1. Similar arguments had been produced before the opposition division in relation to document (3).

In considering inventive step, the appellant firstly indicated document (1) as the closest prior art, which on its own deprived the claimed subject-matter of any inventive merit.

Relying on that part of (1) describing the preparation of sonicated microbubbles, and more specifically the viscous solutions to be subjected to sonication, the appellant stressed that the expression "*and the like*" in the passage on page 4: "*a viscous solution (eg, 70% Dextrose, 50% Dextrose, 70% Sorbitol, Renogratin-76, mixtures of these agents and the like) is subjected to high frequency... ultrasonic energy*" had to be interpreted as referring to the "viscosity" of the solution and not, as asserted by the opposition division, to the chemical nature of the dissolved substance (ie saccharide derivatives). Since the

skilled person was well aware that proteins, such as gelatine, formed viscous and biocompatible solutions, he or she would have considered solutions of the proteins already cited in (1), ie gelatin, albumin or haemoglobin as the first and most obvious candidates to subject to sonication in order to produce smaller and more uniform microbubbles. The same conclusions would also have been reached by the skilled person when considering the combination of document (1) with documents (3) (4) or (6), which all disclosed viscous biocompatible protein solutions (collagen and albumin) already employed in ultrasonic imaging.

In a further line of argument, the appellant alternatively suggested document (4) as possibly the closest prior art. This document disclosed microbubbles obtained by simple mechanical agitation of viscous gelatin solutions but suffering from the drawback of a large size and lack of homogeneity. Thus the skilled person, without any inventive activity, would have submitted the gelatin solution of (4) to the sonication technique described in (1), which, in the appellant's argument, was not only known to be able to solve that kind of problems but, at that time, was the only known technique able to achieve that goal.

V. During the oral proceedings, the respondent defined the meaning of "microbubble" and "encapsulated microbubbles" as it would be understood by the skilled person, and stressed the difference between the "microbubble" of the invention and the "microparticles" or "microspheres" cited in the prior art documents. The respondent specifically emphasised how the different methods of preparation would result in structurally

different products and concluded, on this basis, that neither (1) nor (2), whether taken alone or in combination, nor (3) could result in the claimed microbubbles. Thus none of these documents could affect the novelty of claim 1.

As regards inventive step, the respondent specifically addressed itself to the denaturation of the dissolved protein occurring during sonication of the solution. It maintained that, regardless of whether document (1) or (4) was considered as the closest prior art, neither of them, alone or in combination with any other cited document, could suggest to the skilled person that sonication would have caused protein denaturation to such an extent as to produce a precipitation on the surface of the microbubble thereby forming a "wall" protecting each single microbubble.

VII. The appellant requested that the decision of the opposition division be set aside and the patent be revoked.

The respondent requested that the appeal be dismissed

Reasons for the Decision

1. The appeal is admissible.

2. In the proceedings before the opposition division, the objection of lack of sufficiency of disclosure was raised by the opponent. That objection was not pursued by the appellant during the appeal proceedings.

The Board considers that the invention is disclosed in the patent description (as it was in the original application) in a manner sufficiently clear and complete for it to be carried out by a person skilled in the art. For this reason the objection is not upheld.

3. *Novelty*

3.1 Novelty was firstly objected to by reference to document (1). This document describes a method of ultrasonic imaging with contrast agents. Two main embodiments of the invention are disclosed in (1). In the first, a viscous solution is subjected to high frequency ultrasonic energy resulting in the production of microbubbles, having a diameter of about 6 to 20 micron, to be used as a contrast agent. In the second embodiment, solid or semi-solid biodegradable microparticles formed from an amino acid solid matrix and comprising metallic particles as ultrasound image enhancing material are employed as the contrast agent. For the preparation of these microparticles, the reader is referred to the teaching of document (2), which is incorporated in (1) by reference. As admitted by the appellant, neither of these two embodiments is in itself prejudicial to the novelty of claim 1.

According to independent claim 10 of (1), the microparticles made of an amino acid polymer matrix may comprise, as enhancing material, not only the metallic-particles characteristic of the second embodiment, but also, as an alternative, air, nitrogen or carbon dioxide.

In the appellant's contention, claim 10 in itself represented a further independent embodiment of the invention of (1) different from this aforementioned second embodiment since this latter was expressly said in the description not to contain trapped air (last paragraph of page 8). In order to realise the subject-matter of claim 10 in practice, the skilled person would have taken the step of sonication, thereby obtaining the same microbubbles claimed in the patent in suit.

The Board cannot accept these arguments. In fact, although claim 10 certainly envisages the possibility that a gas be included in the microparticles as an ultrasound image enhancing material, the claim recites each and all of the features of the solid metal-containing microparticles of the second embodiment, indicating that the claim simply relates to a slight variation of this latter. The very word "microparticle" used in claim 10 indicates, in the Board's opinion, a grain of solid or semi-solid material which is not equivalent to a "microbubble", even if comprising some air entrapped in the interstices of its polymeric matrix. That some air or other gas may remain entrapped in the amino acid polymer matrix cited in claim 10 is also evident from the teaching in document (2), which discloses the preparation method for such microparticles. Figure 1 and Example 1 of this prior document illustrate an electron microphotograph of the "microsphere" so obtained, and indicate that a few vacuoles may be present within the solid matrix of denatured albumin. For this reason, the Board is convinced that the microparticles of claim 10 are indeed the same solid microspheres of (2), and that

their solid nature is not changed by the exceptional presence of some vacuoles probably containing gas.

On the other hand the respondent plausibly argued during the oral proceedings that claim 1 of the patent in suit relates to microbubbles encapsulated as single units and that the skilled person would easily be able to distinguish microbubbles from solid microparticles simply on the basis of their physical properties, for example their density. The Board has no reason to dispute these arguments which are thus accepted.

3.2 Document (2) was also relied upon, as independent prior art, for a further novelty objection. The document disclosed, in the appellant's contentions, a sonication process applied to albumin solution to produce generally uniform microcapsules, among which at least some were hollow even though the process also produced solid matrices. Moreover, making reference to experiments allegedly conducted into the sonication process of the opposed patent, it maintained that such a process produced the same mixed population of microcapsules and solid material as obtained in (2). For this reason the product obtained in (2) was comprised in the scope of claim 1 of the patent in suit.

The Board notes, first of all, that the microspheres of (2) are produced by emulsifying a protein solution (human serum albumin according to Example 1) into an oily phase. The water-in-oil emulsion is then treated by sonication at 4°C to reduce the size of the dispersed droplets and homogenize them (see column 5, lines 47 to 54 and Example 1). The oil is then removed

by subsequent washing and the microspheres are hardened by formaldehyde treatment. In the Board's judgement a sonication step intended to reduce the size of the internal aqueous phase of an emulsion, the external being oily, has a very limited efficacy in entrapping air in that internal phase. Therefore it cannot be compared with the sonication of an monophase aqueous solution in order to entrap air, which is in direct contact with such a solution, in the form of microbubbles. Indeed, examination by electron microscopy of the microspheres of (2) confirmed that most microcapsules appeared to have a substantially solid albumin matrix, though only some of them appeared to contain vacuoles (see column 8, lines 27 to 32). It cannot even be ascertained from the document whether or not such vacuoles were filled with air or with droplets of the oily phase.

On the other hand, the appellant did not provide the Board with any results or evidence to substantiate its allegation that the ultrasonic imaging agent of the patent in suit consisted of a solid matrix of denatured protein entrapping some vacuoles, rather than encapsulated individual microbubbles, as appears more likely from the wording of the claim and as was stressed by the respondent. The Board therefore holds that document (2) is not prejudicial to the novelty of claim 1.

3.3 Finally, the novelty of claim 1 was objected to in relation to document (3). This document supplies the same kind of teaching as document (2), with the difference that the disclosed collagen microspheres, obtained as the internal phase of a sonicated water-in-

oil emulsion, are solidified by heat treatment (see Collagen microsphere preparation, page 68). Moreover, the microspheres so produced do not contain scattering centres (metallic particles) or gas bubbles, which are expressly avoided because of the several problems involved in their use (see Introduction). For these reasons document (3) has no relevance for the purpose of novelty.

In view of the foregoing, the Board's decision is that none of the cited document is prejudicial to the novelty of the subject-matter of claims 1 and 2 to 6 which depend thereon.

4. Inventive step

4.1 Document (1) was first indicated by the parties as the most pertinent document, though, in a second line of argument, the appellant also discussed each one of (3), (4) or (6) as a suitable starting point in the examination of inventive step.

Without prejudice to the other documents, which will also be considered later in the decision, the Board shares the opinion that (1) indeed represents the closest prior art.

This document discloses, according to a first embodiment of the invention, that "a viscous solution (eg, 70% Dextrose, 50% Dextrose, 70% Sorbitol, Renogratin-76, mixtures of these agents and the like) is subjected to high frequency (5.000 to 30.000 Hz) ultrasonic energy. As a result, microbubbles having a diameter of approximately 6 to 20 microns are

produced." (see page 4, last paragraph, page 5 first paragraph, page 10, second complete paragraph, page 12 second paragraph). As admitted by the appellant, the document does not explicitly contemplate the use proteins in this embodiment.

As a second embodiment of the invention, the document describes microparticles made from an amino acid polymer matrix containing ultrasound image enhancing material, such amino acid polymer being albumin or haemoglobin.

As indicated in the description of the opposed patent, the microbubbles disclosed in (1) had a short life lasting from a few minutes to a few hours, whereas the stabilized microbubbles of the present invention are said to exist for 48 hours or more (patent disclosure, page 4, lines 49 to 51).

4.2 Accordingly, the underlying technical problem to be solved by the invention was to provide more stable microbubbles as ultrasonic imaging contrast agents. The solution proposed by the patent is the stabilized encapsulated microbubbles of claim 1 obtained by subjecting an aqueous solution of protein or derivative thereof to high frequency sonication.

4.3 No detailed experimental results showing such higher stability have been produced during the proceedings. However, document (7), which is a late published US patent (1989) concerning the preparation of a microbubble-type ultrasonic imaging agent, and not originating from the same proprietor/inventor as the opposed patent (Feinstein Steven), offers a detailed

review of the development of the microbubble-type contrast agents for ultrasonic imaging until 1989. The document acknowledges, in column 1, line 65 to line 20 of column 2, that:

"Using viscous aqueous solutions, such as 70% sorbitol or dextrose, Dr. Feinstein produced a dispersion of microbubbles by high energy sonication of the solutions... The persistence of the microbubbles, although of the order of **a few minutes**, permitted the imaging agent to be prepared and administered intravenously for heart imaging." (Reference is made to US Patent 4,572,203 which corresponds to document (1))

and

"Subsequently, Dr Feinstein sought to improve the persistence of the microbubbles. He found that by sonication of a heat-sensitive protein, such as albumin, microbubbles of improved stability were obtained. ... The microbubbles persisted **for 24 to 48 hours**".

On this basis, the Board is convinced that the technical problem has actually been solved by the claimed subject-matter.

4.4 The Board is thus confronted with the question whether document (1) alone suggests to the skilled person, faced with the above identified stability problem, that the sonication method of the first embodiment, when applied to the solution of amino acid polymer of the second embodiment, will cause denaturation of portions of the dissolved proteins and formation of a "wall" of

denatured material which encapsulates and stabilises each single bubble.

4.5 The appellant put much emphasis on the fact that the essential feature of the solution subjected to sonication was not its chemical nature (saccharide), but rather its viscosity, which was indeed necessary to entrap and stabilise the microbubbles produced within the solution. Since the skilled person was well aware that many proteins, such as gelatin, form viscous solutions, there was no particular prejudice or disincentive for him not to envisage proteinaceous material as an alternative to saccharides. Considering that proteins were the only material mentioned in (1) apart from dextrose, sorbitol or renografin, the choice of a protein would have been the first and most obvious extension of the sonication method of (1).

4.6 The Board cannot accept this argument since it disputes the very foundation and starting point of the appellant's contention. In fact, the technical problem to be solved was not to provide alternative microbubbles - whatever alternative - but to provide microbubbles with improved stability. Thus the inventiveness involved in any possible modification of the known microbubbles has to be evaluated in view of the final effect to be achieved.

If the Board were to accept that the essential feature of the solution subjected to sonication in (1) is, as asserted by the appellant, its viscosity and not its chemical nature, it remains the fact that proteins are not the only alternative to saccharides for preparing viscous solutions, which can also be produced using

other different biocompatible substances such as synthetic polymers or tensides as disclosed in (6); column 5. Thus, in the Board's view, the skilled person faced with the problem to be solved had more than one alternative to investigate, among which proteins certainly did not represent the first and most obvious candidate.

In fact, in addition to document (1) which discloses the sonication of a solution of saccharides, two other prior documents, namely documents (2) and (3), describe processes entailing a sonication step for homogenising protein-containing emulsions. In both cases where proteins were involved, the sonication was carried out under refrigeration conditions: at 4°C in Example 1 of (2), at 15°C in (3). The skilled person was therefore aware from this need to refrigerate, that the sonication technique represented a strong and hazardous treatment very likely to cause heating and/or denaturation of the sonicated proteins. This teaching would have dissuaded the skilled person from subjecting to high energy sonication solutions of substances well known to be heat-sensitive and prone to denaturation, since in the light of the prior knowledge there was no possibility to predict to what extent the protein denaturation would have influenced or affected the viscosity of the solution, the production of microbubbles, if any, and above all the ultimate stability of such microbubbles.

This conclusion of the board is confirmed by the fact that the author of (1), though teaching the use of proteins for one embodiment of that invention, specifically refrained from suggesting the use of

proteins in the sonication method also disclosed in the same document.

Therefore, in view of the foregoing, the Board's judgement is that the closest prior art alone did not make the subject-matter of claim 1 obvious.

4.7 The teaching of the closest prior art was also combined with the teaching of documents (3), (4) or (6), each describing viscous aqueous solutions of proteins used in ultrasonic imaging. The appellant cited these documents to show that those skilled in the art were well aware that proteins, such as collagen and gelatin, gave rise to viscous solutions and were biocompatible. Therefore, the documents had to corroborate the appellant's contention that the skilled person, moving away from the saccharide solutions mentioned in (1), would have selected a protein as a suitable alternative.

These documents, however, go only so far as to corroborate the point, undisputed by the Board, that a viscous solution may be prepared using proteins, but do not support the appellant's argument that an expert in the art would have envisaged the high frequency sonication of such viscous protein solutions in order to improve the stability of the microbubbles already disclosed in (1). In fact, these documents do not offer any additional relevant teaching which could change the Board's opinion already expressed under point 4.6 of this decision.

4.8 In another line of argument, document (4) was suggested as the closest prior art. This document deals with

ultrasonic contrast enhancement and describes gelatin encapsulated microbubbles, having sizes of 12 and 76 microns, as a contrast agent. The document does mention the preparation method of such microbubbles. However, from the passage on page 2, lines 39 to 50 of the patent in suit, where document (4) is apparently acknowledged as prior art, the Board can derive that these microbubbles are prepared mechanically by hand shaking, a conclusion which was not disputed by the parties.

In this case, the problem to be solved by the invention would be to provide a population of microbubbles having a smaller and more uniform size. The later published document (10), written by Feinstein et al. and reporting in the form of a scientific paper the invention of the opposed patent, offers evidence that this problem has been solved. See Figure 2 illustrating the size distribution of the microbubbles obtained.

4.9 The appellant argued that document (1) already offered an obvious solution to both problems of smaller size and higher uniformity.

However, the Board is unable to find among the many arguments submitted by the appellant an answer to the question why the author of (1), who had already correctly recognized the problem of size and uniformity inherent in the gelatin encapsulated microbubbles of the prior art (see page 12, second paragraph), did not unambiguously teach, or at least suggest, the direct application of the sonication technique to such a gelatin solution, rather than simply confine his disclosure to the sonication of the (chemically more

stable) saccharide derivatives.

Indeed, the opinion of the Board is that the author of (1) was well aware, as the skilled person would have been, of all the uncertainty and unanswered questions, discussed above under point 4.6, accompanying the treatment with high frequency sonication of a protein solution. Furthermore, he or she would have found in the available prior art no useful information justifying the prediction that the sonication of a protein solution would have resulted in microbubbles, let alone microbubbles suitable for ultrasonic imaging.

On the contrary, in the light of such uncertainty, the skilled person, faced with the problem of bubble size and uniformity inherent in (4), had no evident motivation to embark upon an attempt to modify the known gelatin microbubbles, an attempt the outcome of which was completely unpredictable, rather than contemplate the simpler and direct use of the saccharide microbubbles disclosed in (1), which had already proved to satisfy all the desired requirements of size and uniformity.

In conclusion, the Board judges that none of the cited prior documents, taken alone or in combination, makes the subject-matter of claim 1, and dependent claims 2 to 6, obvious.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

H. Maslin P. A. M. Lançon