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D E C I S I O N
of 23 March 2000

Case Number: T 0194/97 - 3.4.2
Application Number: 86903608.7
Publication Number: 0230472
IPC: G01N 27/30, G01N 27/416

Language of the proceedings: EN

Title of invention:
Biosensor and method of manufacturing same

Patentee:
MATSUSHITA ELECTRIC INDUSTRIAL CO., LTD.

Opponent:
MediSense, Inc.

Headword:
-

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step - exclusion of hindsight"

Decisions cited:
-

Catchword:
-



Case Number: T 0194/97 - 3.4.2

D E C I S I O N
of the Technical Board of Appeal 3.4.2
of 23 March 2000

Appellant: MediSense, Inc.
(Opponent) 266 Second Avenue
Waltham
Massachusetts 02154 (US)

Representative: Dost, Wolfgang, Dr.rer.nat., Dipl.-Chem.
Patent- und Rechtsanwälte
Bardehle, Patenberg, Dost, Altenburg
Geissler, Isenbruck
Postfach 86 06 20
D-81633 München (DE)

Respondent: MATSUSHITA ELECTRIC INDUSTRIAL CO., LTD.
(Proprietor of the patent) 1006, Oaza Kadoma
Kadoma-shi
Osaka-fu, 571 (JP)

Representative: Bühling, Gerhard, Dipl.-Chem.
Patentanwaltsbüro
Tiedtke-Bühling-Kinne & Partner
Bavariaring 4
D-80336 München (DE)

Decision under appeal: Interlocutory decision of the Opposition Division
of the European Patent Office posted 19 December
1996 concerning maintenance of European patent
No. 0 230 472 in amended form.

Composition of the Board:

Chairman: E. Turrini
Members: S. V. Steinbrener
B. J. Schachenmann

Summary of Facts and Submissions

I. The appellant (= opponent) lodged an appeal against the interlocutory decision of the Opposition Division finding European patent No. 0 230 472 as amended at the oral proceedings before the first instance to meet the requirements of the Convention.

II. The opposition filed by the appellant against the patent as a whole had been based on Article 100(a) EPC since the subject-matter of the patent in suit allegedly lacked an inventive step.

III. In its decision, the Opposition Division held that the subject-matter of independent claims 1 and 5 as amended was inventive with respect to the available prior art comprising (in the numbering of the Opposition Division), *inter alia*, the following documents:

E1: EP-A-0 136 362

E2: EP-A-0 127 958

E3: US-A-4 225 410, and

E4: US-A-3 897 326.

IV. The above documents were again referred to by the appellant in the statement of grounds of appeal.

Furthermore, document

E5: S. Srinivasan et al.(eds.): "Comprehensive Treatise of Electrochemistry", vol. 10 Bioelectrochemistry, Plenum Press, New York,

pages 297 to 346

was for the first time referred to by the appellant in the statement of grounds of appeal.

- V. In a communication pursuant to Article 11(2) of the Rules of Procedure of the Boards of Appeal, the Board drew the appellant's attention to the fact that no evidence had been produced for the publication date of document E5.

Having regard to the issue of inventive step, the Board considered document E1 to come closest to the subject matter of the independent claims, this prior art already anticipating the features set out in the precharacterising portion of claim 1. The subject matter of claim 1 thus differed from the biosensor of E1 in that the electrode system was made primarily of carbon and the surface of at least the measuring electrode was covered with albumin or glucose oxidase by adsorption.

The technical problem solved by these differences might therefore be seen in providing a biosensor of the type disclosed in E1, which was cheap and disposable and had an electrode sensitivity of low dispersion.

At present, it seemed unclear whether this problem would already be realised by a skilled person when trying to put the prior art teaching into practice, or whether its second aspect, i.e. the dispersion of sensitivity, specifically resulted from the use of carbon-based electrodes.

Similar disposable biosensors employing carbon

measuring electrodes seemed to be known, e.g., from document E2, and document E4 related to the aspect of suppressing surface effect problems occurring in electrokinetic studies with platinised platinum electrodes by coating said electrodes with bovine serum albumin (= BSA).

At the scheduled oral proceedings, it should be discussed whether or not a skilled person starting from document E1 in view of the problem posed would consider the remaining prior art, in particular documents E2 and E4, and by doing so, would arrive at the claimed subject matter without exercising inventive skill.

- VI. Oral proceedings which had been arranged at the parties' respective subsidiary requests took place on 23 March 2000. At the end of the oral proceedings, the decision of the Board was given.
- VII. The appellant requested that the decision under appeal be set aside and that the European patent be revoked.
- VIII. The respondent (= patent proprietor) requested that the appeal be dismissed (main request) or, alternatively, that the patent be maintained on the basis of one of the sets of claims filed as auxiliary requests 1 to 5 at the oral proceedings.
- IX. The wording of the independent claims in accordance with the main request reads as follows:

"1. A biosensor of the type which comprises an insulative base (12) having an electrode system which includes at least a measuring electrode (14, 14') and a counter electrode (13, 13') and which is covered with a

perforated body (18) having an oxidoreductase and an electron acceptor in dried state which are soluble in a liquid sample, said perforated body (18) being integrally combined with said electrode system and said base (12), wherein said biosensor electrochemically detects a variation in concentration of a substance occurring during reactions between the oxidoreductase, the electron acceptor and the liquid sample to measure a concentration of a substrate in said liquid sample, characterized in that said electrode system is made primarily of carbon and the surface of at least said measuring electrode is covered with albumin or glucose oxidase by adsorption."

"5. A method for producing a biosensor according to claim 1, said method comprising:

- providing an insulative base,
- printing or applying a carbon paste on said base to form an electrode system including at least a measuring electrode and a counter electrode,
- polishing the surface of the respective electrodes,
- subjecting the polished electrodes to a thermal treatment at a temperature of 60°C to 170°C for 1 to 8 hours,
- covering the electrode system partially with albumin or glucose oxidase by adsorption,
- covering the electrode system with a perforated body having an enzyme and an electron acceptor in dried state therein and
- integrally combining said perforated body with said electrode system and said insulative base."

Claims 2 to 4 and 6 are appended to the independent claims.

X. The appellant advanced the following arguments:

The patent in suit basically relates to the use of a carbon electrode covered with protein for measuring blood sugar levels. The appellant considers document E1 to be the closest prior art corresponding to the preamble of claim 1 of the patent in suit. This document already discloses the same electrode system, albeit consisting of platinum without protein coating. Therefore, as formulated by the Board in its communication, the technical problem may be seen in providing a cheap disposable biosensor having an electrode sensitivity of low dispersion.

In document E2, there is described a biosensor for the same purpose, having a cheap disposable carbon electrode which is produced in the same manner as in the contested patent so that the use of carbon electrodes must be considered to be an obvious solution to the first problem posed. In view of the purpose aimed at, and the effects achieved by the known electrode material, a different electrode construction would not prevent a skilled person from considering an exchange of material with a reasonable expectation of success.

As regards the remaining problem of electrode sensitivity, document E4 discloses a protein coated platinised platinum electrode particularly useful in the study of electrokinetic phenomena by the technique of laser Doppler spectroscopy. The known electrode is exposed to an aqueous sucrose buffer solution containing blood cells, lymphocytes and proteins secreted by the cells or originating from fragmented cells. Moreover, the cells have proteins at the surface

which may attach to other surfaces. Although the specific example in E4 relates to electrophoretic mobility measurements, the prior art is not limited in this respect. Disturbances due to surface effects of adsorbates on the electrode and made visible by a broadening of the Doppler peak after a certain time have been overcome in E4 by coating the electrode with BSA. Such adsorbates are also referred to in the contested patent, proteins being only mentioned as possible species. Since uncoated electrodes cause unreliable measurements, an obvious solution to reproducibility problems would be defined adsorption known from E4 and already practised in E2 (glucose oxidase on carbon).

In this context, a skilled person would readily assume the adsorption process to start from the very beginning of electrode exposure and only to become noticeable in Doppler spectroscopy after five to ten minutes as reported in E4. The fact that no difference of current waveform between coated and uncoated electrodes is observed in the prior art is of no importance since this may also be the case in the patent in suit, which is silent on the current waveform.

As can be seen from document E5, the publication of which has been proven by the result of an Internet search submitted before the oral proceedings, the chemical nature of the electrode material is irrelevant, and adsorption problems affect electrochemical reactions as well. Therefore, starting from document E1 a solution to the remaining problems would be obvious from documents E2 and E4.

Finally, document E3 refers to a disposable array of

chemical sensors, which also includes an electrode system made of carbon and used for the same type of measurement as claimed in the contested patent. Moreover, according to this prior art adsorption problems may affect the electric field. The known measuring electrode is covered by a polymer or gel layer containing glucose oxidase. Reproducibility of electrode sensitivity could be obviously improved by a direct enzyme coating on the electrodes.

The auxiliary requests are not considered admissible since they are late-filed and not justified by exceptional circumstances.

XI. The respondent's argument in support of its requests may be summarised as follows:

It is agreed that document E1 comes closest to the present invention, and that the technical problem solved may be seen in providing a cheap and disposable sensor which, however, is also accurate. Although the claimed coating causes a reduction of the maximum current readings achieved, it also leads to a much more uniform sensor response due to the well-defined adsorption of albumin which can plausibly be considered to suppress subsequent spurious adsorption. This kind of trouble does not occur with the electrode material of E1 since it is less reactive than carbon within the rather short measurement time.

Document E2 relates to a different sensor type employing a carbon electrode as an immobilised enzyme electrode. Such electrodes differ from those used in the contested patent in that the combination of mediator and enzyme is directly fixed to the electrode

material. Moreover, only the measuring electrode may be made of carbon in E2, whereas the counter electrode is a conventional metal electrode. The carbon material may even be dispensed with entirely in E2. Thus, the function of the carbon material in the prior art mainly relates to the fixing of redox-constituents and not to cheap mass production.

Moreover, document E2 is silent on the dispersion of electrode sensitivity, although a lower measuring accuracy must be assumed for the prior art sensor type since it depends on variations of the immobilised and cross-linked layer with respect to thickness, concentration and diffusion properties. As the sensor construction of the patent in suit is subject to much less variation factors, a satisfactory suppression of sensitivity dispersion cannot be achieved in the prior art.

Document E4 would not be taken into account by a skilled person since it does not relate to biosensors, but to electrodes for the study of electrokinetic phenomena fundamentally different from current flow. Furthermore, only platinised platinum electrodes are considered in E4, which are much less reactive than carbon. Although it is correct that document E4 describes problems associated with surface effects, these are not due to adsorption but to specific surface reactions with constituents of the buffer solution. This conclusion is supported by the fact that flushing out the electrodes with water - as reported as a remedy in E4 - would not work in case of protein adsorption. Finally, the type of measurement carried out in E4 relates to the much more time consuming determination of cell mobility in a free buffer solution, any contact

of the cells with the electrodes being irrelevant. The inaccuracy problem mentioned in E4 concerns optical Doppler spectroscopy data having no connection to current measurements. Therefore, even if a skilled person took document E4 into consideration, no incentive to the present invention would be given by this prior art.

Nor would a skilled person consider document E5, the publication date of which is still doubtful. This prior art does not relate to the present problem and merely shows that metal electrodes are conventional.

Finally, the only relevant point in document E3 is the fact that a fully carbon-based electrode system may be used. Apart from that, there are striking differences between the claimed invention and the biosensor of E3 having regard to the sensor construction as the enzyme is immobilised in a polymer layer in E3 so that it would not move to the electrode, and no common perforated body is provided. Nor is there any mediator substance present in E3 using oxygen as a co-reactant. The adsorption effect mentioned in E3 and referred to by the appellant has nothing to do with the invention of E3 nor with the patent in suit. In document E3 as well, there is no indication to make a specific coating on the measuring electrode.

Reasons for the Decision

1. Admissibility of appeal

The appeal meets the requirements of Rule 65 EPC and is therefore admissible.

2. *Article 56 EPC*

2.1 The Board agrees with the parties and the Opposition Division that document E1 comes closest to the subject matter of claim 1 of the main request, which differs from the closest prior art (see E1, in particular claims 1 and 11 and Figures 4 and 7 and associated text) by the features of the characterising portion, i.e. in that

(a) the electrode system is made primarily of carbon, whereas the known measuring electrode consists of platinum, and the known counter electrode consists of platinum or silver/silver chloride (see E1, Figures 4 and 7 and associated text and claim 3); and

(b) the surface of at least the measuring electrode is covered with albumin or glucose oxidase by adsorption, whereas no such coating is disclosed in document E1.

2.2 The electrode system according to feature (a) appears to be considerably cheaper than the known platinum electrodes when disposable sensors are aimed at (see column 1, line 55 to column 2, line 10 of the patent in suit), and the coating according to feature (b) apparently has the effect to reduce dispersion of electrode sensitivity (see Figure 6 and associated text of the patent in suit).

The technical problem solved by these differences may therefore be seen in providing a biosensor of the type disclosed in E1, which is cheap and disposable and has an electrode sensitivity of low dispersion.

The Board is convinced that the first aspect of this problem would readily occur to a skilled person when trying to put the prior art teaching into practice, whereas the situation regarding its second aspect, i.e. the dispersion of sensitivity, seems to be more involved: since no reproducibility problems with precious metal electrodes are mentioned in E1, the respondent plausibly argued that this aspect specifically results from the use of much more reactive carbon-based electrodes and thus could only be realised after a further step, i.e. after replacement of the prior art metal electrodes.

- 2.3 In an attempt to make a biosensor of the known type cheap and disposable, the Board holds the view that a skilled person would obviously try to replace the precious metal electrodes by some cheaper material.

The use of carbon-based electrodes in throwaway biosensors is known from documents E2 (see in particular page 16, lines 1 to 6; page 6, line 10 to page 9, line 3; and Figures 1 and 2 and associated text) and E3 (see in particular column 3, lines 35 to 63; column 5, lines 9 to 13; and Figure 7a and associated text).

- 2.4 In the Board's view, it must, however, be admitted that these biosensors are on one hand of different type so that their electrodes do not have a function identical to that provided in the patent in suit, and on the other hand the electrode material is of more optional character in the prior art.

In document E2, the measuring electrode may consist of carbon, whereas the counter electrode is of

silver/silver chloride type (see page 33, lines 11 to 13). The oxidoreductase and the electron acceptor directly cover the carbon measuring electrode thus belonging to the species of immobilised enzyme electrodes (see also E2, page 37, lines 11 to 14) whereas a perforated body is provided for the redox system in the contested patent. Hence, the reactive carbon electrode must be considered to have a specific function in E2 in that it directly fixes the enzyme/mediator couple as the respondent has pointed out. Cost aspects may therefore be of secondary importance for the selection of carbon material in the prior art. This view is supported by the fact that the counter electrode is not carbon-based in E2, but of precious metal type. Moreover, the carbon material may be dispensed with entirely in E2 (see page 40, lines 10 to 12).

The disposable biosensor chip disclosed in document E3 is similar to the sensor of document E2 in that the enzyme is immobilised in a polymer or gel layer (Figure 7a: layer 71a) directly fixed to the carbon-based measuring electrode (Figure 7a: electrode 72a), and there is no perforated body covering the whole electrode system. Rather the respective electrodes of E3 are covered by separate bodies 71a, 71b, 71c having entirely different properties. Although the whole electrode system (Figure 7a: electrodes 72a, 72b, 72c) may be carbon-based in E3, it appears that the annular layer 71c of the reference electrode 72c (see Figure 7a of E3) is always of Ag/AgCl type (see E3, column 8, lines 63 to 68).

2.5 In view of these differences with respect to prior art sensor design and electrode material options, it cannot

be straightforwardly assumed that a skilled person would have been imparted the teaching to substitute an entirely carbon-based electrode system for the precious metal system of a sensor according to E1 in order to make this sensor disposable.

However, even if such an assumption were made, the average practitioner would not arrive at the claimed invention, but would have to carry out further steps.

First of all, as has been pointed out above (see point 2.2), after exchange of the electrode material he would have to realise that there is a problem of dispersion of electrode sensitivity due to spurious adsorbate built-up on the carbon measuring electrode. Since in documents E2 and E3 the carbon electrodes are of immobilised enzyme type, the enzyme and acceptor (if any) being directly fixed to the carbon electrode, no indication of such undesirable adsorption on the carbon surface can be expected from this prior art. The passage of E3 cited by the appellant in this context (see E3, column 1, lines 61 to 65) relates to adsorption on polymer layers which can affect the electric field at the gates of immuno FET structures and has nothing to do with the present aspect of contamination of carbon measuring electrodes by adsorbates.

- 2.6 Secondly, realising the dispersion problem and tracing it back to adsorption of serum constituents, such as proteins, a skilled person would have to look for a solution to this problem. The Board is not convinced that such a solution would be offered by the teaching of document E4 if hindsight is to be excluded.

Document E4 relates to surface effects in particular affecting the study of electrokinetic phenomena by the technique of laser Doppler spectroscopy (see column 1, lines 5 to 11). Even if a skilled person would be inclined to apply the teaching of E4 in the field of electrochemical electrodes as the appellant tried to prove by referring to textbook E5, there are further major differences with respect to the claimed invention or its preferred embodiments, which, in the Board's view, lead away from such direct application:

- (i) E4 does not relate to carbon electrodes but to platinised platinum electrodes (see the passage cited above);
- (ii) the prior art electrodes are not exposed to a serum sample but to an artificial buffer solution containing lymphocytes and erythrocytes (see E4, column 1, line 66 to column 2, line 4);
- (iii) the undesirable surface effects become apparent within a few minutes when using Doppler spectroscopy (see E4, column 2, lines 5 to 12) whereas the measurement time for a sensor according to the patent in suit is of the order of 10 seconds, the measurement being carried out two minutes after serum addition (see column 3, lines 30 to 49 of the patent in suit);
- (iv) the prior art surface effects can be removed by flushing out the electrodes with distilled water (see E4, column 2, lines 12 to 15) whereas according to the same prior art protein adsorbates, as e.g. adsorbed BSA, apparently cannot be removed by washing with water (see E4,

column 3, lines 14 to 17 and column 3, line 30 to column 4, line 2);

- (v) the surface effects are caused by a surface reaction of the sucrose buffer solution with the metal electrode since replacement of cells by polystyrene spheres leads to the same problems in the prior art (see E4, column 2, lines 15 to 21); and
- (vi) no difference of current waveform is observed between untreated and BSA coated electrodes in E4 (see column 2, lines 57 to 60) whereas according to Figure 6 of the patent in suit at least a reduction of the maximum current amplitudes is observed.

All of these aspects would oblige a skilled person to make further assumptions or to surmount further barriers in order to arrive at the claimed invention. The Board in particular considers points (i), (iii), (iv) and (v) to prevent a skilled person from applying the teaching of E4 to a modified biosensor of the type known from E1 because no adsorption problems with platinum electrodes have been observed in the closest prior art, at least not within the rather short measurement time. Therefore, a surface effect apparent on platinum electrodes after a few minutes only would not be considered relevant by a skilled person in the present context. Moreover, if the reproducibility problems are believed to originate from spurious protein adsorption, no remedy would be expected from measures against surface effects that can simply be washed off with distilled water.

2.7 Finally, even if document E5 is considered to be prepublished, which in the Board's view must be assumed on the basis of the appellant's submissions, then this general textbook only mentions that protein adsorption, in particular cytochrome c adsorption, has been detected on some metal electrodes like silver and platinum, but not on others like gold, whereby different detection methods have been applied (see E5, in particular page 326, second to fourth paragraph). There is, however, no indication in this prior art that intentional adsorption of albumin or glucose oxidase on carbon-based electrodes avoids reproducibility problems of biosensors of the type known from document E1.

2.8 In consequence, the subject-matter of claim 1 of the main request involves the inventive step required by Articles 52(1) and 56 EPC, and claim 1 is accordingly allowable.

An analogous finding holds good for independent claim 5 concerning a method of producing the biosensor according to claim 1.

Dependent claims 2 to 4 and 6 relating to specific embodiments of the subject matter of the independent claims also meet the requirements of the EPC.

3. *Auxiliary requests*

In view of the allowability of the main request, auxiliary requests 1 to 5 need not be regarded.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar:

The Chairman:

P. Martorana

E. Turrini