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D E C I S I O N
of 12 September 1995

Case Number: T 0639/93 - 3.4.2

Application Number: 82201107.8

Publication Number: 0075353

IPC: G01N 21/75, G01N 33/53

Language of the proceedings: EN

Title of invention:
Method and apparatus for the determination of species in solution with an optical wave-guide.

Patentee:
BATTELLE MEMORIAL INSTITUTE

Opponent:
FISONS plc

Headword:
-

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step - no"

Decisions cited:
-

Catchword:
-

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Beschwerdekammern | Boards of Appeal | Chambres de recours

Case Number: T 0639/93 - 3.4.2

D E C I S I O N
of the Technical Board of Appeal 3.4.2
of 12 September 1995

Appellant: (Proprietor of the patent)
BATTELLE MEMORIAL INSTITUTE
7 route de Drize
CH-1227 Carouge/Genève (CH)

Representative:
Savoye, Jean-Paul
Moinas Kiehl Savoye & Cronin
7, route de Drize
CH-1227 Carouge (CH)

Respondent: (Opponent)
FISONS plc
Fisons House
Princes Street
Ipswich IP1 1QH
Suffolk (GB)

Representative:
Jones, Stephen Anthony
E. N. Lewis & Taylor
144 New Walk
Leicester LE1 7JA (GB)

Decision under appeal: **Decision of the Opposition Division of the European Patent Office dated 13 May 1993 revoking European patent No. 0 075 353 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: E. Turrini
Members: C. Black
L. C. Mancini

Summary of Facts and Submissions

I. European patent No. 0 075 353 (application No. 82 201 107.8) was revoked by a decision of the Opposition Division on the ground that the subject-matter of its Claims 1 to 19, although novel, did not involve an inventive step.

II. The present appeal lies against this decision. In the grounds for the appeal the Appellant (Patentee) requested in effect that the decision under appeal be set aside and that a patent be granted on the basis of Claims 1 to 19 of the published specification with the amendments to Claims 1, 2 and 15 proposed in the Opposition Division's communication dated 21 June 1991.

In response to a communication from the Board, setting out its provisional opinion which in substance agreed with the decision of the Opposition Division, the Appellant submitted as the basis of an auxiliary request an amended set of Claims 1 to 16, wherein Claim 1 results from the combination of Claims 1, 2 and 9 of the granted patent, with consequent deletion and/or renumbering of the remaining claims, and Claim 13 is identical with Claim 15 of the granted patent.

As a further auxiliary measure, at least in respect of the main request, the Appellant proposed that "matches or exceeds" in Claim 1 be replaced by "practically matches without being below".

The Respondent (Opponent) requested that the appeal be dismissed.

III. Independent Claims 1 and 15 according to the main request read as follows:

"Claim 1. A method of determining an analyte in a solution, in which method

- the solution is brought into contact with a reactant specific to said analyte so as to form a layer of a reaction product of said analyte and said reactant on the surface of a waveguide core exposed to said solution and having a refractive index n_1 higher than the refractive index n_2 of the solution,
- light of wavelength λ is propagated through the waveguide core by multiple internal total reflection at a reflection angle θ,
- the effect of the reaction product layer on said light is determined by measuring the modification of a parameter of said light,
- the analyte is determined from said modification,

the method being characterized in that the ratio n_1/n_2 and θ are so selected that the depth Z at which the electric field E of the evanescent waves, occurring outside the waveguide core due to the total reflection of the light guided inside and penetrating into the surroundings of the waveguide core, is at least 0.1 Eo, matches or exceeds the thickness of said reaction product layer, Eo being the electric field at zero depth."

"Claims 15. An apparatus for carrying out the method of Claim 1, comprising:

- a light source (8; 43; 70; 107),
- an optical waveguide (1; 41; 71; 101) having a core section which may be exposed to a solution containing an analyte to be measured, said core section having a refractive index n_1 higher than the refractive index n_2 of said solution and carrying a coating of a reactant specific to the analyte,

- optical elements (9, 10, 11; 47-52; 70; 105) arranged to inject light from said light source into said waveguide so as to enable propagation of the injected light through the core section by multiple internal total reflection at a reflection angle θ,
- light detecting means (12; 58; 78; 124) arranged to receive light emerging from said waveguide and to convert it to an electrical signal reflecting a modification which a parameter of the injected light may have suffered in response to the penetration of the propagating light into a reaction product layer forming on the surface of the core section as a result of a reaction between the reactant and the analyte,
- circuit means (13, 14; 55; 61; 81-86; 115-117) form determining, from said electrical signal, data representative of said reaction, the apparatus being characterised in that,
- the optical elements (9, 10, 11; 47-52; 70 ;105) are arranged to inject said light into said waveguide (1; 41; 71; 101) at a front face thereof extending transversely to the direction of light propagation inside the waveguide,
- the light detecting means (12; 58; 78; 114) are arranged to collect said light emerging from the waveguide at a rear face thereof also extending transversely to the direction of light propagation, and
- the ratio n_1/n_2 and θ are so selected that the depth Z at which the electric field of the evanescent waves, occurring outside the waveguide core due to the total reflection of the light guided inside and penetrating into the surroundings of the waveguide core, is at least o.1 Eo, matches or exceeds the thickness of said reaction product layer, Eo being the electric field at zero depth."

The independent Claims 1 and 13 according to the auxiliary request do not contain the amendments effected in Claims 1 and 15 according to the main request and mentioned in paragraph II above. Otherwise Claim 1 differs from that of the main request in that at the end there is added the wording "said modification of the light parameter concerns the generation of a fluorescent light signal, characterized in that said signal is measured at an end of said waveguide core".

Claim 13 is the same as Claim 15 of the granted patent.

IV. In its decision the Opposition Division relied on the following documents:

Document A: J. Immum. Meth., 8(1975) pages 235 to 240,

Document B: Internal Reflection Spectroscopy, by N. J. Harrick, Interscience Publishers, New York 1967,

Document C: US-A-4 050 895.

The Opposition Division found that all of the features of Claim 1 were known from document A except for the requirement that the light is propagated by **multiple** total internal reflection. The use of multiple internal reflection to increase the sensitivity in internal reflection spectroscopy was however known from document B, and its application in the process disclosed in document A was therefore obvious. The subject-matter of apparatus Claim 15 was also obvious from a combination of documents A and B taking into account common general knowledge. The features of the dependent claims added nothing inventive to the subject-matter of Claim 1 and 15, document C being cited in respect of Claims 12, 14 and 18.

V. The gist of the Appellant's argumentation is that the Opposition Division was wrong in finding that all but one of the features of Claim 1 were known from document A. The Opposition Division had rather interpreted document A with knowledge of the patent in suit and had used ex post facto analysis.

As regards the auxiliary request, the subject-matter of Claim 1 differs further from the cited prior art in that it is the propagated fluorescence which is detected at the end of the wave guide rather than the attenuation of the incident light as in document B. Moreover, as compared with document A, which discloses detection of fluorescence at right angles to the wave guide, detection at the end of the wave guide permits greater measurement sensitivity because of the concentration of the fluorescence signal within the wave guide.

The Respondent was content to counter the Appellant's argumentation contained in the grounds for the appeal point by point.

Reasons for the Decision

1. The appeal is admissible.

2. The Opposition Division found that the amended claims then under consideration did not infringe Article 123(2) of (3) EPC, and the Board can agree with this conclusion, which is moreover also valid for the claims according to the Appellant's main and auxiliary requests, as is apparent from the indication in paragraph II above of the origin of these claims.

3. As will become apparent from the following discussion of inventive step, novelty of the subject-matter of the claims according to the main and auxiliary requests can be acknowledged, even when account is taken of the fact that the wording of Claim 13 according to the auxiliary request has been restored to that of granted Claim 15.

4. *Main request*

4.1 Document A is the most appropriate prior art for the assessment of inventive step. It discloses a method of determining an analyte, specifically an antibody, in an aqueous solution, in which method the solution is brought into contact with a reactant specific to said analyte (an antigen or hapten-protein conjugate) so as to form a layer of a reaction product of said analyte and said reactant on the surface of a quartz slide exposed to said solution and having a refractive index n_1 (that of quartz) higher than the refractive index n_2 of the solution, light of wavelength λ = 442 nm (see description with reference to Figure 1) is propagated through the quartz slide at a reflection angle θ = 70°, an effect of the reaction product on said light is determined by measuring the modification of a parameter of said light and the analyte is determined from the said modification. The disclosed modification is the change in wavelength of the evanescent wave resulting from fluorescence induced thereby in the reaction product layer. The first part of Claim 1 differs from this disclosure, at least in words, in that the layer of reaction product is formed on the surface of a waveguide core rather than a quartz slide and light is propagated through the waveguide core by multiple internal total reflection. However no difference which might contribute to an inventive step can be seen here. Firstly the quartz slide in which a single total internal reflection takes place is just as much a wave guide as a medium in

which multiple total internal reflections take place. Further document B, which is a standard text book on internal reflection spectroscopy, and is referred to in document A on pages 235 and 236, describes both single reflection and multiple reflection elements and states on page 100 that for many bulk materials, thin films, and particularly monomolecular layers, spectra of sufficient contrast cannot be obtained using a single reflection and thus multiple reflections must be employed to enhance the contrast and that a variety of IRE's have been developed for this purpose. It is therefore obvious to use multiple internal reflections rather than a single internal reflection for very thin layers such as is the case in the patent in suit (several Ångstroms to several hundreds of Ångstroms as disclosed on page 4, lines 59).

The copy of extracts from Document B at the Board's disposal does not bear an indication of a publication date, but it has not been disputed that the date 1967 is valid and the Board accepts this.

The wording of the characterising portion of Claim 1 is somewhat involved, but what the claim requires can be expressed as follows. Firstly the thickness of the reaction product layer should be the same as ("matches") or be less than the depth Z at which the electric field E of the evanescent wave is 0.1 Eo or more. Further the ratio n_1/n_2 and the reflection angle θ are to be selected so that this condition is satisfied. The required relationship between reaction product layer thickness and depth Z is derivable from document A, because on page 236 it is stated that the evanescent wave extends a few thousand Ångstroms, and this depth of penetration is an order of magnitude greater than the thickness of an antibody-antigen reaction layer quoted in the patent in suit, even when the words "or more" (page 4, line 59)

are taken into account. Further, on page 237 it is stated that at the concentrations used, it can be shown that the number of molecules in the evanescent wave region, but not on the surface, is normally small compared to the number on the surface, which demonstrates that the evanescent wave extends beyond the reaction product layer.

The evanescent wave field in fact decays exponentially in the rarer medium and accordingly extends an infinite distance into it. What we are talking about however is an effective depth of penetration beyond which the evanescent wave field has decayed to such an extent that its effect is negligible. Document B, page 30, defines depth of penetration as the distance required for the electric field amplitude to fall to e^{-1}, that is about one third, of its value at the surface and gives a formula for this depth which is reproduced at the top of page 4 of the patent in suit. This depth was a feature of Claim 2 of the patent as published and indicated as being the preferred depth which had to match or be greater than the thickness of the reaction product layer. The range of penetration depths now embraced by Claim 1 extends to that at which the field has decayed to o.1 Eo, which is, as stated on page 4, line 22 of the description, a depth where the electric field magnitude is still a reasonable fraction of Eo, that is, where the amount of energy for interacting with the product is still significant. However the selection of a suitable depth of penetration which is sufficient to extend at least as far as the thickness of the reaction product layer but not so far into the solution under test that interaction with its constituents occurs is something which can be arrived at, without using inventive ingenuity, by the average skilled person having knowledge of the disclosure in document A and familiar with the theory of internal reflection spectroscopy from

document B. In this respect it will be recalled that from document A page 237 it can be derived that the evanescent wave field extends beyond the reaction product layer. In addition to this, document A, on page 236, states that tagged antibody beyond the evanescent wave region is not excited by the light and thus does not fluoresce. The obvious conclusion that the person of average skill in the art derives from these passages is that the evanescent wave region should embrace the thickness of the reaction product layer but not extend unduly beyond it, that is, document A recognises the desirability of limiting the evanescent wave to a region close to the reaction product layer.

As regards the feature that the ratio n_1/n_2 and θ have to be selected to achieve the desired relationship between penetration depth and reaction product layer thickness, it is known from document B that the depth of penetration depends inter alia on n_1/n_2 and θ - see Figure 11 on page 31 (reproduced in the patent in suit as Figure 2) and the formula on page 30 (reproduced in the patent in suit at the top of page 4). The average skilled person must of necessity select these parameters so that a proper depth of penetration for the purpose of internal reflection spectrometry is obtained.

4.2 To the various points raised by the Appellant in the Statement of Grounds for the appeal the Board observes the following:

Paragraph 1.

Here the Appellant seeks to show that in document A a layer of reaction product is not formed, as is required by Claim 1 - see the wording "so as to form a layer". The reasoning is apparently that in document A sites on the quartz slide not occupied by e.g. antigen molecules

are not filled with BSA as is disclosed in the patent in suit (page 14, lines 39 to 46) so that presumably isolated antigen molecules are attached to the slide rather than a layer thereof. The Board cannot follow this argumentation. Document A discloses antigen-coated quartz on page 236 (four lines from foot of page) and refers to the antigen-coated side of D (page 237, description of Figure 2), and there is no reason to consider that this coating is not a layer. Because the measure described in the patent in suit is not employed, it may not be a perfect layer, but it is nevertheless a layer. The said measure is, and was at the priority date of the patent in suit, in fact standard practice in immunoassays where one partner in the antigen-antibody reaction is immobilised on a surface, and seeks to avoid non-specific interaction of the partner which is not immobilised with the said surface by blocking sites where this might occur. In the Board's opinion this does not require documentary evidence, but it is noted that it is disclosed in document C, column 8, lines 6 to 8.

Paragraph 2

The Appellant argues that the method employed to clean the slide in document A will lead to a rough surface and that light will thus penetrate the rarer medium by scattering and not as in the patent in suit. However document A clearly relates to internal reflection spectroscopy and refers to the evanescent wave **inter alia** on pages 235, 236 and 237. The Board can in any case agree with the Respondent that the cleaning method would result in a surface which was optically smooth and that the fact that the antigen is attached to the surface by physical absorption is consistent with the surface being optically smooth.

Paragraph 3

The argument that there is a distinction between a quartz slide permitting one total internal reflection and a wave guide permitting multiple reflections has already been dealt with.

Paragraph 4

The Appellant argues that the angle of injection of the light disclosed in document A (70°) is so close to the critical angle (66°) that light will leak out into the solution, that is, the internal reflection will not be total. The Board can agree with the Respondent that at 4° above the critical angle, internal reflection will be substantially total.

Paragraph 5

The Appellant seeks to draw a distinction between the method of the patent in suit and that disclosed in document A in that in the patent the modification of a parameter of the light is measured directly at the output of the waveguide core whereas in document A the modification is indirectly measured (fluorescence excited by the evanescent wave). In the first place this distinction is not brought out in the wording of Claim 1, because in document A it is still a modification of a parameter of the light which is being measured. Moreover it is clear that document B contemplates measuring a modification of a parameter of the light directly - see page 100: "focus the exit aperture (of the IRE) on the detector element". This is also disclosed in document C (Figure 3 and the corresponding description, column 4, lines 5 to 35). In the Board's opinion this is a general teaching which can be derived from document C, that is, the said measure is

not seen as being restricted to particular embodiments disclosed in document C. Finally the patent in suit also embraces indirect measurement - see Figure 16 and the corresponding description on page 12, lines 20 to 41 relating to operation in the side-pickup mode (line 33). The two methods are therefore obvious alternatives for the average skilled person. As regards the further objection that the signal resulting from only one total reflection would not be measurable, the Board can agree with the Respondent that this only requires use of the correct equipment. In any case, as stated above it is known from document B, page 100, to employ multiple reflections to increase contrast.

Paragraph 6

This concerns the selection of n_1/n_2 and θ as required by Claim 1 and this has already been dealt with. Appellant returns to the point raised in paragraph 2 and argues in respect of document A that on the etched porous glass surface there will be antigen absorbed into the recesses of the pores and therefore it is not possible to refer to the thickness of a reaction layer since this does not exist. As stated above however the Board sees no reason to conclude that the quartz slide employed in document A is other than optically smooth. The Appellant refers to the passage in document A on page 237 which states that at the concentrations used, it can be shown that the number of fluorescent molecules in the evanescent wave region is small compared to the number on the surface, and interprets this an meaning that the concentration is adapted to minimise the influence of fluorescent molecules in the evanescent wave region but not on the surface. This is repeated in paragraph 8. The Board cannot follow this and as stated above is of the opinion that for the average skilled person the interpretation

is that the evanescent wave region does not extend so far beyond the reaction product layer that it interacts significantly with molecules in the bulk test solution.

Paragraph 7

The Appellant states that the Opposition Division's calculation of the depth of penetration of the evanescent wave field in document A (150 nm) is incorrect and that this should be 218 nm. Whichever is correct, it is greater than the thickness of the reaction product layer which according to the patent in suit may be from several Ångstroms or more, so that the disclosure in document A meets the requirement of Claim 1 in this respect.

Paragraph 8

The Appellant suggests that the use of a filter before the PMT in the apparatus disclosed in document A confirms that only scattered light is involved. The Board can agree with the Respondent that the presence of the filter does not mean that light scattering is high but is simply good experimental practice. It is noted that in Figure 16 of the patent in suit operating in the side-pickup mode, filter 76 is employed to screen off all other wavelengths including scattered light just as in document A.

4.3 The Appellant's argumentation has therefore not convinced the Board that the subject-matter of Claim 1 involves an inventive step.

4.4 Apparatus Claim 15 falls for substantially the same reasons as does Claim 1. It is restricted to the embodiment where the modification of the light is detected at the output of the waveguide, but as set out

in the Board's observations regarding paragraph 5 of the grounds of the appeal, this does not contribute to inventivity.

4.5 Dependent Claims 2 to 14 and 16 to 19 fall because they are appended to claims found not to be allowable. The Board can in any case agree with the finding of the Opposition Division at least in respect of Claims 2 to 8, 10 to 14 and 16 to 19 (point 4.3 of the decision). The features of Claim 9 have been incorporated into Claims 1 and 2 of the auxiliary request.

4.6 As regards the Appellant's proposal to replace "matches or exceeds" by "practically matches without being below" in Claim 1, the Board shares the doubts of the Respondent as to compliance of this amendment with Article 123(2) EPC. However it is not necessary to go into this question, because the Board also agrees with the Respondent that the alternative wording does not have any material effect on the conclusion drawn. As previously stated, the Board found that the obvious conclusion that the person of average skill in the art derives from pages 236 and 237 of document A is that the evanescent wave region should embrace the thickness of the reaction produce layer but not extend unduly beyond it. This is all that the expression "substantially matches without being below" is saying.

4.7 Purely by way of corroboration, since the Board's decision does not rely on this document, reference is made to US-A-3 939 350, referred to on page 7, lines 31 to 39 of the patent in suit, in which the inventors are the authors of document A. It would moreover not be disputed that it is describing substantially the same process and apparatus as is disclosed in document A. This confirms that the method operates by total internal reflection - see column 2, lines 48 to 51: "The angle of

irradiation provides total internal reflection, so that fluorescence can occur within only a few hundred Ångstroms of the surface". Later, in column 3, lines 30 to 33 it is stated: "Under conditions of total internal reflection, only fluorescent molecules within a few hundred Ångstroms of the surface will be activated and fluoresce". Then in column 3, line 59 to column 4, line 2: "Some light energy does in fact penetrate the second medium over relatively short distances, usually not exceeding about 1,000 Å. Depending upon the variables involved, the distance of penetration of light energy can be diminished to as little as 500 Å. If a fluorescing molecule is positioned at the interface in the second medium, so as to be within the range of light energy which penetrates the second medium, when the wavelength of the light is within the adsorption peak of the fluorescing molecule, the fluorescing molecule will fluoresce. Fluorescing molecules that are outside this narrow band will not fluoresce".

The variables referred to in column 3, lines 61, 62 are known, as stated previously, from document B to include n_1/n_2 and θ.

These passages confirm the Board's conclusion that in carrying out internal reflection spectroscopy, the average skilled person will seek to achieve conditions in which the evanescent wave field will be able to interact with the whole of the thickness of the reaction product layer but not with the constituents of the test solution not forming part of said layer, and knows from document B which variables to adjust with this in view.

5. *Auxiliary request*

5.1 As noted above, Claim 13 has the same wording as Claim 15 of the granted patent and its wording differs from that of the corresponding Claim 15 of the main request particularly in the absence of the feature that the depth Z is at least 0.1 Eo. The claim is accordingly broader in scope than Claim 15 of the main request and must fall for the same reasons (see paragraph 4.4 above).

5.2 Since the auxiliary request therefore includes at least one claim (Claim 13) which is not allowable, this request must be rejected. Nevertheless the Board has elected to examine Claim 1 in order to establish whether the subject-matter of this claim might form the basis of an allowable auxiliary request.

5.3 Claim 1 requires that the modification of a parameter of the light should involve the generation of a fluorescent light signal and that the signal is measured at an end of the wavelength core. As noted in connection with the main request, in document A, the said modification also involves fluorescence, but the signal is measured at right angles to the wave guide. Document B, on the other hand, does not mention fluorescence, but does disclose measuring the modification of a parameter, namely attenuation of the propagated light at an end of the wave guide. The question to be answered therefore is whether the addition of the step of measuring the fluorescent light signal at the end of the wave guide would contribute inventivity to the method according to the Claim 1 of the main request which has been shown to be obvious.

5.4 In the Board's opinion, since fluorescence is a readily observable phenomenon, it would be apparent to the average skilled person carrying out the method disclosed in document A that fluorescence was being propagated to the end of the wave guide (quartz slide). It would therefore be obvious to him that this was a modified parameter of the light of wavelength λ which could be measured. Accordingly in making the combination, shown in connection with the main request to be obvious, of the teachings of documents A and B, he would also arrive at the subject-matter of Claim 1 of the auxiliary request without using inventive ingenuity. The advantages mentioned by the Appellant, namely concentration of the fluorescent signal within a small angular range and accumulation of the fluorescence on the detector are the automatic result of measuring at the end of the waveguide and cannot be seen as surprising.

Moreover, the Board would draw attention to the document "Multiple Internal Reflection Fluorescence Spectrometry", by Harrick and Loeb in Analytical Chemistry, vol. 45, No. 4 (1973) pages 687 to 691, which was cited in the European Search Report on the present case, but not mentioned in the examination or opposition proceedings. This document shows that Harrick, author of document B, recognised in 1973 the role fluorescence could play in internal reflection spectroscopy, and discloses propagation of the emitted fluorescence to a narrow edge of the element (wave guide), where it emerges concentrated into a narrow aperture - see e.g. the abstract, which also mentions separation of the emitted radiation from the exciting beam, considered by the Appellant to be another unexpected effect in the present case. This document is cited merely to corroborate the Board's conclusions and has not been relied upon the Board in coming to its decision.

Nevertheless it does demonstrate that the Appellant was not correct in saying "until now, nobody has foreseen the above explained amplification effect ... when this emitted fluorescence is measured at an end of a wave guide" (page 3 of the communication dated 19 April 1995) because such amplification is clearly disclosed on page 687, left hand column, paragraph beginning "In the recording ..." and right hand column, paragraph beginning "Excitation of Fluorescence".

5.5 The feature of Claim 2, although commented upon by the Appellant, has not been made the basis of a further auxiliary request. However, in the Board's opinion it would be obvious to the average skilled person when operating in the side pick up mode if the opacity of the test solution was causing deterioration of the fluorescence signal, and equally obvious that this could be overcome by detecting the signal at the opposite face of the wave guide.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

P. Martorana E. Turrini