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**D E C I S I O N**  
of 17 October 1997

**Case Number:** T 0291/94 - 3.3.4  
**Application Number:** 84305286.1  
**Publication Number:** 0140489  
**IPC:** G01N 33/543  
**Language of the proceedings:** EN

**Title of invention:**

Method for stabilizing immunologically active substances immobilized on an insoluble carrier and their use in the preparation of reagents for measuring physiologically active substances

**Patentee:**

WAKO PURE CHEMICAL INDUSTRIES, LTD.

**Opponent:**

Unilever PLC / Unilever N.V.  
Behring Diagnostics GmbH

**Headword:**

Stabilizing/WAKO

**Relevant legal provisions:**

EPC Art. 54, 56

**Keyword:**

"Main request - novelty (yes) - suitable disclaimer"  
"Inventive step (no)"  
"First to third auxiliary requests - inventive step (no)"  
"Fourth auxiliary request - not allowed - late filed and not meeting the formal requirements"

**Decisions cited:**

-

**Catchword:**

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Boards of Appeal

Chambres de recours

Case Number: T 0291/94 - 3.3.4

**D E C I S I O N**  
of the Technical Board of Appeal 3.3.4  
of 17 October 1997

**Appellant I:**  
(Opponents 01)

Unilever PLC  
Unilever House, Blackfriars  
Jacobplein 1  
London EC4 4BQ GB/3000 (GB)  
and

Unilever N.V.  
Burgmeesters'  
DK Rotterdam (NL)

**Representative:**

Butler, David John  
Unilever PLC  
Patent Division  
Colworth House  
Sharnbrook  
Bedford MK44 1LQ (GB)

**Appellant II:**  
(Opponent 02)

Behring Diagnostics GmbH  
Postfach 1149  
35001 Marburg (DE)

**Respondent:**  
(Proprietor of the patent)

WAKO PURE CHEMICAL INDUSTRIES, LTD.  
10, Doshomachi-3-chome  
Higashi-ku  
Osaka (JP)

**Representative:**

Wotherspoon, Hugh R.  
Langner Parry  
52-54 High Holborn  
London WC1V 6RR (GB)

**Decision under appeal:**

Interlocutory decision of the Opposition Division  
of the European Patent Office posted 1 March 1994  
concerning maintenance of European patent  
No. 0 140 489 in amended form.

**Composition of the Board:**

**Chairwoman:** U. M. Kinkeldey  
**Members:** L. Galligani  
S. C. Perryman

## Summary of Facts and Submissions

I. The appeals were lodged by appellants I and II (opponents 01 and 02) against the interlocutory decision of the opposition division issued on 1 March 1994 whereby the European patent No. 0 140 489, which they had opposed under Article 100(a) and (b) EPC, was maintained on the basis of amended claims 1 to 10.

Claims 1 and 5 thereof read as follows:

"1. A process for stabilising an immuno active substance on a carrier, comprising the steps of immersing the immuno active substance on the carrier in a treating solution and subsequently drying the carrier wherein the immuno active substance is immersed in a treating solution containing a sugar and a protein before being air dried, provided that the process wherein the immuno active substance is rabbit anti-human chorionic gonadotropin is hereby disclaimed.

5. A reagent for measuring a physiologically active substance comprising an immuno active substance immobilized on a carrier and stabilised with a treating solution wherein the immuno active substance is stabilised by being immersed in said treating solution containing a sugar and a protein before being air dried provided that the reagent comprising rabbit anti-human chorionic gonadotropin is hereby disclaimed."

Dependent claims 2 to 4 and 6 to 10, which were as granted, concerned specific embodiments of claims 1 and 5, respectively.

The opposition division had considered that the subject-matter of the amended claims fulfilled the requirements of novelty and non-obviousness having regard to the following citation:

- (8) DE-A-2 651 139, which corresponds to US-A-4 003 988.

II. On 14 August 1997, the board issued a communication stating the issues to be discussed. In this communication the attention of the parties was drawn inter alia to the following document:

- (2) GB-A-2 016 687, which was cited in the specification and was equivalent to DE-A-2 901 707 cited during the opposition proceedings.

III. In reply thereto, appellants I made further submissions and introduced into the proceedings additional documents.

IV. The respondents (patentees) filed on 17 September 1997 five auxiliary requests (I to V) and with letter dated 3 October 1997 requested that the introduction of the new documents by the appellants I be refused. With letter dated 16 October 1997, the respondents filed new auxiliary requests IV and V in substitution of the respective requests filed on 17 September 1997.

V. Oral proceedings took place on 17 October 1997. At the beginning of these, appellants I declared that they would not rely on the late-filed evidence. During oral proceedings, the respondents withdrew auxiliary requests IV and V on file and submitted - as a new **auxiliary request IV** - claims 1 to 4, of which claim 1 read as follows:

"Use of a treating solution in a process for stabilising an immuno active substance on a carrier, comprising the steps of immersing the immuno active substance on the carrier in the treating solution and subsequently drying the carrier wherein the immuno active substance is immersed in the treating solution consisting of an aqueous solution of a sugar and a protein before being air dried, provided that optionally said treating solution is buffered in the range pH 6.8-7.2, and further provided that the process wherein the immuno active substance is rabbit anti-human chorionic gonadotropin is hereby disclaimed, said treating solution being used for the provision of a synergistic stabilizing effect."

In summary, the claim requests before the board were as follows:

- **main request:** claims 1 to 10 as maintained by the opposition division;
- **auxiliary request I,** whose claims differ from the claims of the main request in that the features of granted claims 2 and 6, respectively, have been incorporated into independent claims 1 and 5 (now 4);
- **auxiliary request II,** whose claims differ from those of the main request in that the disclaimer is directed generally to anti-human chorionic gonadotropin;
- **auxiliary request III,** which corresponds to auxiliary request I with a disclaimer directed generally to anti-human chorionic gonadotropin;
- **auxiliary request IV:** claims 1 to 4 filed at oral proceedings.

VI. The appellants essentially submitted that in the main request and auxiliary request I the disclaimer was not sufficient to confer novelty to the claimed subject-matter over the disclosure of document (8). Furthermore, they submitted that the subject-matter of all requests lacked an inventive step having regard to documents (2) and (8) as well as to common general knowledge. This was because it was well known to the skilled person that the combination of a sugar and a protein had a stabilising effect on proteins, thus also on immuno reagents such as antigens and antibodies which are proteins. As for the alleged synergistic effect relied upon by the respondents, they submitted that the skilled person expected the combination of a sugar and a protein to provide an additive or, in some cases, even a synergistic effect on stabilisation. Thus, an inventive step had to be ruled out in respect of the claimed subject-matter.

VII. The respondents replied that novelty had been restored by disclaiming the subject-matter of document (8) and that obviousness was not shown by the appellants. Document (8) was technically speaking far from the claimed subject-matter because the disclosure therein was the finding of a suitable buffering system, not a method for stabilising an immunoreagent. When construed without hindsight, this document neither taught a process for stabilising an immuno active substance on a carrier nor air-drying nor the synergistic effect of sugars and proteins on stabilisation. Nor could such a synergistic effect be expected on the basis of the common knowledge on the stabilising effect of proteins in combination with the teachings of document (2). In fact, on the basis of the results presented in the latter document, the skilled person would have doubted whether sugars had a stabilising effect, when air-dried.

VIII. The appellants requested that the decision under appeal be set aside and that the patent be revoked.

The respondents requested as main request that the appeal be dismissed and that the patent be maintained, and as auxiliary requests that the decision under appeal be set aside and that the patent be maintained as amended in the first or second or third auxiliary requests filed with letter dated 17 September 1997 or the fourth auxiliary request filed at the oral proceedings on 17 October 1997.

### **Reasons for the Decision**

#### *Main request*

#### *Article 123(2) and (3) EPC*

1. The only difference between the amended claims and the claims as granted is the disclaimer in respect of rabbit anti-human chorionic gonadotropin (HCG) introduced in claims 1 (process) and 5 (reagent). This does not result in an extension of the protection conferred compared with the claims as granted because the effect of the disclaimer is to exclude from protection subject-matter that was previously covered. Thus, the requirements of Article 123(3) EPC are complied with. Furthermore, the disclaimer of background art is not an addition of subject-matter and is thus permitted under Article 123(2) EPC.

*Novelty (Article 54 EPC)*

2. The appellants consider that the disclaimer in claim 1 is not sufficient to establish novelty over document (8) because in their view the teaching of the said document goes beyond the specific example given. In this respect, they refer to the introductory part of the document in which reference is made generally to anti-serum to HCG (see eg in the US version column 1, lines 39 to 40, 52 to 53 and 64).
  
3. In the board's view, the disclosure in document (8) is not broader than the disclaimer because, for the purpose of novelty, the skilled reader has no reasons to derive a more general teaching from the specific experimental details given in the examples in relation to the preparation of dried disposable slides containing the latex particles with rabbit anti-HCG stabilised with bovine serum albumin (BSA) and sucrose. In this respect, it should also be noted that the claims of both the German and English version of document (8) refer specifically only to rabbit anti-HCG. This demonstrates that precisely rabbit anti-HCG was meant in the document, not any anti-serum to HCG. Thus, in the board's judgement, the introduction of the disclaimer into claims 1 and 5 as granted has cured lack of novelty in respect of document (8).

*Inventive step (Article 56 EPC)*

4. The board sees in document (2) the most appropriate starting point for the evaluation of inventive step. This document describes the stabilisation of an immuno active reagent on a carrier such as eg polystyrene beads, by way of treatment with a sugar solution and subsequent air-drying. The experimental results therein show that a variety of sugars protect and preserve the activity of the immuno active substance on the carrier

before and after heat stress (cf. Tables II to IV). In this respect, the slight loss in activity in comparison with the wet carrier to which the respondents have referred as being dissuasive, is immaterial as the correct comparison is made with the dry carrier without sugars (cf. Table II).

5. In the light of this document the problem to be solved is the further improvement of the stability of an immuno active reagent on a carrier.
6. As a solution thereto, the patent in suit proposes in the claims a process in which a further treatment of the immuno active reagent with a protein is carried out before air-drying (claims 1 to 4), the resulting reagent (claims 5 to 9) and use thereof (claim 10), with the exclusion of the process and reagent already known from document (8). The data reported in the patent in suit demonstrate that the proposed solution indeed results in an improved stability as measured by the activity retention rate (%) after 2 and 4 weeks. These data are also indicative of more than just an additive effect.
7. The relevant questions in relation to inventive step are what measure(s) the skilled person, starting from the document (2), would have considered adopting and whether these would have included a process covered by claim 1 and a reagent covered by claim 5 in the expectation of an improved stabilisation effect.
8. The stabilising effect of proteins such as BSA on biologically active molecules such as enzymes and immunoreagents is common wisdom for a skilled person. The stabilising effect of the combination of a protein with a sugar was equally well known to the skilled person from the art, eg from the art of lyophilisation.

In this respect, reference was made by the appellants to the following document already cited during the opposition proceedings:

(a) EP-A-0 037 110 (cf in particular the passage bridging pages 20 and 21).

9. Thus, in the board's judgement, the skilled person, faced with the stated technical problem, would have readily adopted a step of treatment of the immuno active reagent on a carrier with a protein solution in addition to the treatment with a sugar before drying. On the basis of the disclosure in document (8), which the skilled person would have taken into account as it was concerned with an immuno active substance (rabbit anti-HCG) immobilised on an insoluble carrier, the skilled person would have expected such a treatment to provide an improved stability. In fact, document (8) explicitly concludes that: " The addition of the sucrose in bovine serum albumin (Example III) was found not only to make a better preparation for distinguishing positive from negative reactions, **but it also allows the reagent to be placed on cards and dried, thus providing even longer stability of reagents at room temperature.**" (emphasis added) (cf. column 3, lines 10 to 15 of the US version)
10. As for the arguments put forward by the respondents in respect of an allegedly unexpected synergistic effect of the sugar and the protein, the board, while not denying that the data reported in the patent in suit are indicative of such an effect, disputes that such an occurrence would have been unpredictable and surprising for the skilled person. Both sugars and proteins were known for the skilled person as stabilisers of biologically active, in particular immuno active substances. The technical effect of stabilisation is not always exactly predictable **in quantitative terms** as

many variables (concentration, time, molecular interactions etc.) can affect measurements. Thus, when using two known stabilisers in combination, the skilled person knows that from a quantitative point of view, eg when measuring the retention activity (%), he or she can expect to obtain values which, while being better than those of each individual component, can reflect an effectiveness lower or higher than the merely additive effectiveness. Thus, a more than additive effect of a sugar and a protein on stabilisation would not be surprising for a skilled person. In any event the skilled person would expect an improvement from the combination, which would lead him or her to choose the combination.

11. For these reasons, the board considers that both the process of claim 1 and the reagent of claim 5 were obvious for the skilled person having regard to the cited prior art. Consequently, the main request is refused under Article 56 EPC.

*Auxiliary request I*

12. Claims 1 and 4 of this request differ from the corresponding claims of the main request only in that they specify the feature "the carrier is a synthetic polymer material or an inorganic substance". However, this feature per se cannot confer an inventive step to an otherwise non-inventive method and reagent because both documents (2) and (8) were also concerned with the use of a synthetic polymer material as a carrier, namely polystyrene beads. Thus, this request is refused under Article 56 EPC for the same reasons given above in respect of the main request.

*Auxiliary request II*

13. This claim request differs from the main request in that the disclaimer is of a broader scope, namely "anti-human chorionic gonadotropin". This, however, does not affect the finding of a lack of an inventive step. Thus, for the reasons given above in respect of the main request also this request is refused under Article 56 EPC.

*Auxiliary request III*

14. In addition to a disclaimer of broader scope (cf. auxiliary request II), claim 1 and 4 of this request contain the feature "the carrier is a synthetic polymer material or an inorganic substance" (see auxiliary request I). This request is thus refused under Article 56 EPC for the same reasons given above in respect of auxiliary request I.

*Auxiliary request IV*

*Allowability of the request*

15. During oral proceedings, in view of the interim decisions taken on the pending requests, the respondents submitted a new **auxiliary request IV** wherein claim 1 was centred on the feature "use .. for the provision of a synergistic stabilizing effect" (see Section V supra). No reason was given why this new request had not been filed within the time limit set in the communication accompanying the summons. The introduction of new requests at such a late stage is regarded by the board as detrimental to the smooth progression of the procedure. Such requests, if not

prompted by the fact that the subject-matter of the proceedings has changed - which is not the case here - are only acceptable if it is immediately apparent that they fulfil the formal requirements of Article 123(2), (3) EPC as well as those of Article 84 EPC.

16. In the present case, firstly, the new feature which characterises the subject-matter now claimed, is neither explicitly nor implicitly derivable from the application as filed. The fact that the data of the specific examples given are indicative of a synergistic effect (see point 6 supra) is immaterial because there is no generalised teaching in respect of this technical effect. Furthermore, the new feature creates doubt as to what is meant by "for the provision of a synergistic stabilizing effect". Thus, the amended claim is unclear and does not meet the requirements of Article 84 EPC. Consequently, the Board refused to allow this newly filed request into the proceedings.

## Order

### **For these reasons it is decided that:**

1. The decision under appeal is set aside.
2. The patent is revoked.

The Registrar:

The Chairperson:

D. Spigarelli

U. Kinkeldey

