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Bezeichnung der Erfindung:
Title of invention:
Titre de l'invention : Covalently bonded high refractive index particle reagents and their use in light scattering immunoassays

Klassifikation / Classification / Classement : G01N 33/545, C07K 15/00

ENTSCHEIDUNG / DECISION

vom / of / du 25 October 1990

Anmelder / Applicant / Demandeur :

Patentinhaber / Proprietor of the patent /
Titulaire du brevet : E.I. Du Pont de Nemours and Company

Einsprechender / Opponent / Opposant : Behringwerke Aktiengesellschaft

Stichwort / Headword / Référence :

EPÜ / EPC / CBE Article 56

Schlagwort / Keyword / Mot clé : "Inventive step (confirmed)"

Leitsatz / Headnote / Sommaire

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Case Number : T 85/89 - 3.4.2

D E C I S I O N
of the Technical Board of Appeal 3.4.2
of 25 October 1990

Appellant : (Opponent)
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Decision under appeal :
Decision of Opposition Division of the European Patent Office dated 25 November 1988 rejecting the opposition filed against European patent No. 0 073 611 pursuant to Article 102(2) EPC.

Composition of the Board :

Chairman : E. Turrini
Members : C. Black
M. Lewenton

Summary of Facts and Submissions

I. European patent No. 0 073 611 was granted on the basis of European patent application No. 82 304 384.9.

II. Claims 1, 4, 11 and 13 of the granted patent read as follows:

"1. A particle reagent having high refractive index comprising a polymer particle having an inner core and an outer shell to which is covalently bound a compound of biological interest, its antigen or its antibody, characterised in that the inner core of the said particle is a polymer having a refractive index of not less than 1.54 as measured at the wavelength of the sodium D line, and the outer shell of the said particle is a polymer of

(1) an ethylenically unsaturated monomer having a functional group selected from epoxy, carboxyl, amino, hydroxyl and formyl, capable of reacting with a compound of biological interest, its antigen or its antibody;

(2) optionally, other ethylenically unsaturated monomers;

(3) not more than 10 percent by weight of the outer shell of the monomers of the inner core, said outer shell having been formed by polymerization in the presence of said inner core; and

(4) said inner core and outer shell comprising a polymer particle having a diameter in the range of 0.03-0.1 μm.

4. A method for measuring a compound of biological interest comprising the steps of

(A) incubating

(1) a particle reagent as claimed in Claim 1, 2 or 3

(2) a liquid suspected of containing the compound of biological interest; and

(3) an agglutinating agent; and
(B) photometrically measuring increased particle size resulting from agglutination.

11. A particle reagent having high refractive index comprising a polymer particle as defined in Claim 1 or 2 to which a (sic) covalently bound an antigen of a compound of biological interest.

13. A method for measuring a protein comprising the steps of
(A) incubating
(1) a particle reagent as claimed in Claim 1, 2 or 3 in which the polymer particle is covalently attached to the antibody of the protein; and
(2) a liquid suspected of containing the protein; and
(B) photometrically measuring increased particle size resulting from agglutination."

The remaining claims are dependent claims relating to particular embodiments of the reagent or method according to the above claims.

III. The patent was opposed by Behringwerke Aktiengesellschaft on the ground that the subject-matter of the claims did not involve an inventive step having regard to the disclosure in the following:

DE-A-2 833 510 (D1)
DE-A-2 907 794 (D2)
DE-A-2 840 768 (D3)

The opposition was rejected by a decision of the Opposition Duivision.

IV. An appeal was lodged against this decision. The Appellant (Opponent) requested that the decision under appeal be set aside and that the patent be revoked in its entirety. In the grounds for the appeal are cited, in addition to D2, DE-A-2 736 805 (D4) and Automated Immunoanalysis, Part 1, ed. R.F. Ritchie, Marcel Dekker, Inc. New York and Basel (1978) (D5). The gist of the Appellant's argumentation can be summarised as follows. The Opposition Division, in its decision rejecting the opposition, found that the main difference between the subject-matter of Claim 1 and the disclosure in D2 lay in the size of the polymer particles. However at the priority date of the patent in suit the criteria for choosing particle size in immunoassay in relation to the wavelength of the measuring radiation belonged to the general knowledge of the skilled person, as evidenced by D4 and D5. From the Mie theory of scattering, in particular the Rayleigh-Debye equation (D5, page 14) can be derived that for particles whose diameter is 0.1-1 times the wavelength of incident light, the intensity of scattered light goes through a maximum which is related to the ratio of particle radius to wavelength. This relationship between particle size and wavelength is moreover the basis of D4, which discloses a ratio of wavelength to particle size of more than 1.5, preferably more than 2. Since it also discloses a preferred particle size of 200 to 800 nm and a preferred radiation wavelength of 1000 to 1400 nm it can be seen as disclosing a preferred ratio of wavelength to particle size of 5 to about 1.8. This teaching, applied to the chosen wavelength range of 320 to 400 nm in the patent in suit, results in a particle size range of 60 to 200 nm, which does not materially differ from the range of 30 to 100 nm required by Claim 1. The Opponent notes moreover that in most of the examples the wavelength is 340 nm, the particle size 69 nm, that is, a ratio of about 5, corresponding to that taught by D4.

Further, from Rayleigh's scattering law (D5), for constant particle size the intensity of scattered radiation increases with decreasing wavelength. It follows therefore that if one wants a maximum intensity of scattered light using light of low wavelength, one will use correspondingly small particle sizes, because the maximum intensity is related to the ratio of particle diameter to wavelength.

V. The argumentation of the Respondent (Patentee) is essentially as follows.

The subject-matter of Claim 1 requires a combination of the five features identified in the decision rejecting the opposition as features (a) to (e) (in effect the feature of Claim 1 that the inner core has a refractive index of not less than 1.54 together with features (1) to (4) of the claim). None of the documents D2, D4 or D5 suggests this subject-matter, whether these documents are considered separately or together.

D2 is not concerned with sensitive light-scattering immunoassays and does not disclose particles which combine very small particle size, high refractive index and a shell which contains not more than 10% by weight of the monomers of the core.

D4 is not concerned with core-shell particles and discloses particle sizes of 0.1 to 1 μm, preferably 0.2 to 0.8 μm. A size of 0.234 μm is considered to be relatively small, cf. Figure 3 and column 7, lines 12,13 of the equivalent US-A-4 118 192 (D4'). The particle material may have relatively low refractive index, and in the examples the means of attachment of the compound of biological interest is by simple physical adsorption, with the attendant problem of risk of desorption. Moreover example 9 of D4 refers to an assay time of 3 hours, whereas the assay time according to the patent in suit is of the order of minutes.

As to D5 it is not disputed that the Mie light scattering theory has long been known; however the equations are complex and have been solved only for a limited number of cases, involving ideal, homogeneous systems with spherical particles, and therefore cannot be used to predict the behaviour of the particle reagents according to the patent in suit. The equations are moreover not relevant because they relate to the total amount of light scattered, whereas the patent in suit relies on the change in turbidity on agglutination. The Appellant's combination of the teachings of D4 and D5 in order to substantiate his contention that it was obvious to optimise the particle size in the range set out in Claim 1 is therefore contrived and based on hindsight.

Reasons for the Decision

1. The appeal is admissible.

2. **Novelty**

2.1 In examining Claim 1 for novelty, feature (2), which is optional, is to be left out of consideration. Feature (3), up to the words "inner core" is also to be treated as optional. The claim does not require the presence in the outer shell of monomers of the inner core, but is saying that if such monomers are present, they should constitute no more than 10 percent by weight of the outer shell. Feature (4) is not, as the wording of the claim suggests, a fourth feature of the monomer of which the outer shell is a polymer, but is an additional feature of the particle reagent itself. However no real confusion results from this.

2.2 D1 discloses a particle reagent of particle size 0.03 to 5 μm, comprising a core of vinyl or diene polymers carrying carboxylic and/or sulphonate groups and a peripheral layer (shell) of vinyl polymer with terminal aminophenylthio groups. A compound of biological interest may be chemically, therefore covalently, bonded thereto.

The shell is prepared by polymerising a vinyl monomer, in the presence of the core polymer and a chain transfer agent containing the aminophenylthio group. That is, the shell is not prepared from monomers already containing a functional group as required by Claim 1. Moreover, although the particle size range overlaps that required by Claim 1, all but one of the examples disclose particle size ranges of from 0.195 to 0.355 μm. The exception, example 9, differs further from the subject-matter of Claim 1 in that the core polymer is essentially polyvinyl acetate which has a refractive index of 1.46, therefore significantly lower than 1.54 as in Claim 1. Most of the examples have polystyrene as core material, which therefore has the required refractive index. However in these cases the subject-matter of Claim 1 is further excluded in that the shell material is derived from styrene monomers in an amount much greater than 10% by weight.

D2 discloses a particle reagent having high refractive index comprising a polymer particle having an inner core and an outer shell to which is covalently bound a compound of biological interest (for example, HCG), the inner core being a polymer having a refractive index of not less than 1.54 as measured at the wavelength of the sodium D line (the list of suitable monomers on pages 7,8 includes styrene, and the examples use polystyrene), and the outer shell of the particle is a polymer of an ethylenically

unsaturated monomer having an epoxy functional group capable of reacting with the compound of biological interest (see the list of monomers on pages 8,9, in particular glycidyl esters as in the patent in suit), and other ethylenically unsaturated monomers (styrene), the outer shell having been formed by polymerisation in the presence of the inner core. The disclosure in D2 therefore differs from the subject-matter of Claim 1 in that the particle size range is 0.15 to 1 μm and the shell contains more than 10% by weight of monomer of the inner core.

D3 discloses core-shell particle reagents to which a compound of biological interest of particle size 0.01 to 0.9 μm, overlapping that required by Claim 1. The core material may be polystyrene or carboxylated copolymers of styrene and butadiene, therefore should have a refractive index greater than 1.54. The shell material is a polyhydroxy compound such as a polysaccharide which is covalently bonded to the core, possibly using a coupling agent. There is no suggestion of an ethylenically unsaturated monomer as required by feature (1) of Claim 1, and the shell material is not formed by polymerisation in the presence of core particles.

D4 does not disclose core-shell particle reagents. The particle material may be polystyrene, but also may be silica or other inorganic material, therefore with refractive index lower than that required by Claim 1. The particle size should be less than 1.6 μm, but as disclosed is, in ascending order of preference, 0.1 to 1 μm, 0.2 to 0.8 μm and 0.3 to 0.6 μm. As pointed out by the Respondent, a particle size of 0.234 μm is stated to be relatively small (see D4': column 6, lines 39 to 49 and column 7, lines 12 to 17).

D5 surveys light-scattering theory but does not disclose specific particle reagents, let alone those of the core-shell type.

The subject-matter of Claim 1, and consequently also of Claims 4, 11 and 13, is therefore novel.

3. **Inventive step**

3.1 In the Board's view D2 represents the closest prior art to the subject-matter of Claim 1 (see paragraph 2.2 above) even though it refers to the use of the reagent for diagnostic tests rather than immunoassays and the problem underlying the patent in suit can be seen as seeking to improve the particle reagent disclosed in D2 so that it is suitable for light-scattering immunoassays. According to Claim 1, as interpreted by the Board, if the outer shell contains monomers of the inner core, these should constitute not more than 10% by weight. This is not the case in D2 where, having particular regard to the examples, the outer shell contains considerably more than 10% by weight of monomers of the inner core. However it is not apparent in what way this difference contributes to inventivity. According to page 7, lines 46 to 48 of the patent in suit, when the monomer (of the shell) is one which contains an epoxy group, it is preferred that the shell polymer be a homopolymer although, as a practical matter, monomers of the inner core, up to 10 parts by weight of the outer shell, can be present. This is in substance repeated in lines 60 to 63. The requirement in Claim 1 seems therefore to be merely reflecting a preference, and the description contains no indication of any deleterious effect associated with more than 10% by weight of core monomers. In any case, since this feature is optional in Claim 1, it does not require consideration in assessing inventive step.

Therefore in assessing inventive step, the difference which has to be taken into consideration is that involving the particle size of the reagent, that is, would the person of average skill in the art, starting from the D2 disclosure, be led to investigate smaller particle sizes, in particular those required by Claim 1.

D2 discloses a particle size range of 0.15 to 1.5 μm, preferably 0.45 to 0.90 μm. Page 2, lines 55,56 (see equivalent GB-A-2 041 940 (D2')) states that the smaller particles will have a greater surface area and consequently more total reaction sites than the larger particles. What is being discussed here, however, is not the criteria for selection of particle size, but the effect of using a latex containing various particle sizes and in view of the preferred range as stated above, the skilled person would derive no incentive from D2 to investigate even lower particle sizes.

D1, D3 and D4, although in other respects more remote from the subject-matter of Claim 1, disclose particle size ranges which overlap or touch the range required by Claim 1. However in the Board's opinion none of these documents contain any teaching which would induce the skilled person to replace the size range of 0.15 to 1.5 μm disclosed in D2 by the size range of 0.03 to 0.1 μm required by Claim 1. D1 and D3 contain no criteria in respect of the choice of particle size and as indicated in paragraph 2.2 above, the examples in D1 employing core material meeting the requirements of Claim 1 disclose particle sizes from 0.195 μm to 0.355 μm. D3, apart from stating the range of 0.01 to 0.9 μm, says no more about particle size and no examples are given.

D4 relates the particle size to the wavelength of light used for the assay, and discloses a wavelength of 0.6 to 2.4 μm, particle size not greater than 1.6 μm, the wavelength being at least 1.5 ties the particle size. Although a first preferred particle size range of 0.1 to 1 μm is disclosed, that is particle sizes under 0.1 μm are not excluded, the general teaching of the document points to particle sizes higher than those required by Claim 1 of the patent in suit. The sizes disclosed in the examples range from 0.234 μm to 0.481 μm and as pointed out in paragraph 2.2 above, 0.234 μm is considered to be relatively low. Further, page 29 of D4 refers to two methods A and B for carrying out the assay, method A measuring the absorption of the test sample and method B the reaction rate, therefore the rate of change of absorption, the latter being the preferred method in the patent in suit.

For method A a particle size range of 0.3 to 0.6 μm is recommended (page 30 of D4) and for method B particles having a relatively large average diameter page 31 of D4). Therefore also from D4 the skilled person derives no suggestion of the particle size range required by Claim 1.

There remains therefore the disclosure in D5, possibly in combination with D4 as put forward by the Appellant in the grounds for the appeal. There can be no doubt that D5 is a document of which the person of average skill in the art can be assumed to be aware, because it is an extract from a publication dealing with Immunoanalysis, and the question to be answered is what teaching he derives from D5.

He learns that the light scattering behaviour of particles varies according to whether their size is less than 0.1 times the wavelength of the radiation, 0.1 to 1 times the wavelength and greater than the wavelength. The second of these possibilities is of most interest, because according

to D5, page 10, many of the smaller aggregates, formed as a result of immunological reactions, are typical examples. From page 10 he learns also that the dissymmetry ratio, which is 1 for very small particles (D5, page 7), increases with particle size. On pages 14 to 16 Raleigh-Debye scattering (applicable to the particles under consideration) is discussed, with the conclusion (page 15) that the intensity of light scattered by a large particle is the same as would be obtained from a small particle of equal molecular weight. This however does not seem to provide a criterion for selection of particle size for particle reagents, but rather is stated to provide a means of measuring the radius of gyration which is an effective particle size for non-spherical particles. A further conclusion (page 16, first paragraph) is that the formation of aggregates in an antibody-antigen reaction can be followed by measuring the changing dissymmetry ratio, but here again no particle size selection criterion can be derived.

All in all, the Board can find nothing in D5 which would provide an incentive for the average skilled person to modify the teaching of D2 and use particle sizes in the range 0.03 to 0.1 μm the more so because he would give more weight to the practical information content of D1, D3 and D4 than to the theoretical information content of D5. The Appellant has developed equations (1) and (11) in D5 to derive equations (1a) and (11a) and substituted these in equation (9) and seeks thereby to demonstrate that the intensity of scattered light goes through a maximum which is dictated by the ratio of particle size to wavelength. The Respondent doubts in effect whether this elaboration of equations (1), (9) and (11) falls within the competence of the person of average skill in the art of immunoassays. It does not seem to be necessary to decide on the latter point, because in the Board's view D5 provides the skilled

person with the conclusions to be drawn from the equations but not with any hint that they would be of any use in the investigation of optimisation of particle size. Moreover in the discussion of Raleigh-Debye scattering one assumption made is that the index of refraction of the particle is close to that of the surrounding medium, which does not apply to the disclosure in D2. It is true that the skilled person can be assumed to know that scattering intensity varies in accordance with the inverse fourth power of the radiation employed. This is not seen as an incentive to operate at very short wavelengths, because maximum scattering intensity does not mean optimum assay sensitivity; in particular in turbidimetry, which measures attenuation of transmitted radiation, it would seem to be self-evident that not too much of the radiation should be scattered, but that an optimum amount should be transmitted to the detector. Further, even if the skilled person were to elect to operate at short wavelengths, it does not follow from the teaching of D4 that he would be led to use particle sizes in the range required by Claim 1. The required ratio of wavelength to particle diameter of at least 1.5 applies to wavelengths of 0.6 to 2.4 μm and, as shown above, to particles which are much larger than required by Claim 1. There is no suggestion that this may be generalised to cover other wavelengths and sizes. Even if, following the Appellant's line, the preferred particle size range of 0.2 to 0.8 μm (Claim 3 of D4) and preferred radiation wavelength of 1 to 1.4 μm (Claim 15 of D4) is taken, this would cover a ratio range of 1.5 (since it may not be less) to 7 (1.4/0.2). Applying this to the wavelengths disclosed in the patent in suit (0.32 to 0.4 μm) a particle size range of 0.046 to 0.266 μm can be derived. The skilled person still has to elect to operate at the lower end of this range, and in view of the teaching in D1 to D4 pointing towards higher particle size ranges, there is no reason for him to do so.

Finally, it is not disputed by the parties that the scattering theories set out in D5 have been known for a long time. However no-one would appear to have been led by these theories to particle reagents having the features required by Claim 1. The subject-matter of Claim 1 therefore involves an inventive step as required by Article 56 EPC and Claim 1 is therefore allowable.

4. Claims 4, 11 and 13 are so linked to Claim 1 as to stand or fall with it and are therefore also allowable. For the same reason the dependent claims are also considered to be allowable.

5. The Grounds of Opposition mentioned in Article 100(a) EPC do not therefore prejudice the maintenance of the European patent as granted.

6. Only the Respondent filed a request for oral proceedings, this request being conditional on the Board's being minded to allow the appeal. Since this was not the case, it was unnecessary to appoint oral proceedings.

Order

For these reasons, it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

P. Martorana E. Turrini