Internal distribution code:

(A) [-] Publication in OJ
(B) [-] To Chairmen and Members
(C) [-] To Chairmen
(D) [X] No distribution

Datasheet for the decision of 7 May 2026

Case Number: T 0939/24 - 3.3.02

Application Number: 18780149.3

Publication Number: 3692101

IPC: C09B11/20, A61K49/00, C07C309/49, G01N33/68, C07C309/46

Language of the proceedings: EN

Title of invention:
BRILLIANT BLUE (BBG) DYE DERIVATIVES AND STAINING COMPOSITIONS COMPRISING THE SAME FOR SELECTIVELY STAINING BIOLOGICAL SUBSTRATES

Patent Proprietor:
Alfa Instruments S.r.l.

Opponent:
D.O.R.C. Dutch Ophthalmic Research Center (International) B.V.

Headword:

Relevant legal provisions:
EPC Art. 54, 56, 83

Keyword:
Novelty
Inventive step
Sufficiency of disclosure

Decisions cited:
G 0001/03, T 0012/90

Catchword:

Europäisches Patentamt
European Patent Office
Office européen des brevets

Beschwerdekammern

Boards of Appeal

Chambres de recours

Boards of Appeal of the
European Patent Office
Richard-Reitzner-Allee 8
85540 Haar
GERMANY
Tel. +49 (0)89 2399-0

Case Number: T 0939/24 - 3.3.02

D E C I S I O N
of Technical Board of Appeal 3.3.02
of 7 May 2026

Appellant: (Opponent)	D.O.R.C. Dutch Ophthalmic Research Center (International) B.V. Scheijdelveweg 2 3214 VN Zuidland (NL)
Representative:	V.O. P.O. Box 87930 2508 DH Den Haag (NL)
Respondent: (Patent Proprietor)	Alfa Instruments S.r.l. Via Fratelli Bandiera NCC 80026 Casoria (NA) (IT)
Representative:	Porta & Consulenti Associati S.p.A. Via Winckelmann, 1 20146 Milano (IT)
Decision under appeal:	**Interlocutory decision of the Opposition Division of the European Patent Office posted/ electronically transmitted on 10 May 2024 concerning maintenance of the European Patent No. 3692101 in amended form.**

Composition of the Board:

Chairman	M. Maremonti
Members:	S. Bertrand
	M. Blasi

Summary of Facts and Submissions

I. The appeal by the opponent ("appellant") is against the interlocutory decision of the opposition division, according to which European patent No. 3 692 101 ("the patent"), as amended in the form of the main request comprising the set of claims filed on 6 July 2023, was found to meet the requirements of the EPC.

II. The patent concerns a dye of a specific chemical formula and compositions comprising said dye for selectively staining biological substrates, in particular structures of the posterior segment of the eye and proteins (paragraph [0001] of the patent).

III. The following documents are referred to in the present decision:

D1	US 2014/0065526 A1
D2	JPH0894826A
D3	English translation of JPH0894826A
D4	WO 2006/062233 A1
D8	US 5,132,439
D13	Experimental Report of Pharmpur GmbH dated 30 June 2023
D14	Spadaro et al., "*New Brilliant Blue G Derivative as Pharmacological Tool in Retinal Surgery*", Frontiers in Pharmacology, May 2020, vol. 11, pages 1 to 10
D17	Experimental report of the University of Catania: "*Evaluation of protein affinity of BBG-derivatives according to European*

Patent EP 3 692 101 vs. compounds BBG and methyl-BBG" dated 30 June 2023

D18 Kohno et al. "*Immunofluorescent Studies of Fibronectin and Laminin in the Human Eye*", Investigative Ophthalmology B Visual Science, 1987, vol. 28, pages 506-14

D21 Peynshaert et al., Current Eye Research, "*Morphology and Composition of the Inner Limiting Membrane: Species-Specific Variations and Relevance toward Drug Delivery Research*", 2019, 44(5), pages 465-75

D22 Halfter et al., "*Origin and turnover of ECM proteins from the inner limiting membrane and vitreous body*", Nature Eye 2008, 22, pages 1207-13

D23 Experimental report "*Development of a* [sic] *SPR-assay for/and quantitative interaction analysis of three Brilliant Blue G dyes binding to FN (R&D grade)*" dated 22 February 2024

A31 Statement from Biaffin, Biaffin GmbH & Co KG dated 5 September 2024

IV. In the impugned decision, the opposition division's conclusions included that the invention defined in the claims of the main request was sufficiently disclosed and that the subject-matter of claim 1 of the main request was novel over the disclosure in each of D1, D2 and D4 and involved an inventive step starting from D4 as the closest prior art.

V. In its statement of grounds of appeal and subsequent letters, the appellant contested the opposition division's findings and argued that the subject-matter of claim 1 of the main request lacked novelty over D1,

D2 or D4 and did not involve an inventive step starting from any of D1, D2 and D4. Moreover, the claimed subject-matter was insufficiently disclosed. Together with the statement of grounds of appeal, the appellant filed the above-mentioned document A31 (denoted D31 by the appellant, new numeration introduced by the board).

VI. In its reply to the grounds of appeal and subsequent letters, the patent proprietor ("respondent") disputed the appellant's arguments.

VII. The board summoned the parties to oral proceedings as per their requests and issued a communication under Article 15(1) RPBA.

VIII. Oral proceedings before the board were held by videoconference on 7 May 2026 with both parties present.

IX. The parties' requests relevant to the present decision were as follows.

The appellant requested that the appealed decision be set aside and that the patent be revoked in its entirety.

The respondent requested that the appeal be dismissed, thereby implying that the interlocutory decision of the opposition division be upheld (main request).

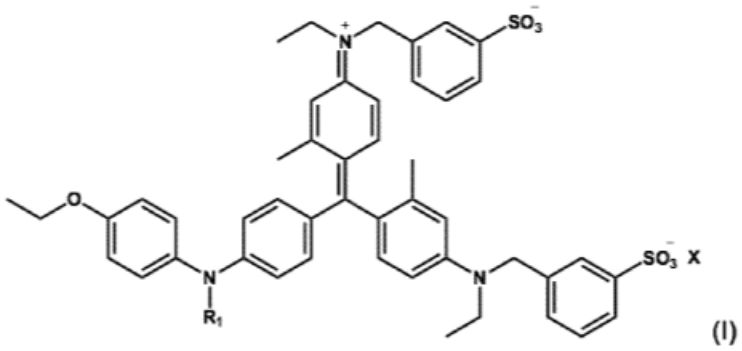
X. The parties' submissions that are relevant to the decision are referred to in the reasons for the decision below.

Reasons for the Decision

Main request

1. Novelty - claim 1 - Article 54 EPC

1.1 Claim 1 of the main request relates to a dye molecule having the following formula (I)

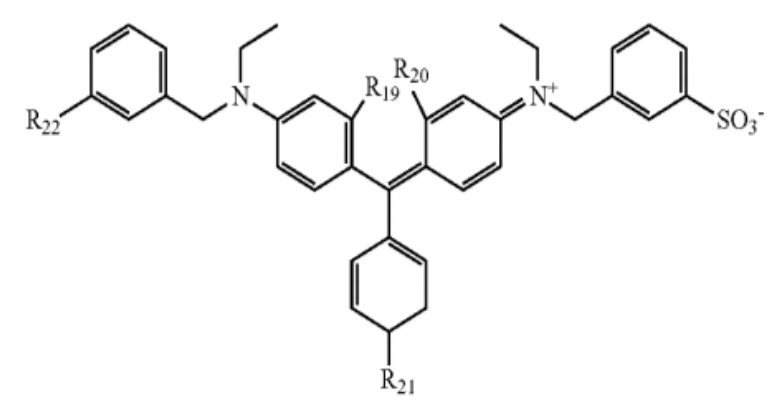


(I)

wherein X represents an organic or inorganic cation and R_1 represents a specific substituted or unsubstituted benzyl group selected from a list of specific groups.

1.2 The appellant submitted that the subject-matter of claim 1 of the main request lacked novelty in view of each of D1, D2 and D4.

1.3 D1 discloses compositions comprising a dye C. Dye C may be a triarylmethane dye C-2 (paragraph [0009]), with dye C-2 including compounds having formula (I) or (II) described in paragraph [0042]. Formula (II) is reproduced below:



R_{19}, R_{20}, R_{21} and R_{22} are defined in paragraphs [0045] and [0046] of D1.

According to the established case law of the Boards of Appeal, a specific combination of elements requiring the selection of elements from two known groups/lists in the absence of a pointer to this selection cannot be regarded as having been disclosed in the prior art and therefore remains novel.

To arrive at a compound falling within claim 1 of the main request, the following selections must be made from the definitions of the substituents of formula (II) in D1 (references in brackets refer to the corresponding passages in D1):

- R_{19} and R_{20} are methyl groups (paragraph [0045]: "*R_{19} and R_{20} each is independently selected from the group consisting of a hydrogen atom, a halogen atom and a* ***C_1*** *to C_5 alkyl group*", emphasis added by the board);

- R_{22} is sulfonyl ($-SO_3^-$) (paragraph [0045]: "*R_{22} is selected from the group consisting of a hydrogen atom and* ***$-SO^{3-}$*** [sic]", emphasis added by the board);

- R_{21} is $—NR_{17}R_{18}$ (paragraph [0045]: "*R_{21} is selected from the group consisting of a hydrogen atom, $-SO^{3-}$*

[sic], *a carboxyl group, a C_1 to C_3 alkyl group, a C_1 to C_3 alkoxyl group and* ***$-NR_{17}R_{18}$***", emphasis added by the board);

- R_{17} is p-ethoxyphenyl (paragraph [0046]: "*the phenyl group comprising the C_1 to C_3 alkoxyl group substituted at a p position thereof of R_{17} and R_{18} is p-methoxyphenyl,* ***p-ethoxyphenyl*** *or p-propoxyphenyl group*", emphasis added by the board);

- R_{18} is benzyl (paragraph [0044]: "*R_{17} and R_{18} each is independently selected from the group consisting of a hydrogen atom, a C_1 to C_5 alkyl group,* ***benzyl,*** *phenyl group and a phenyl group that comprises a C_1 to C_3 alkyl group or a C_1 to C_3 alkoxyl group substituted at a p position thereof*", emphasis added by the board).

D1 contains no pointer to this specific combination of selections. In the absence of any such pointer, D1 does not directly and unambiguously disclose the subject-matter of claim 1 of the main request.

This conclusion was disputed by the appellant.

According to the appellant, the case law concerning selection inventions relating to individual compounds had to be distinguished from cases concerning the selection of a second family of compounds partially covering a broader family disclosed in the prior art. Referring to decision T 12/90, the appellant submitted that novelty could only be acknowledged for a selected family of compounds if the selection provided a new technical teaching, for example through the identification of a specific chemical group which, although encompassed in general terms by the prior-art disclosure, had not been individually disclosed

therein. The appellant argued that claim 1 of the main request did not constitute a novel selection from D1 because it related to a family of compounds rather than to an individual compound and because the claimed family did not provide any new technical teaching over the prior-art disclosure. According to the appellant, none of the claimed structural features had been selected from a generic disclosure in a manner that resulted in subject-matter not already individualised in the prior art.

The board does not find the appellant's reliance on T 12/90 persuasive.

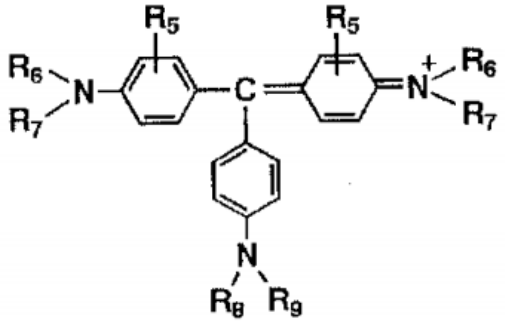
The circumstances of the present case differ from those underlying T 12/90. That decision concerned the assessment of the novelty of a first family of compounds defined by a general structural formula over prior art disclosing a second family of compounds likewise defined by a general structural formula, the first family being encompassed by the second one. The competent board addressed the conditions under which such a first family could constitute a novel invention. In particular, the board emphasised that the assessment of novelty for a selected family of compounds defined by a general formula differed from that applicable to the selection of individual compounds, and required consideration of whether the selected family provided a new technical teaching (see points 2.8 and 2.9 of the Reasons; see also Case Law of the Boards of Appeal, 11th edition, 2025, I.C.6.2.2).

In the present case, however, as submitted by the respondent, claim 1 of the main request defines a list of individualised compounds and not a family of compounds defined by a general formula. Moreover, the appellant has not shown that the claimed subject-matter

constitutes a selected sub-group wholly contained within a broader group disclosed in D1. Rather, the appellant has identified only a limited overlap between claim 1 and the general disclosure of D1 referred to above. More specifically, such an overlap arises only when R_1 in claim 1 of the main request is benzyl, resulting in a salt of a single compound falling within formula (I) of claim 1. The appellant has not identified any further R_1 substituent encompassed by claim 1 that would also fall within the disclosure of D1.

Thus, unlike the situation considered in T 12/90, the present case does not concern the novelty of a selected family of compounds defined by a general formula within a broader family also defined by a general formula disclosed in the prior art. Instead, the appellant has established at most that one individual embodiment falling within the scope of claim 1 may overlap with the disclosure of D1, this embodiment however requiring several selections to be made within the disclosure of D1 as set out above. The board therefore concludes that the considerations set out in T 12/90 are not applicable to the present case and the appellant's objection based on this decision cannot succeed.

1.4 D2 discloses ink compositions for producing a colour filter comprising a triphenylmethane-based compound of formula (3) (paragraphs [0018] and [0019] of D3, the English translation of D2). Formula (3) is reproduced below:

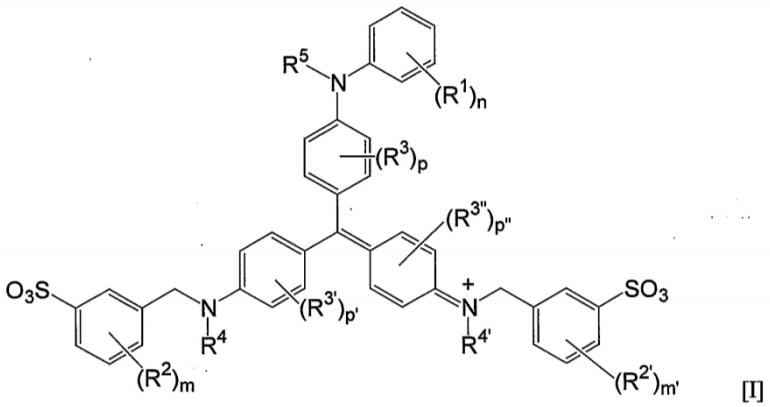


R_5 to R_9 are defined in paragraphs [0019], [0020] and [0034] of D3.

According to paragraph [0034] of D3, R_7 may be a sulfonylbenzyl group. However, D3 does not directly and unambiguously disclose that the sulfonylbenzyl group is a meta-sulfonylbenzyl group as required by claim 1 of the main request. Furthermore, while paragraph [0034] discloses that R_9 may be a phenyl group bearing a C_1–C_6 alkoxy substituent, D3 does not specify that the alkoxy group is ethoxy, let alone that it is in the para position as required by claim 1 of the main request.

For these reasons alone, D2 is not prejudicial to novelty of the subject-matter of claim 1 of the main request.

1.5 D4 relates to a staining composition for staining an ophthalmic membrane comprising a Brilliant Blue G (BBG) derivative or a pharmaceutically acceptable salt thereof. The BBG derivative is represented by formula [I] in paragraph [0012], reproduced below:



[I]

R^5 in formula [I] of D4 corresponds to R_1 in structure (I) in claim 1 of the main request. The substituents of formula [I] are defined in paragraph [0012].

Paragraph [0012] of D4 discloses that R^1 may be *inter alia* a C_1–C_{10} alkoxy group. However, D4 does not directly and unambiguously disclose ethoxy (required to arrive at a compound of formula (I) according to claim 1 of the main request) as a specific embodiment, even if encompassed by the generic alkoxy group. Furthermore, D4 does not specify that the alkoxy group is in the para position.

For these reasons alone, D4 does not anticipate the subject-matter of claim 1 of the main request.

1.6 The appellant's novelty objections in view of D2 and D4 were based on the same arguments as the objection in view of D1, in particular referring to decision T 12/90 discussed above. For the reasons set out previously, however, these arguments are not convincing.

1.7 In view of the above, the board concludes that the subject-matter of claim 1 is novel in view of the disclosure in any of D1, D2 and D4 (Article 54 EPC).

2. Inventive step - claim 1 - Article 56 EPC

2.1 The appellant objected that the subject-matter of claim 1 of the main request lacked an inventive step starting from any one of D1, D2 or D4.

2.2 Starting from D4

2.2.1 The appellant relied on the individual dye compound BBG disclosed in paragraph [0019] of D4, i.e. a compound of formula (I) of claim 1 of the main request wherein R_1 is hydrogen, as the starting point for assessing inventive step.

2.2.2 It was common ground that the distinguishing feature of claim 1 of the main request resided in the definition of the R_1 substituent, namely that R_1 represents a specific substituted or unsubstituted benzyl group.

2.2.3 Technical effect and objective technical problem

The respondent relied *inter alia* on example 8 of the patent (and the application as filed) and documents D14 and D17.

Example 8 of the patent reports dyeing tests of internal limiting membrane (ILM) and retina with, *inter alia*, BBG (comparative, in accordance with D4) and benzyl-BBG (in accordance with claim 1 of the main request). Table 5 of the patent (page 17), which summarises the amounts of the dye molecule in the ILM and retina, is reproduced below:

Table 5

Tested structure	Reference composition including BBG	Reference composition including methyl-BBG		Composition according to the invention including		Benzyl-BBG according to Example 4	
	mean	sd	mean	sd		mean	sd
ILM	74.26	35.64	36.47	22.77		214.88	53.79
Retina	402.50	246.92	219.18	123.80		19.69	37.37

Paragraph [0170] of the patent states that the dye level detected in eyes treated *ex vivo* with benzyl-BBG (approx. 215 ng/mg tissue) was significantly higher than in eyes treated with BBG (approx. 74 ng/mg tissue). Furthermore, benzyl-BBG at the same time exhibits lower retinal migration of the dye compared with the reference composition according to the prior art and including BBG (paragraph [0172] of the patent).

Figure 3 of D14 compares the protein-binding ability of, *inter alia*, BBG ("control BBG", comparative) and benzyl-BBG ("PBB", in accordance with claim 1 of the main request). One of the proteins tested is fibronectin, one of the proteins composing the ILM (see paragraph [0007] of the patent). As shown in figure 3, benzyl-BBG ("PBB") exhibits a higher binding affinity for fibronectin than BBG. The reported differences are statistically significant ($p < 0.05$).

Figure 6 of D14 shows the retinal ILM and the underlying retinal layers after exposure to benzyl-BBG ("Pure-Brilliant Blue G") and two commercial formulations of BBG ("Brilliant Peel" and "ILM Blue"). Benzyl-BBG shows a higher ILM selectivity and a negligible retinal absorption compared with the two commercial formulations of BBG.

D17 is an experimental report on the evaluation of the binding affinity for fibronectin of benzyl-BBG and seven compounds according to claim 1 of the main request (compounds Q1 to Q7) compared with BBG.

Compounds Q1 to Q7 are respectively the following compounds: 4-(trifluoromethyl)benzyl-BBG, 3,5-bis(trifluoromethyl)benzyl-BBG, 4-tert-butylbenzyl-BBG, 3,5-di-tert-butylbenzyl-BBG, 4-chlorobenzyl-BBG, 4-fluorobenzyl-BBG and 3,4-dichlorobenzyl-BBG.

As shown in figure 3A of D17 (see pages 14 to 16), benzyl-BBG and compounds Q1 to Q7 in accordance with claim 1 of the main request have a higher binding affinity for fibronectin, quantified as staining intensity through SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis), than BBG.

Example 8 of the patent, D14 and D17 thus show better and more selective staining of ocular membranes as achieved by the claimed compounds compared with BBG.

This conclusion was contested by the appellant.

The appellant first argued that the results reported in example 8 were not reliable due to deficiencies in the experimental design. In particular, the staining compositions compared in the experiment differed not only in the dye employed but also in the presence and concentration of excipients. As disclosed on pages 15 and 16 of the patent, the benzyl-BBG composition used a different buffer system from the other dye compositions and the BBG composition contained PEG. According to the appellant, these differences prevented any observed effect from being unequivocally attributed to the dye structure itself. Moreover, D13, D23 and A31 demonstrated that no meaningful difference as regards the staining ability existed between benzyl-BBG and BBG.

The board is not persuaded by the appellant's submissions.

The board acknowledges that the staining composition comprising benzyl-BBG of example 8 comprises a mixture of sodium phosphate monobasic dihydrate and sodium phosphate dibasic dihydrate and no excipient (paragraph [0153] of the patent), while the comparative staining composition comprising BBG (paragraph [0156] of the patent) comprises a mixture of sodium hydrogen phosphate dodecahydrate and sodium dihydrogen phosphate dihydrate and polyethylene glycol (PEG) as an excipient.

As submitted by the respondent, however, it is common general knowledge that excipients such as PEG are pharmacologically inactive, non-toxic and inert substances. As such, they would not be expected to interact with the active ingredient or with the other excipients in a manner that would alter, let alone adversely affect, the staining properties of the dye. The same considerations apply to the nature of the buffer. In the absence of any experimental evidence demonstrating that the differences in excipients or buffer composition had an impact on the staining properties of the dyes tested, the board sees no reason to conclude that these differences in the dye formulation invalidate the comparison or affect the reliability of the results obtained.

Table 3 of D13 (page 12), reproduced below, presents the dyeing results of benzyl-BBG, a purified BBG and a commercial BBG (ILM-Blue):

Table 3: Average quantified dye amount from tissue extracts in ng/sample

Tested structure	Benzyl-BBG		Purified BBG		ILM-Blue	
	mean	SD	mean	SD	mean	SD
ILM	14	26	15	16	9	6
Retina	1	2	4	2	4	4

As shown in table 3 of D13, the average amounts recovered from tissue extracts are 14 ng/sample for ILM stained with benzyl-BBG, 15 ng/sample for ILM stained with purified BBG and 9 ng/sample for ILM stained with ILM-Blue (BBG).

However, the experimental variability reported in table 3 of D13 is substantial. In particular, for benzyl-BBG, the standard deviation (26 ng/sample) exceeds the reported mean value (14 ng/sample). Likewise, the standard deviations reported for purified BBG and ILM-Blue are of the same order of magnitude as the corresponding mean values. As submitted by the respondent, in the absence of any statistical analysis demonstrating that the observed values are significant, the data do not permit any reliable conclusion as to whether benzyl-BBG and BBG differ in their staining property. At most, D13 shows that, under the specific experimental conditions employed therein, the reported measurements do not permit any reliable conclusion regarding a difference between benzyl-BBG and BBG. This is not equivalent to proving that no difference exists.

Furthermore, the board is not persuaded by the appellant's reliance on D23. D23 analysed the interaction of *inter alia* BBG and benzyl-BBG with fibronectin, and determined the apparent equilibrium dissociation constant (KD) for this interaction. In the experiments, fibronectin was covalently immobilised on sensor chips. The immobilisation was carried out on two surfaces representing two different surface densities, namely 210 and 980 RU (see point 4.1 on page 10). In table 3 on page 13, D23 reports apparent average KD values of $6.47 \pm 0.89 \times 10^{-6}$ for BBG and $6.97 \pm 2.09 \times 10^{-6}$ for benzyl-BBG, demonstrating, according to the appellant, the absence of any effect of benzyl-BBG over BBG. However, as submitted by the respondent, in view

of the large standard deviation reported for benzyl-BBG, these values substantially overlap. D23 does not report any statistical analysis demonstrating that the observed values are significant or, conversely, that benzyl-BBG does not exhibit improved binding affinity for fibronectin compared with BBG.

Finally, the board is not persuaded by the appellant's reliance on A31, which is an experimental report reproducing the experiment described in D23. Table 1 on page 2 of A31 reports apparent KD values of $6.50 \pm 0.65 \times 10^{-7}$ for BBG and $8.78 \pm 1.01 \times 10^{-7}$ for benzyl-BBG, demonstrating, according to the appellant, an affinity of BBG for fibronectin higher than the affinity of benzyl-BBG. However, A31 merely reproduces part of the experimental protocol described in D23 by reporting the apparent KD values obtained for BBG and benzyl-BBG on only one of the two fibronectin surfaces used in D23 (see above), namely the surface with an immobilisation level of 210 RU (table 1 of A31). As correctly pointed out by the respondent, the experimental design of D23 is based on measurements performed on two fibronectin surfaces differing in their immobilisation levels, and table 3 of D23 expressly reports apparent average KD values obtained by combining the results from both surfaces. The board agrees with the respondent that neither D23 nor A31 provides any convincing technical justification for disregarding the measurements obtained on the 980 RU surface and considering only those obtained on the 210 RU surface as done in A31. Nor does A31 explain the origin of the considerable experimental variability reflected in the apparent average KD values reported in table 3 of D23. Accordingly, A31 cannot be regarded as correcting or superseding the experimental results reported in D23.

Consequently, none of D13, D23 or A31 cast doubt on the technical effect established by the patent (and application as filed) and corroborated by the results reported in D14 and D17.

The appellant further argued that it was entirely unclear why compounds Q1 to Q7 of D17 should be regarded as a representative selection of the compounds falling within the scope of claim 1 of the main request. In particular, although Q1 to Q7 were selected to cover substituents of different steric bulk, ranging from a single fluorine atom (very small) to a tert-butyl group (comparatively large), such a selection did not adequately reflect the breadth of claim 1. The claimed compounds encompassed substantially larger and structurally more complex substituents, such as phenoxybenzyl and 4'-methyl-2-biphenylcarbonitrile groups. Compared with these substituents, a tert-butyl group could not reasonably be considered representative of the upper end of the steric range covered by claim 1 of the main request. Consequently, the appellant submitted that the results obtained for compounds Q1 to Q7 could not be extrapolated to the entirety of the claimed compounds.

The board is not convinced.

It is true that claim 1 encompasses substituents that are bulkier and structurally more complex than tert-butyl, such as phenoxybenzyl and biphenylcarbonitrile groups. However, as submitted by the respondent, the appellant has not provided any evidence showing that these structural differences would be expected to alter the relationship between fibronectin binding affinity and staining properties, or that the technical effect demonstrated for the tested compounds would not also be achieved by compounds bearing such substituents. The

appellant's submission therefore amounts to mere speculation and is not sufficient to cast doubt on the representativeness of the experimental evidence provided by the respondent.

Furthermore, the appellant submitted that the application as filed did not disclose that binding of BBG derivatives to fibronectin was relevant, let alone essential, for the staining properties of the claimed compounds. According to it, the application contained no teaching establishing a causal link between fibronectin binding and improved staining properties. The appellant additionally argued that the significance of fibronectin affinity for the staining of ocular structures was questionable. In this respect, reference was made to the scientific literature, which allegedly provided highly divergent descriptions of the composition of the human ILM. The appellant further contended that more recent publications such as D18, D21 and D22 did not identify fibronectin as a major constituent of the human ILM. Consequently, enhanced binding affinity for fibronectin could not be regarded as a reliable indicator of improved staining of ocular tissues. Finally, the appellant emphasised that the claims were not restricted to staining of the ILM. Even if fibronectin were assumed to play a role in ILM staining, this would not demonstrate an effect across the full scope of the claims, which encompassed the staining of ocular structures more generally. According to the appellant, the staining results obtained with bovine serum albumin (BSA) in the patent and D17 were therefore also relevant to the assessment of the alleged technical effect and should likewise be taken into consideration.

The appellant's submissions are not persuasive.

First, contrary to the appellant's allegation, as submitted by the respondent, the application as filed expressly discloses fibronectin as a component of the ILM (see application as filed, page 1, lines 22 and 23 and corresponding passage of the patent, paragraph [0007] "*the ILM is composed of collagen fibers, glycosaminoglycans, laminin, and fibronectin*."). The selection of fibronectin is therefore not arbitrary, but is directly based on the teaching contained in the application as filed and the patent itself.
Moreover, the relevance of fibronectin is corroborated by the scientific literature relied on by the respondent. In particular, D14, referred to above, expressly refers to fibronectin as "one of the main components of ILM" (page 2, first full sentence), while D18 (relied on by the appellant) discloses that fibronectin and laminin are present in the human ILM and may play a role in the attachment of the vitreous to the ILM and of the ILM to the Müller cell processes (abstract). Therefore D14 and D18, contrary to the appellant's submission, support the use of fibronectin as a relevant model protein for assessing the staining properties of the claimed compounds. The board is not convinced by the appellant's reliance on D21 and D22 either. The fact that these documents refer to laminin and collagen IV (D21, page 466, under the heading "Composition of the inner limiting membrane"; D22 abstract) as major constituents of the ILM does not imply that fibronectin is absent or irrelevant. Nor does the absence of an explicit reference to fibronectin in a particular document constitute evidence that fibronectin is not present in the ILM. Rather, the available evidence shows that fibronectin is one of several proteins present in the ILM and that it is of relevance in the context of retinal membranes.

Consequently, D21 and D22 do not cast doubt on the suitability of fibronectin as a test protein. The rationale underlying the selection of fibronectin as a test protein is therefore supported by both the patent and the state of the art.

Finally, the board does not accept the appellant's submission that the BSA results should be regarded as determinative for assessing inventive step. The respondent relied on fibronectin because of its recognised relevance to the ILM, whereas no comparable relevance of BSA to the staining of ocular tissues has been established. Under these circumstances, the board considers the fibronectin data to be the more appropriate basis for assessing the alleged technical effect.

The appellant further argued that figure 3A of D17 showed that compounds Q4 and Q6 did not exhibit higher binding affinity for fibronectin than BBG. According to the appellant, statistically significant differences relative to BBG were denoted in figure 3A by a single cross, and no such significance marker was present for compounds Q4 and Q6. The appellant therefore concluded that the binding affinity for fibronectin of these compounds was not different from that of BBG, and that their staining behaviour was consequently comparable to that of BBG.

The board is not persuaded by this argument either.

As correctly submitted by the respondent, the results reported in D17 demonstrate that compounds Q1 to Q7, all of which fall within the scope of claim 1 of the main request, exhibit a stronger binding affinity for

fibronectin than BBG (see paragraph bridging pages 14 and 15 of D17).

More specifically, figure 3A shows that all the compounds Q1 to Q7 display staining intensities numerically higher than that of BBG. While for compounds Q4 and Q6 the increase is more limited than for the remaining compounds Q1 to Q3, Q5 and Q7, the results in figure 3A show a consistent trend across all the tested compounds Q1 to Q7 towards increased binding affinity for fibronectin relative to BBG. The data therefore support, rather than contradict, the respondent's position that the introduction of a substituted benzyl group leads to enhanced binding affinity for fibronectin and enhanced staining properties.

Furthermore, the board notes that the appellant has not provided any experimental evidence demonstrating that any of compounds Q1 to Q7, and particularly compounds Q4 and Q6, perform worse than, or even identically to, BBG. In the absence of such evidence, the appellant's arguments cannot call into question the technical effect established by D17.

It follows that the objective technical problem can be formulated as the provision of a dye having improved staining properties with respect to proteins from the ILM.

2.2.4 Obviousness

The appellant relied in particular on D8, submitting that D8 (column 7, lines 33-41) explicitly taught that increasing the hydrophobicity of the group corresponding to R_1 of formula (I) of claim 1 of the

main request enhanced the ability of the dye to stain proteins and increased the affinity for proteins over gel or solid supports. It submitted that a skilled person would have had no difficulty in selecting more-hydrophobic groups from the alternatives listed in D4, such as a benzyl or a hydrophobic substituted benzyl group. Therefore the subject-matter of claim 1 lacked inventive step in view of D4 in combination with D8.

The board is not convinced by the appellant's argument.

D8 relates to the detection of proteins using synthetic dyes of formula (I) (paragraph bridging columns 3 and 4).

D8 does indeed teach that increasing the hydrophobicity of the substituent R^7 corresponding to the R_1 position according to formula (I) of claim 1 of the main request may enhance protein staining and affinity for proteins (column 7, lines 33 to 41). However, as submitted by the respondent, D8 does not suggest achieving this objective by introducing a benzyl or substituted benzyl group at that position. On the contrary, the modification specifically proposed in D8 consists in introducing an acyl substituent at the corresponding position (column 4, lines 26 to 35). Thus, although D8 provides a general incentive to increase hydrophobicity, it directs the skilled person towards a different structural modification from that claimed.

The appellant has not identified any teaching in D8, or in the cited prior art, that would have prompted the skilled person to depart from the specific modification proposed in D8 and instead to replace the hydrogen atom at the R_1 position by a benzyl or substituted benzyl group. The conclusion that the skilled person would have selected such a substituent is therefore based on

hindsight knowledge of the claimed invention rather than on the teaching of the prior art.

Accordingly, the combination of D4 and D8 does not render the subject-matter of claim 1 of the main request obvious.

2.3 Inventive step starting from the generic disclosure of D1, D2 or D4.

The appellant submitted that the claimed compounds merely constituted a selection from the generic disclosures of D1, D2 and D4. According to the appellant, no technical effect had been shown for this selection over the broader class of compounds disclosed in those documents. In particular, no comparison had been provided with compounds representative of the generic disclosures of D1 or D2. The appellant therefore argued that the claimed selection could not support an inventive step, since a selection invention was only inventive if the selected subject-matter was associated with a particular technical effect.

The board is not persuaded by the appellant's submissions.

As regards D4, the appellant's argument is not convincing because, as set out above, the comparative data provided in the patent and corroborated by D14 and D17 credibly demonstrate that the claimed compounds exhibit improved staining properties compared with BBG, a specific compound disclosed in D4. Thus a technical effect associated with the claimed selection has been established.

The same conclusion applies to D1 and D2. Although these documents disclose generic formulae encompassing a broad class of compounds, those generic formulae also

encompass BBG, which was used as the comparative compound in the experimental evidence relied upon by the respondent. Since the generic formulae of D1 and D2 encompass BBG, the comparison with BBG demonstrates a technical effect over at least one embodiment falling within those generic disclosures. The appellant has not shown why any other embodiment of D1 or D2 would have constituted a more appropriate starting point than BBG for assessing inventive step.

Accordingly, the board sees no reason to conclude that the alleged technical effect has not been demonstrated with respect to the generic disclosures of D1 and D2. The appellant's objections must therefore fail.

2.4 In view of the above, the subject-matter of claim 1 of the main request, and consequently that of dependent claims 2 to 6, involves an inventive step within the meaning of Article 56 EPC. The same applies to method claims 7 to 12 concerning the use and the synthesis of the dye of claim 1 (Article 56 EPC).

3. Sufficiency of disclosure - Article 83 EPC

3.1 The appellant raised an objection of sufficiency of disclosure against the claimed subject-matter. In particular, the appellant submitted that:

(a) it was debatable whether all the claimed compounds were able to stain biological structures; hence the skilled person would allegedly be unable to carry out the invention across the full scope of the claims; and

(b) for claims 8 to 12 of the main request, the synthesis methods using microwave irradiation were not disclosed in sufficient detail to enable the skilled person to prepare the claimed compounds.

Specifically, it was argued that step b) of claim 8 could not be performed over the entire range of microwave power recited in the claim, especially at the lower end (as low as 5 W). Furthermore, no conditions regarding reaction temperature or reaction time were specified in the claim.

3.2 The board is not persuaded by the argument that it was debatable whether all the compounds encompassed by claim 1 of the main request were capable of staining biological structures. No evidence has been submitted showing that any compound falling within the scope of claim 1 was incapable of staining biological structures. In the absence of such evidence, the objection remains speculative and is insufficient to establish insufficiency of disclosure. Furthermore, the board notes that the objection concerning the capability of the claimed compounds to stain biological structures amounts to an allegation of non-working embodiments. However, a technical effect — let alone the staining of biological structures — is not a feature of claim 1 of the main request. Consequently, whether such an effect is achieved across the entire breadth of the claim is a matter that was assessed above under Article 56 EPC and is not relevant to the question of sufficiency of disclosure under Article 83 EPC (see G 1/03, OJ EPO 2004, 413, Reasons 2.5.2).

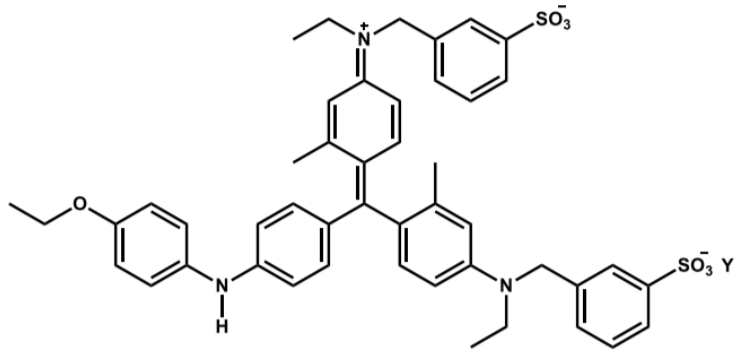
3.3 With regard to the method of claims 8 to 12, the board points out that the burden of proof lies with the opponent/appellant to establish that the skilled person, even when using common general knowledge, would be unable to carry out the claimed invention on the basis of the information provided in the patent.

Claim 8 of the main request reads as follows:

"*8. Method of synthesis of a dye molecule having structure (I) as defined in any one of claims 1 or 2,*

the method comprising

a. forming a reaction mixture having a pH from 8 to 14 comprising a protic solvent and a molecule having structure (III)



(III)

wherein Y represents an organic or inorganic cation; and

b. irradiating the reaction mixture resulting from step a. with microwave radiation comprising electromagnetic waves having power of from 5 W to 300 W in presence of an alkylating agent having structure (IV)

$$R_1\text{-}Z \qquad (IV)$$

wherein R_1 represents a substituted or unsubstituted benzyl group as defined in claim 1 and Z represents a leaving group; so as to obtain said dye molecule having structure (I), and

wherein the alkylating agent is added to the reaction mixture in one or more aliquots in the course of step b."

As to the appellant's argument that step b) of claim 8 of the main request could not be performed over the full range of microwave power recited in claim 8, the decisive question is whether the skilled person — when applying microwave irradiation to N-alkylation of amines, and assuming *arguendo* an initial failure — would find in the patent sufficient guidance to remedy such failure and achieve the desired product. In the board's view, in the light of example 4 of the patent, this question must be answered in the affirmative.

Example 4 discloses the synthesis of a dye molecule of structure (I) wherein R_1 is benzyl using a method involving microwave irradiation. It provides explicit operating conditions, including microwave power (150 W), temperature (80°C) and irradiation time (10 min). These details inform the skilled person of suitable reaction parameters and enable them to adapt the reaction conditions appropriately.

3.4 In view of the above, the board concludes that the invention as claimed in the main request is sufficiently disclosed, and hence the claims of the main request comply with Article 83 EPC.

Conclusions

4. None of the appellant's objections is convincing.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar: The Chairman:

U. Bultmann M. Maremonti

Decision electronically authenticated