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DECISION
of 5 December 2002

Case Number: T 0658/01 - 3.4.2

Application Number: 96938675.4

Publication Number: 0838032

IPC: G01N 30/50

Language of the proceedings: EN

Title of invention:
High performance liquid chromatography method and apparatus

Patentee:
Cohesive Technologies Inc.

Opponent:
Waters Corporation

Headword:
-

Relevant legal provisions:
EPC Art. 56

Keyword:
"Inventive step (yes)"

Decisions cited:
-

Catchword:
-

Case Number: T 0658/01 - 3.4.2

DECISION
of the Technical Board of Appeal 3.4.2
of 5 December 2002

Appellant:
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Decision under appeal: **Decision of the Opposition Division of the European Patent Office posted 18 April 2001 revoking European patent No. 0 838 032 pursuant to Article 102(1) EPC.**

Composition of the Board:

Chairman: E. Turrini
Members: A. G. M. Maaswinkel
G. E. Weiss

Summary of Facts and Submissions

I. The appellant (proprietor of the patent) lodged an appeal, received on 13 June 2001, against the decision of the opposition division, dispatched on 18 April 2001, revoking the European patent No. 0 838 032 (application No. 96 938 675.4). The fee for the appeal was paid on 13 June 2001. The statement setting out the grounds of appeal was received on 8 August 2001.

Opposition had been filed against the patent as a whole on the basis of Article 100(a) EPC in combination with Article 56 EPC and on the basis of Article 100(b) EPC.

The opposition division did not follow the objection pertaining to lack of sufficiency of disclosure (Art. 100(b) EPC) but held that the ground of the opposition under Article 100(a) EPC prejudiced the maintenance of the patent, having regard *inter alia* to the following documents:

(A3) US-A-4 208 284
(A4) US-A-3 493 497
(D1) Stuart Winston Churchill, "Viscous Flows, The Practical Use of Theory", pp. 504 to 507, Butterworths, Boston (1988).

II. In a communication pursuant to Article 11(2) of the Rules of Procedure of the boards of appeal the board referred to the following textbook for the definition of technical terms:

TB1: N.A. Parris: "instrumental liquid chromatography", 2nd edition, Elsevier, Amsterdam, NL, 1984, pages

43 to 50. In addition during the oral proceedings reference was made to Table 3.1 on page 38 of this textbook.

III. Oral proceedings were held on 5 December 2002 at the auxiliary request of the appellant.

During the appeal proceedings the respondent (opponent) made reference to the following documents:

(A14) Tony Edge, "The Application of Turbulent Flow Liquid Chromatography to High Speed Analysis", CAST March/April 2002;

(A17) G.A. Howard and A.J.P. Martin, "The Separation of the C_{12}-C_{18} Fatty Acids by Reversed-phase Partition Chromatography", Biochem. J., Vol. 46, pp. 532 to 538 (1950).

IV. The appellant requested that the decision under appeal be set aside and that the patent be maintained as granted.

V. The respondent requested that the appeal be dismissed.

VI. The wording of claim 1 reads as follows:

"Chromatography apparatus comprising a chromatographic body (20) including a pressure-packed bed of a multiplicity of static, substantially uniformly distributed, rigid, solid, porous particles (22) with chromatographically active surfaces, characterized in that
said particles (22) have average, substantially uniform diameters lying in a range between about 30 µm, to about 500 µm, the interstitial volume (24) between said

particles being greater than about 45% of the total volume of said body; and
said apparatus includes means (26, 28) for loading said surfaces with at least one solute that is reactive with said surfaces, by flowing a liquid mixture containing said solute through said body at a velocity sufficient to induce flow of said mixture within at least a substantial portion of said interstitial volume at a reduced velocity greater than about 5,000."

The wording of claim 14 reads as follows:

"A method of performing liquid chromatography characterized in
forming a body (20) of pressure-packed, substantially uniformly distributed, static multiplicity of rigid, solid, porous particles (22) with chromatographically active surfaces, so as to pruvide [*sic*] an interstitial volume between said particles of greater than about 45% of the total volume of said body, said particles having substantially uniform, average diameters lying in the range of between about 30 µm and about 500 µm;
loading said surfaces with at least one solute that is reactive with said surfaces, by flowing a liquid mixture containing said solute through said body at a velocity sufficient to induce flow of said mixture within at least a substantial portion of said interstitial volume at a reduced velocity greater than about 5,000."

Claims 2 to 13 and 15 to 32 are dependent claims.

VII. The appellant's arguments may be summarised as follows.

The closest prior art for the subject-matter of independent claims 1 and 14 is disclosed in document A4 in which a chromatography apparatus and a related separation process are disclosed. The main focus of the teaching in this document is not the issue of turbulence, but the use of a particular type of packed beds, namely expanded non-fluidised particle beds, see the Abstract of A4, in particular column 1, lines 31 to 34. According to A4 these beds result in a lowered resistance to the flow in the forwarding phase and lower working pressure. The document proposes a particular application of this principle for chromatographic separation in pipelines, for instance in the petrochemical industry.

In the decision under appeal it was stated that the subject-matter of the independent claims differs from the disclosure in A4 in that the bed is pressure packed; in the different ranges of the particles sizes; in the restrictions to the interstitial volume; and in the values of the reduced velocity. However, there are two more differences to the device disclosed in this document, namely the requirements that the particles are static and that they are porous. The problem solved by the invention is to provide a genuine workable pressure-packed chromatography apparatus and method using particular types of particles operating at lower pressures. By defining a range of values for the reduced velocities the claimed subject-matter enables a dramatic enhancement in the speed and the capacity of both analytical and preparative chromatography for both small and large, for instance biological, molecules. In contrast, document A4 does not disclose any numerical value for the mobile phase velocity and, moreover, it teaches that the answer to reducing the plate height is by creating turbulent conditions in the mobile phase.

As can be seen in the patent, this view is not correct, because Figure 9 of the patent shows the dramatic dependence of the reduced plate height on the size of the molecule at the same molecular phase velocity. This behaviour, which in the patent is reflected by the claimed requirement for the reduced velocity, could not have been predicted from the disclosure in A4. Furthermore example 10 of the patent illustrates that by selecting particles of a comparatively large diameter it is possible to attain small values of reduced plate height also for small molecules like acetone. This is a further proof that the relevant parameter is not merely the creation of turbulence as taught by document A4, but that reduced phase velocity is the relevant parameter in conjunction with interstitial volume and particle diameters in a column of porous particles.

For the issue of inventive step it is important to point out that document A4 was published in 1970, 25 years before the priority date of the patent in suit, and its teaching never found any commercial application because of long-standing technical prejudices in the art. In fact, although turbulent flow liquid chromatography using packed columns was known in theory, there were no practical applications of this technique because of a general belief that it would require impractically high pressures. Therefore, documents A4 and A3 were considered rather as theoretical papers. In this respect parts of the disclosure in A4 are speculative, for instance the embodiment shown in Figure 5, proposing to modify a long pipeline for effecting chromatographic separations. This also holds for the Examples in this document. In particular, Example 1 deals with the separation of palmitic acid and stearic acid by partition or liquid-liquid chromatography in which the liquid stationary phase is coated onto a support particle. In partition chromatography the particles should not be porous or have blind pores, see Example 1b, where glass beads are used. Since this Example is opposed to Example 1a, in which the separation is carried out in a "conventional packed column", it is concluded that the "packed column" used in Example 1b must be of the expanded non-fluidised bed type as shown in Figure 2 of A4. In addition, it appears that Example 1b cannot be performed either as disclosed or at all. The respondent's argument that for the interpretation of the experimental conditions of Example 1b the skilled person would have consulted the publication A17 is not persuasive, since there was no legitimate reason why he would have done so, the more because not only the particles were different (kieselguhr versus silica beads) but also the respective packing methods.

Comparing the disclosure of document A4 with the teaching of the patent it is observed that A4 contains three pointers leading away from the invention. In column 7, lines 10 to 16, and column 8, lines 36 to 39, it discloses that the use of an expanded non-fluidised bed is preferable to wall-strengthened high pressure columns. In addition, there is no disclosure of an embodiment involving a pressure-packed bed at all. In contrast, claims 1 and 14 of the patent define that the particles should be pressure-packed and static. Furthermore, in the same column 8, lines 22 to 35 of A4, it is recommended that the minimum particle size should be in the vicinity of 0.5 mm, and more advantageous, a powder at least of the order of 1 mm. This large particle size is required in order to reduce the pressure drop. Although for the single value of 500 µm there is, in a mathematical sense, an overlap between the particle size in A4 and the range in the independent claims of the patent, the recommended range in A4 is irreconcilable with the range of between about 30 µm to about 500 µm in the patent, which furthermore teaches that the actual particle diameter should be selected having regard to the size of the molecules to be separated (example 10). In this respect it is noted that in the textbook TB1, page 38, Table 3.1, it is disclosed that in 1984 it was "current practice to use particles of less than 10 µm diameter", which equally teaches away from the range defined in the independent claims. Thirdly in column 5, lines 28 to 48 document A4 teaches that the contribution of the retarding phase should be as low as possible, and that therefore non-porous particles or at least not having blind pores such as silica or alumina should be used. The independent claims of the patent define the use of porous particles. Since all these requirements from A4 are opposite to the features defined in the independent claims of the patent, a modification of the chromatography device to the claimed device is not obvious. In addition, there is no teaching in A4 at all concerning interstitial volumes, even including the passages cited by the division in this respect, and a combination of the teachings of A4 and A3 is not possible, since the latter document teaches the use of giant honeycomb structures, which is irreconcilable with an expanded non-fluidised bed disclosed in A4. Finally the division's view that the use of a reduced velocity greater than about 5,000 is known per se and that this would be suggested in A4, because it mentioned turbulence in the forwarding phase, is erroneous, because the reduced velocity depends not only on establishing turbulence conditions but as well on the diffusion of the solute in the forwarding phase, on which issue document A4 is silent. Also the opponent's assertion that there is a simple proportional relationship between the Reynolds number and reduced velocity, and that in order to establish turbulent conditions document A4 would teach to increase the Reynolds number and thereby the value of the reduced velocity, is unfounded. This is because the Reynolds number contains the superficial velocity in the nominator and the viscosity of the forwarding phase in the denominator, whereas the reduced velocity contains mobile phase velocity in the nominator and the diffusion coefficient of the solute in the denominator, therefore these dimensionless quantities are not comparable. In any case, the value of the Reynolds number to indicate the transition from laminar to turbulent flow including the superficial velocity is only valid for open tubes, and there is no consensus in the scientific community about the relation between Reynolds number and the creation of turbulence for a flow through interstitial volumes. Furthermore whereas the reduced velocity takes the porosity of the particles into account, the Reynolds number does not and cannot, which again proves that these quantities are not comparable.

VIII. The respondent's arguments may be summarised as follows.

Document A4 forms the closest prior art for the subject-matter of claims 1 and 14. This document relates to a high performance liquid chromatography apparatus and method and discloses that, for obtaining the minimum reduced plate height, the condense packing of small particles is not the only optimum but that a second optimum may be obtained if an arrangement with turbulent flow and radial mixing to enhance mass transfer is selected. A4 teaches that by using larger particle sizes the requirement of a large pressure drop is relaxed, see, for instance Example 1b of A4. This example differs from the requirement in claim 1 of the patent in suit in that it does not *explicitly* disclose that the bed should be pressure-packed, but the skilled person would immediately realise that this example was carried out under the same experimental conditions as the famous palmitic and stearic acid separation experiment by Howard and Martin in reference A17, in which, similar as in Example 1b in A4, the retarding phase consisted of paraffin and the particles had been treated with dichlorodimethyl silane. Hence he would realise that the author of document A4 would have packed his column by a slurry technique using a plunger to exert pressure as disclosed in A17, which results in a packed column, as indicated in Example 1b of A4. It is added that the terms "packed bed" or "pressure-packed bed" are used as synonyms, and that any packing at higher than atmospheric pressure would fall under the definition of "pressure-packing". As to the particle size, document A4 discloses in column 8, line 14 a range of 10 to 500 mesh ASTM, which corresponds to sizes of 2400 μm to 50 μm and therefore overlaps with the range of the independent claims of the patent in suit. Furthermore in column 8, line 30, A4 discloses the use of a particle diameter of 0.5 mm. It is added that although the advantage of using smaller particles - namely the totally larger active surface of the bed - was known, at the time of the writing of document A4 particles having a uniform diameter smaller than of 0.5 mm were not generally available, which explains the use of particles of 1 mm diameter in Example 1b of A4. According to column 5, lines 33 to 35, porous particles like alumina and silica gel can be used, which substance is also employed in Example 3b and 4b. With respect to the interstitial volume, document A4 does not disclose an explicit value, but the document emphasises that it is desirable that the turbulence of the forwarding phase should extend over the entire column width (column 5, lines 52 to 53), which also implies that it should extend over the interstitial channels. Since A4 teaches that a loose packing of the particle bed in order to obtain turbulence is favourable, a value greater than about 45% of the interstitial volume is a direct consequence of such loose packing and the claimed range is automatically obtained, even when using spherical particles, as was discussed in point 2.2 of the Decision under appeal and mentioned in document D1, Table 19.1. Also the authors of A4 emphasised in document A3 the relevance of a high interstitial volume. Finally A4 does not give an explicit value for the reduced velocity. However, the main issue of document A4 is selecting a sufficiently high relative velocity between the phases so that turbulence is established and a second optimum of the theoretical plate height curve can be obtained as shown in Figure 1. The relevant phenomenon is therefore turbulence, which can be either addressed in terms of Reynolds number or equally by reduced velocity. This correlation between turbulence and Reynolds number also for packed columns has been acknowledged in the recent publication A14 by the appellant's company, according to which "it is generally accepted that the onset of turbulence occurs at Reynolds number between 3 - 7 in a packed column". See also the patent specification, page 9, lines 9 and 10; and claim 9 of the patent. By means of the value of the separation time between the species in the Examples of document A4 a value of the chromatographic velocity can be calculated which allows a conservative estimation of the velocity of the mobile phase. Such an estimation leads to values of the reduced velocities above 5,000.

Therefore the only differences between what is known from document A4 and the independent claims is that the latter *explicitly* define values for the interstitial volume and for the reduced velocity. As was discussed in the Decision under appeal, page 8, second paragraph, the underlying problem addressed by defined ranges is to achieve a substantially greater mobile phase velocity i.e. to provide a turbulent flow, and the skilled person would know that the conditions for turbulence could, instead in terms of Reynolds number, equally be defined in terms of reduced velocity, which parameter was known in the art. Furthermore he would know from document A3 that for creating turbulent flow, interstitial channels larger than 45% were advantageous, and document D1 taught the skilled person how to achieve such conditions.

IX. The board gave its decision at the end of the oral proceedings.

Reasons for the Decision

1. The appeal is admissible.

2. *Objection under Article 100(b) EPC*

The objections under Article 100(b) EPC made by the opponent in the notice of opposition have not been repeated during the appeal procedure. Therefore it appears that this issue is no longer under dispute between the parties.

3. *Inventive step*

3.1 There is agreement amongst the parties that document A4 discloses the closest prior art. This document discloses a number of chromatographic separation processes and apparatuses of widely varying configurations (open columns, packed columns, pipelines, see Figures 2 to 5 and the Examples in A4), which have in common that these are designed in order that the relative velocity between the forwarding and the retarding phases is higher than that corresponding to the onset of turbulence in the forwarding phase, thereby decreasing the resistance to mass transfer in the forwarding phase and reducing the theoretical plate height. According to A4, column 4, lines 18 to 32, care has to be taken not to obscure the aimed effect by keeping the contribution to plate height by the retarding phase low, which is achieved by choosing the lowest possible film thickness in the retarding phase and by the selection of non-porous materials or porous material devoid of blind pores (column 5, lines 28 to 38).

In the following Sections, the differences in the features of the embodiments in document A4 and the claimed apparatus and method are addressed.

3.2.1 Since the independent claims of the patent in suit define a chromatography apparatus and method employing a *pressure-packed* bed, those parts of the disclosure in document A4 relating to "packed beds" appear to be of interest in the discussion of inventive step:

3.2.2 In column 7, lines 11 to 20, A4 discloses that high flow rates may be attained in packed columns with modified wall strengths dimensioned to withstand the higher pressures needed, and that the particles size should at least be 0.5 mm in diameter and preferably at least 1 mm.

3.2.3 In a subsequent passage, starting on line 21 of this column, A4 discusses modifications of the column in the form of a "loose bed", wherein the bed is prepared by first introducing the forwarding phase at a rate sufficient for bringing the packing in a fluidised condition and the particles are in thoroughly turbulent movement, and, thereafter, by reducing this rate, settling the bed at a lower bulk density and higher porosity than in its loosest condition in the absence of any flow of the forwarding phase. In column 8, lines 36 to 39, document A4 refers to this bed as an "expanded non-fluidised bed" which contrasts to a "firmly packed bed" in that the pressure drop needed for attaining a given flow rate may be as much as twenty times less. As to the nature of the packing material it is recommended (see column 8, lines 5 to 16) to use generally spherical particles with size "usually in the range 10 to 500 mesh ASTM" (according to the respondent corresponding to approximately 2400 µm to 50 µm), and for a particular case of gas chromatography a minimum favourable particle size "in the vicinity of 0.5 mm" and more advantageous "a powder at least of the order of 1 diameter" (column 8, lines 24 to 35).

3.2.4 A third series of embodiments referring to "packed columns" is in the context of Example 1b, further "packed columns" being referred to in Examples 3, 4 and 5. As to the nature of the packed columns, the description of these examples does not offer further information, apart from the fact that the packed column in Example 1b apparently is a counterexample to the "conventional packed column" referred to in Example 1a. In Example 1b the particles are glass beads of 1 mm diameter and the flow is turbulent, whereas in Example 1a the particles consist of kieselguhr and the flow is laminar.

3.2.5 With respect to the question whether the packed column in Example 1b involves a *pressure-packed* column, the parties have different views.

According to the appellant, since Example 1b is a counterexample to the one in Example 1a and the latter involves a "conventional" packed column, it follows that the column in Example 1b should be construed in accordance with the embodiment of the invention of document A4, and therefore as involving an "expanded non-fluidised bed", which by its nature is not "pressure-packed". Furthermore the appellant expressed its doubts concerning the feasibility of the separation process in this Example which in his opinion was purely speculative. The respondent argued that Example 1b involves the repetition of the classic chromatography experiment disclosed in document A17 of the separation of the same substances palmitic and stearic acid. Therefore the experimental conditions should also be as in A17, which involved preparing the particle bed by slurrying and using a plunger, thereby pointing towards pressure packing.

3.2.6 The board in its assessment of the nature of the packed column in Example 1b of A4 is unable to follow the argument by the respondent. Firstly in document A4 there is no reference to the publication A17 or mentioning of this experiment. Furthermore it appears that the kind of particles used in Example 1b (glass beads, 1 mm diameter) and those employed in A17 (kieselguhr) is different. Finally the dimensions of the columns are different (standard columns of 20 cm length and 12 mm diameter in A17, a column of 2.5 m length in Example 1b). It is therefore not plausible why the column and the particle bed in Example 1b of A4 would have been prepared using the slurrying technique used in document A17. Furthermore, according to the textbook TB1, page 46, lines 3 to 27, a slurrying technique is employed for packing columns with particle materials of less than 20 µm diameter, whereas for larger particles a "dry-packing" method using a bouncing technique is more suitable (page 44 and 45 of TB1). Therefore it would be expected, that glass beads with 1 mm diameter would not be slurried or pressure-packed as the kieselguhr particles in document A17.

3.2.7 On the other hand, since, according to A4, column 10, lines 63 to 65, the examples directly following this passage show "applications of the invention", and since Examples 1a and 1b apparently compare a conventional experimental set-up (1a) and a further set-up (1b), it appears highly probable that the packed column in Example 1b was prepared following the procedure disclosed in column 7, line 28 to column 8, line 45 according to which the column in accordance with the embodiment of the invention shown in Figure 2 was prepared (column 4, lines 6 to 8). This view is further supported by the fact that the particles used (glass beads, 1 mm diameter) are recommended for this procedure (lines 28 to 35 of column 8) and that the resulting column is used under turbulent conditions.

3.2.8 Therefore the board finds that apart from the "conventional" packed columns which need higher wall strengths (column 7, lines 11 to 16) and for which document A4 does not provide any further details, the columns disclosed in A4 in the embodiments of Figures 2 to 4 and in the Example 1b all include "expanded non-fluidised beds".

3.3.1 With respect to the particles, the independent claims define that these should be "static" and "porous"; and they have average substantially uniform diameters lying in a range between about 30 μm, to about 500 μm.

3.3.2 In document A4, see the embodiments of Figure 2 and Example 1b (see the discussion in points 3.2.7 and 3.2.8 *supra*), the particles are in an expanded non-fluidised bed, which bed has "free flowing properties" (column 7, lines 52 to 54), therefore it appears that the particles are not "static" as defined in the claims.

3.3.3 The feature "porous" particles can be found in column 5, lines 33 to 35 and column 8, line 17, of document A4 disclosing the possible use of silica gel and alumina (with the restriction that these particles should be devoid of blind pores). Alumina particles are equally used in the patent in suit (page 11, lines 5 to 19 and further Examples). On the other hand, although in

Example 1b of A4 glass beads are disclosed, it is noted that in Example 10 of the patent in suit equally silica particles are used (page 13, line 28). Therefore it would appear that the same kind of porous and non-porous particles as in the patent are disclosed in document A4.

3.3.4 As to the particle diameter ranges the respondent has referred to column 8, line 14 of A4, in which is disclosed that powder particles will "usually be in the range 10 to 500 Mesh ASTM", which according to the respondent corresponds to sizes of 50 µm to 2400 µm. In the interpretation of the board this citation has to be read together with the following paragraph in lines 22 to 35 in the same column, in which the range is further restricted by the information that the minimum favourable particle size is in the vicinity of 0.5 mm, and that more advantageous will be a powder at least of the order of 1 mm in diameter. Furthermore the glass beads employed in Example 1b of A4 equally are 1 mm in diameter.

The respondent has argued that the use of larger particles in document A4 had been motivated because at the time of the original filing of A4 (1966) smaller particles of uniform sizes would not have been generally available. The respondent did not provide any material supporting this submission. On the other hand the appellant had referred to textbook TB1, Table 3.1 on page 38, from which it follows that at least at the publication date of this book (1984) particles of 10 µm diameter size had been available. According to the references in this Table, particles with mean diameter below 10 µm had been available in 1972. Therefore it appears to the board that the recommendation in A4, column 8, of choice of particles size is not so much motivated by lack of availability of smaller particles, but because a larger particle size enables to obtain turbulent conditions with a smaller pressure drop. In any case the upper value of "about 500 µm" defined in the independent claims of the patent in suit appears to be disclosed in the embodiment of Figure 2 of A4.

3.3.5 In the independent claims it is furthermore defined that the interstitial volume between the particles should be greater than about 45% of the total volume. In this respect the respondent has argued that according to the literature in document D1 a value of the void fraction for packing spheres is in the range of 0.3781 to 0.468, and that this textbook reports a value of 0.412 for glass spheres. The board observes that since, according to document A4, in the embodiment of Figure 2 the bed is loosely packed, which condition would also apply to Example 1b of A4, it appears that these respective beds have an interstitial fraction of "greater than about 45%", the more because the range limits in the claims are not sharply defined by the use of the term "about".

3.3.6 Finally the independent claims define that the reduced velocity of the mixture containing the solute should be greater than about 5,000. It is noted that the parties agreed that document A4 is silent about this condition. On the other hand, the respondent has submitted the result of calculations in support of his contention that document A4 would anticipate implicitly the claimed range of the reduced velocity. However, as submitted by the appellant, in case of packed columns there appears to be no unambiguous and unique correlation between the reduced velocity, the Reynolds number and the setting of a turbulent flow through interstitial volumes that would support the indirect estimation of the reduced velocity carried out by the respondent on the basis of the information content of document A4. In the absence of clear and conclusive evidence, the respondent's submission that the claimed range of the reduced velocity would be inherently anticipated by the disclosure of this document has not been sufficiently proven to the board's satisfaction.

3.4 From the above analysis it is concluded that the subject-matter of the independent claims differ from the disclosure in A4, in particular the embodiment of Figure 2 and Example 1b in the following features:

i) the bed is pressure-packed;

ii) the particles are static;

iii) a reduced velocity of greater than about 5,000.

3.5 In the opinion of the board, the technical problem addressed by these differences may be seen in the provision of a chromatography apparatus and method which enable to obtain optimised reduced plate heights for both small and large molecules and at lower pressures.

3.6 The solution to this problem is by the *combined* features defining the nature of the particles, their size, the way of packing, their interstitial volume and the requirements to the reduced velocity. Because these features are interrelated and cooperate with each other (*for instance, the conditions "pressure packing", "interstitial volume" and "static and porous* *particles"; or "particle size" and "reduced velocity", both affecting the reduced plate height and the required pressure drop*), in the assessment of inventive step it should be avoided to consider each feature in isolation.

3.7.1 Having regard to the embodiment of Figure 2 in A4, it does not appear obvious why the skilled person *would* modify the loosened bed with free flowing particles to a pressure-packed bed with static particles, because according to document A4 the expanded non-fluidised bed offers the advantage of providing turbulence conditions at lower pressure drops. Therefore the skilled person would not find an incentive to modify the apparatus against this teaching. Furthermore such an incentive is also not found in any other prior art document.

3.7.2 Since, if interpreting the operational conditions of Example 1b in the light of the teaching of document A4 as a whole, the particles in this Example are packed as in the embodiment of Figure 2, the above conclusion equally applies to this embodiment.

3.8.1 Furthermore no teaching or suggestion was found in document A4 in which way other parameters that may affect the chromatographic performance, such as the reduced velocity, would have to be selected in order to provide an optimum separation as a function of the size of the molecules. The appellant's argument that the relevant quantity is the reduced velocity which not only includes the velocity of the mobile phase, but also the particle diameter and the diffusion coefficient of the solute in the mobile phase, as illustrated in the patent in suit, appears credible.

3.8.2 The emphasis in document A4 is on creating conditions in which turbulence is more easily established. The value of the particle size is only addressed in relation to the achievement of a low pressure drop, and the recommendation in A4 for obtaining such conditions is in the direction of particle diameters greater than 0.5 mm, which would lead away from the range defined in the claims. Finally the document does not discuss the effect of the diffusion coefficient of the solute in the mobile phase.

3.9 The opposition division had based its conclusion that the claimed subject-matter was obvious in the light of the teaching of document A4 on the assessment that A4 disclosed packed columns, and that the use of pressure packing would be common practice to the skilled person, whence this feature did not contribute to inventive step. That, furthermore, the skilled person at the priority date of the patent would have used particles with sizes below 500 µm, because they were available then (unlike the situation at the filing date of A4). And that the use of a reduced velocity greater than 5,000 is known per se for the same purpose and/or at least obvious for the skilled person in order to solve the problem posed. The board does not share this view, as may be seen from the above paragraphs. Rather it is of the opinion that, even if some of the claimed features might be anticipated or rendered obvious by the prior art when assessed *in isolation*, the objective assessment of the inventive step of the claimed subject-matter should take into account the technical effects associated with the cooperation between the different claimed features and thus requires the assessment of the inventive step of the *overall combination of all features* defined in the independent claims.

4. Claims 2 to 13 and claims 15 to 32 are dependent on claims 1 and 14 and, therefore, their subject-matter also involves an inventive step.

Order

For these reasons, it is decided that:

1. The decision under appeal is set aside.

2. The patent is maintained unamended.

The Registrar:	The Chairman:
P. Martorana	E. Turrini