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D E C I S I O N
of 13 January 2006

Case Number: T 0112/04 - 3.3.05

Application Number: 90903657.6

Publication Number: 0442977

IPC: B01D 15/08

Language of the proceedings: EN

Title of invention:
Chromatography method

Patentee:
PERSEPTIVE BIOSYSTEMS, INC.

Opponent:
Amersham Biosciences AB

Headword:
Chromatography/PERSEPTIVE

Relevant legal provisions:
EPC Art. 100(b), 54, 56

Keyword:
"Sufficiency of disclosure: yes"
"Novelty: yes"
"Inventive step: yes"

Decisions cited:
T 0225/93

Catchword:
-

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Case Number: T 0112/04 - 3.3.05

D E C I S I O N
of the Technical Board of Appeal 3.3.05
of 13 January 2006

Appellant: (Opponent)
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Decision under appeal: **Interlocutory decision of the Opposition Division of the European Patent Office posted 14 November 2003 concerning maintenance of European patent No. 0442977 in amended form.**

Composition of the Board:

Chairman: M. Eberhard
Members: B. Czech
H. Preglau

Summary of Facts and Submissions

I. The appeal is from the decision of the opposition division concerning the maintenance of European patent No. 0 442 977 in amended form, on the basis of the "2nd amended auxiliary request 3" presented during the oral proceedings on 13 March 2003.

II. The independent claims 1, 12, 23 and 24 according to this request read as follows:

"1. A chromatography method comprising passing a liquid mixture of solutes comprising biological molecules, and subsequently a liquid eluant, through a chromatography matrix to load onto and thereafter to elute said solutes from said matrix,

said matrix comprises interconnected first and second throughpore sets, the ratio of the mean diameter of the first throughpore set to the mean diameter of the second throughpore set being small enough such that intraparticle flow enhances mass transport in the second throughpore set at liquid velocities above 1000 cm/hr through the matrix bed, the members of said first throughpore sets having a greater mean diameter than the members of the second throughpore set, the second throughpore set being in fluid communication with solute interactive regions which interact reversibly with said solutes to effect chromatographic separation thereof,

the liquid mixture or eluant is passed through the matrix at a fluid velocity sufficient to

(i) induce a convective fluid flow through said throughpores,

(ii) provide that the fluid flow velocity is greater through the first throughpore set than is the fluid flow velocity through the second throughpore set, and (iii) induce a convective fluid flow through the second throughpore set at a rate which is greater than the rate of diffusion of a said solute through said second throughpore set,

whereby there exists a range of liquid velocities above 1000 cm/hr through the matrix bed, wherein bandspreading is substantially constant, wherein the eluant or liquid mixture is passed through the said matrix at a bed velocity greater than 1500 cm/hr and wherein the matrix comprises packed particles having a mean diameter greater than 8 μm, said second throughpore set comprises throughpores within the particles having a mean diameter greater than 200nm (2000Å), and the ratio of the mean diameter of the particles to the mean diameter of the throughpores is less than 70."

"12. Use of particles defining a pore structure at least bimodal in its distribution which form a chromatography matrix, in the chromatography method of claim 1, wherein the matrix in use exhibits substantially constant bandspreading over a range of flow velocities greater than 1000 cm/hr through the matrix bed, involving a hybrid mass transfer system of convective and diffusive transport wherein the velocity of convective transport exceeds the velocity of diffusive transport in throughpores permeating the particles,

wherein said particles have a mean diameter greater than 10 μm and comprise a rigid solid defining (1) solute interactive regions derivatised with

chemical groups which interact reversibly with biological solutes to effect chromatographic separation thereof, (2) a plurality of throughpores for convective mass transport, and (3) a plurality of smaller pores in communication with the throughpores for diffusive mass transport to said interactive surface regions,

the ratio of the mean diameter of the particle to the mean diameter of the throughpores being less than 70, and having throughpores with a mean diameter greater than 200 nm (2000 Å) and wherein the flow velocities through the matrix bed are greater than 1500 cm/hr."

"23. Use of a matrix for conducting adsorptive liquid chromatography comprising use of particles according to any one of claims 12 to 22."

"24. Use of a chromatography system including use of a chromatography matrix according to claim 23, disposed in a column and a pump for passing liquids through said matrix."

III. The references cited in the course of the opposition procedure include the following:

R1: N. B. Afeyan et al., "Flow-through particles for the high-performance liquid chromatography separation of biomolecules: perfusion chromatography"; Journal of Chromatography, 519, 1990, pages 1 to 29

R5: Summary by C. Herbertsson (and translation thereof into English) of:

F.E.Regnier, "Macroporous Divinyl Benzene-Based Media for Protein Separations", presented at: "HPLC-88, 12th International Symposium on Column Liquid Chromatography", Washington DC, USA, 19-24 June 1988

R6: L. L. Lloyd et al., "Affinity and ion exchange chromatographic supports for high performance biological separations"; presented at: International Conference on Separations for Biotechnology, University of Reading, UK, 15-18 September 1987

R7: L. L. Lloyd et al., "Influence of pore size/ionic capacity on the separation of small and large biomolecules when using polymeric anion exchange media"; presented at: Seventh International Symposium on HPLC of Proteins, Peptides and Polynucleatides, Washington DC, USA, University of Reading, UK, 2-4 November 1987

R8: L. L. Lloyd et al., "Polymeric Anion Exchange Columns for the HPLC Analysis of Large Biological Solutes (Proteins); presented at: 39th Pittsburgh Conference and Exposition on Analytical Chemistry and Applied Spectroscopy, New Orleans, USA, 22-26 February 1988

R9: L. L. Lloyd et al., "Application of Polymeric Packings in Bio-HPLC"; presented at: International Symposium on Biomedical Applications of Liquid Chromatography, Bradford, UK, 23-25 March 1988

R13: A "Memorandum and Order", Civil Action No. 93-12237-PBS, US District Court, District of Massachusetts, March 31, 1997

R15: Extracts from the thesis of R. W. Stringham, "Selective Non-Adsorption Preparative Chromatography of Proteins", 1989, kept confidential up to May 1991

R18a: Polymer Laboratories, Price List effective from 1st April 1989

R18b: Undated Polymer Laboratories brochure "High Performance Columns and media for Today's Life Scientist", pages 1 to 10

R19: Fax from F. Warner to N. Afeyan dated 7 October 1988 (3 pages of 4)

R101: US-A-3 782 075

R102: Amendment, Response and Interview Summary Record for US Patent Application Serial No. 376 885 received by the USPTO on 28 August 1990; pages 1 to 19

IV. In the contested decision, the opposition division held that the patent in the amended form according to the "second amended auxiliary request 3" complied with the requirements of Articles 123(2) and (3), 83, 54 and 56 EPC. Concerning sufficiency of the disclosure, the opposition division concluded that claim 1 as maintained contained sufficient matrix structure related information together with specific fluid flow

related information so that a skilled person could without undue burden carry out the method of claim 1 in the whole area claimed. Furthermore, the patent contained enough guidance for the skilled person on how to produce the required particles containing the necessary throughpores. It considered R8 to represent the closest prior art. Taking into consideration also R18a and R19, it concluded that the documents cited during the opposition period did not suggest a chromatographic method as claimed.

V. In its statement of grounds of appeal the appellant (opponent), referring to R1 and to decision T 0225/93 and relying on the two further references

R201: M. Rhodes, "Introduction to Particle Technology", 1998, John Wiley & Sons, Chichester, UK, pages 55-66; and

R202: J. Seville et al., "Processing of Particulate Solids", 1997, Blackie Academic & Professional, London, UK, pages 1-52

objected to the sufficiency of the disclosure of the claimed invention. It considered the subject-matter of claim 1 to lack novelty in view of either of references R9 and R6, and argued that a more complete R19 might be relevant for claim 12. Referring to R5, R6 and R9, it also considered the subject-matter of claim 1 to be obvious. The subject-matter of claim 12 lacked an inventive step in view of the teachings of R9 and R18b.

VI. In its reply, the respondent (proprietor of the patent) rejected all the objections raised as unfounded. It discussed the references relied upon by the appellant.

VII. With its last submission of 13 December 2005, the appellant submitted copies of two further documents:

A1: a US court opinion, and

A2: A sheet labelled "Protein Capacity Determination", allegedly the fourth page missing from R19.

The appellant objected to the clarity of claim 1 and, referring also to R18a, it upheld its objections as to the sufficiency of the disclosure. It dropped its novelty objection based on R6 but still considered the claimed subject-matter to lack novelty in view of R9 or at least inventive step in view of R5 to R9. In discussing some statements of the respondent, it also referred to R13, R15, R18b and R101.

VIII. With its last submission of 9 January 2006, the respondent submitted a "further auxiliary claim set" comprising a further amended claim 1 (in part), and reminded the board of other auxiliary requests filed before the opposition division.

IX. Oral proceedings took place on 13 January 2006. In the course of these oral proceedings, the respondent submitted three complete sets of amended claims as auxiliary requests 1 to 3. The appellant filed the two following references:

R301: S. K. Bathia et al., "Geotextile characterization and pore-size distribution: Part III. Comparison of methods and application to design"; Geosynthetics International, 1996, Vol. 3, No. 3, pages 301 to 328,

R302: A print-out of 26 slides allegedly presented at PREP2004 by L. M. Bryntesson et al., "Analysis of Chromatographic Media using a Pore Network Model".

X. The appellant requested that the decision under appeal be set aside and the patent be revoked.

The respondent requested that the appeal be dismissed (main request) or, in the alternative, that the decision under appeal be set aside and that the patent be maintained on the basis of one of the auxiliary requests 1 to 3, submitted at the oral proceedings.

XI. The essential arguments of the parties concerning the respondent's main request can be summarised as follows:

Sufficiency of the disclosure

The appellant alleged that the patent lacked vital information about the total porosity of the particles. Since there was no mention in the patent about what proportion of the particle volume was made up by the throughpores having a mean diameter of more than 200nm, the skilled person could not find out without undue burden which particles would be suitable for performing the method of claim 1. Moreover, the patent did not specify any definition of the term "mean diameter" of the particles. Referring to R201 and R202 it argued

that there were several different ways of calculating a mean diameter and several methods for measuring the particle size, which led to different results. Moreover, Figure 4A of the patent did not show particles having a mean diameter of 10µm as indicated in the corresponding section [0069] of the description. Choosing a different mean diameter definition would not always lead to the same results as those obtained in the patent. Hence in the absence of any guidance in the patent, it was not possible for the skilled person to measure the mean diameter of particles that it may wish to use in a method according to claim 1 and to reproduce the latter without undue burden. In this connection, it also referred to decision T 0225/93. The appellant considered that there was no clear definition in the patent of the different throughpore sets and of the mean diameter of the throughpores. Moreover, the patent contained no information on how to measure the mean diameter of the first and second throughpore sets. The appellant did not accept the validity of the "rule of thumb" mentioned in the patent in suit, according to which the mean diameter of the pores defined by the interstices among roughly spherical particles was about 1/3 of the diameter of the particles (designated as "1/3 rule" hereafter). No measuring method was mentioned. According to R1 throughpore sizes could only be inferred, but not measured. It was not apparent from section [0066] of the patent how the respondent arrived at a throughpore mean diameter differing from the mean pore diameter indicated by the supplier of the particles. The throughpore diameters could thus not be clearly and reliably determined by objective procedures which were usual in the art. The four different methods for measuring the pore characteristics of the particles

referred to by the respondent, i.e. SEM (scanning electron microscopy), TEM (transmission electron microscopy), mercury intrusion porosimetry and size exclusion chromatography (as referred to in R7), were not mentioned in the patent in suit. Referring to R301 and R302, the appellant argued that all of these methods would lead to different results based on subjective interpretation of the data measured. A TEM technique for identifying and measuring the diameter of throughpores would be particularly laborious and time consuming and hence represent an undue burden for a skilled person trying to carry out the invention. The appellant also argued that it was impossible for a skilled person to carry out the claimed invention since it would be unable to find a range of liquid velocities above 1000 cm/hr wherein band spreading was "*substantially constant*", i.e. essentially unchanged. None of the results in the patent supported this feature. On the contrary, figures 14 and 15 showed that plate height, and hence also band spreading, increased with increasing linear velocity. The expression "*substantially constant*" was not mentioned in the corresponding parts of the description. The said expression had no clear, generally accepted meaning. Therefore, in the absence of any illustrating example, the skilled person could not determine what subject-matter was covered by claim 1, or whether or not a band spreading "*substantially constant*" over a range of liquid velocities above 1000 cm/hr was achieved.

The respondent argued that the patent contained sufficient information to carry out the subject-matter of claim 1 regardless of total porosity. It was of the opinion that the reference to the mean diameter of the

particles gave no difficulty to a skilled person. Commercially available particles were usually sold under a certain particle size which was an average size. For the skilled person, there was no obstacle to the measurement of the mean diameter of the particles or the reproduction of the method of claim 1. In a bed of packed particles, the interstices between the particles belonged to the first throughpore set and intraparticle throughpores of a size and location permitting convective flow of liquid therethrough when practising chromatography under the conditions mentioned in claim 1 belonged to the second set. Generally, the mean diameter of throughpores was a function of the size of the particles among which interstices constituting throughpores existed. A "1/3 rule" was repeatedly mentioned in the patent and sections [0061] to [0078] taught how to make matrix materials having the necessary throughpores for performing the method of claim 1. The existence and characteristics of throughpores could be determined by the skilled person using available techniques, including mercury intrusion porosimetry, electron microscopy techniques including TEM. Throughpore diameter measurements using TEM, mercury intrusion porosimetry and size exclusion chromatography gave practically the same results. Furthermore, the respondent was of the opinion that the expression "substantially constant" was well understood by the skilled person and was used relative to the performance of prior art particles. The expression was supported by Figures 14, 15A and 15B and discussed in sections [0099] and [100] of the patent. At the oral proceedings, the respondent pointed out curve A of Figure 14 which showed this feature.

Novelty

The appellant considered that the method of claim 1 lacked novelty over R9, which referred to the use of "PL-SAX", "PLRP-S" and "PL-AFC" particles in bio-chromatographic separations. R9 described stability tests performed on particles "d. 4000Å 10µm" at flow rates of up to 2880 cm/hr. The use of an "eluent" composed of ACN and water in these tests implied that a biological sample had been previously loaded onto the matrix.

The respondent pointed out that R9 was silent about the presence of throughpores as defined in claim 1. The described tests were only performed to evaluate the stability of some new particles being presented. The term "eluent" did not imply that a solute was run through the column. The highest flow rate disclosed in R9 in connection with actual separations was 2 ml/min.

Inventive step

At the oral proceedings, the appellant presented an attack based on R101 which disclosed chromatographic separations at speeds much higher than 1500 cm/hr and with very good resolutions. It considered R101 to disclose all the features of present claim 1 except for the solutes being biological molecules. The internal pores of the particles used as packing material were necessarily throughpores. The ranges indicated in R101 with respect to the average particle and pore diameters covered the ranges of present claim 1. Starting from R101 as closest prior art, and considering the various types of molecules separated according to the examples

in R101, the skilled person would consider it as obvious to apply the methods disclosed therein to the separation of biological molecules such as proteins.

The appellant considered R6 to R9 to be just one piece of prior art since they had two authors in common. At the oral proceedings, it did not rely on R5 or present any arguments based on R13, R15 or A1. Instead, it presented objections based on a combination of R9 with R6, and on R6 alone. The appellant argued that the PL-SAX and PLRP-S particles with an indicated pore size of 1000 or 4000Å referred to in the publications from Polymer Laboratories Ltd. ("PL" hereafter) actually had throughpores dimensioned as required by present claim 1, and that this was confirmed by sections [0066] to [0068] of the patent in suit. The stability tests on page 6 of R6 showed that 8µm PL-SAX 1000Å particles could also be used at flow rates exceeding 1500 cm/hr, and that higher flow rates were possible in shorter columns. The chromatograms and their evaluation shown on page 6 were identical to the ones on page 3 of R8, which more clearly indicated which type of particles had been used. The chromatograms showed that even with an 8-fold increase in speed adequate resolution was obtained. Considering that R6 was concerned with the speed of chromatographic separations and also referred to 10µm particles, a further increase in speed was obvious. The particles were sufficiently stable and high speed chromatography was known from e.g. R101. Referring to Figures 20D and 20F of the patent in suit, it argued that according to some examples thereof, there was also a loss of resolution at high flow rates. In writing, having regard to claim 12 according to the main request, the appellant has also invoked a combination of R9 and

R18b, arguing that the use of particles with a mean diameter of more than 10µm, such as the media disclosed in R18b as being suitable for low and medium pressure chromatography, was an obvious measure for reducing back-pressure in the column. At the oral proceedings, the appellant did not, however, rely on R18b in connection with the main request.

The respondent argued that R101 did not mention throughpores, and did not suggest using particles having pores that were large compared to the size of the particles. Moreover, R101 did not suggest the separation of biological molecules using an adsorptive chromatography method. Example 3 of R101 concerned a size exclusion chromatography method requiring no solute interactive regions. Hence, there was no obvious way from R101 to the claimed invention. R6 to R9 were to be considered as separate references when assessing inventive step. The information given in these publications concerning the nature of the particles of PL Ltd. was not sufficient to conclude that they had all the features required by present claim 1. There was no evidence that any of the PL particles used to generate the chromatograms in the prior art had the required throughpores. It argued that at the time of the invention, not all batches of PL particles were identical. R19 showed that PL-SAX particles of different particle and pore sizes exhibited variations in their properties from batch to batch. R9, R6 and R19 were silent about the presence and geometry of intraparticle throughpores. The mechanical stability tests described in R6 and R9 were merely performed to evaluate the properties of some new particles being presented. The term "eluent" did not imply that a

solute was run through the column. Neither R9 nor R6 suggested the use of particles as defined in claim 1 or operating a chromatographic method at liquid velocities above 1500 cm/hr. Such high velocities were not usual although possible in terms of stability. R6 did not indicate the particles actually used in the separation of STI and OVA illustrated on page 6. Moreover, these experiments showed a loss in resolution of more than 30% when the flow rate was increased from its optimum to 1444 cm/hr. This actually taught away from further increasing the liquid velocity. R5 provided too little detail concerning the chromatographic separation actually performed. Claim 12 was patentable for similar reasons as given for claim 1. The respondent was of the opinion that R18b was not prior art and did not disclose the necessary intraparticle throughpores.

Reasons for the Decision

Main Request

1. *Allowability of the amendments*

The appellant has not raised objections under Article 123(2)(3) EPC against the present claims. The board has no reason to depart from the positive finding of the opposition division concerning the allowability of the amendments in the present claims.

2. *Sufficiency of the disclosure*

2.1 To be able to carry out the claimed invention, the skilled person must dispose of the suitable particles

for forming the chromatographic matrix. Particles suitable for being used according to the invention must inter alia have a mean particle diameter greater than 8µm, internal throughpores (second throughpore set) with a mean diameter greater than 200 nm, the ratio of the former to the latter being less than 70, and a mean diameter of the first throughpore set greater than the mean diameter of the second throughpore set.

2.1.1 The patent in suit not only describes commercially available particles that were found to be suitable and mentions PL Ltd. as a supplier who sold such particles before the priority date, see sections [0066] to [0069], [0073] last sentence, and [0094]. As pointed out in the contested decision, it also contains information on how particles having the structure required by present claim 1 may be prepared by means of polymerisation and agglomeration techniques. More particularly, sections [0063], [0064], [0065] and [0073] contain information concerning techniques that can be used to produce suitable particles consisting of agglomeration of smaller polymeric particles and in particular of substantially spherical "porons". Sections [0028] to [0030], [0037], [0046], [0071] and [0074] contain additional indications concerning the desirable dimensioning of the smaller particles and ultimate "porons" making up the particles forming the chromatographic matrix and defining the average size of the pores between the particles. In discussing dimensioning issues, the patent in suit refers repeatedly, explicitly or implicitly, to a rule of thumb according to which the mean diameter of the pores defined by the interstices among roughly spherical particles will approximately be 1/3 of the particle

diameter, see e.g. page 5, lines 49 to 51, page 7, lines 26 to 27, page 8, lines 45 to 46, page 12, lines 46 to 47, page 13, lines 20 to 23, page 14, line 2.

2.1.2 Only at the oral proceedings, the appellant argued that in view of some of the language used in the quoted parts in the patent in suit (e.g. the use of "might" in section [0074]) the indications in the patent in suit concerning the preparation of suitable particles were rather theoretical. Moreover, it alleged that since the patent contained no example of a preparation of particles it was not detailed enough to enable a reproduction thereof. However, the fact that a preparation might be theoretical does not allow the conclusion that when putting into practice the theoretical preparation, it would not be possible to obtain the desired product. Furthermore the presence of an example containing all the details of the preparation is not necessary if the patent gives sufficient instructions or guidance to the skilled person on how to prepare the product. As pointed out above, the patent refers to known techniques which permit the production of substantially spherical "porons" by polymerisation and to known techniques which enable the preparation of variously sized particles. The appellant did not indicate any particular kind of information which, although required for enabling the preparation of the desired product, was missing in the patent in suit. Therefore, in the absence of evidence to the contrary, the board does not accept the appellant's allegation and considers that the passages quoted in point 2.1.1 provide sufficient information to enable the skilled person to prepare suitable particles.

2.1.3 In writing, the appellant referred to Figure 5B of the patent in suit, from which it gathered that the maximum possible width of the interstice between multiple touching particles was less than 1/3 of the particle diameter. The appellant argued that based on "simple geometry", it was thus unclear how the mean diameter of the interstice between multiple touching particles could be as high as 1/3 of the diameter of the particles. At the oral proceedings, it however presented another, three-dimensional geometrical model. It argued that in the case of the closest possible packing of spherical particles, the void volume of the packing was 25.95%. All interstices between the spheres being connected, they formed one pore. The diameter of a cylinder corresponding to this void pore volume could be computed to be 0.58 times the diameter of the particles. In the case of a less perfect packing, the pore diameter would even be higher than that, and hence substantially higher than the one according to the "1/3 rule" mentioned in the patent. However, the appellant has not provided any evidence showing that the "1/3 rule" used in the patent in suit would not be applicable under "real life" conditions in the particular context of the preparation methods described in the patent. In the absence of such evidence, and in view of the contradictory conclusions the appellant drew from its two different approaches for evaluating the average pore size geometrically, the board sees no reason not to accept the validity of the "1/3 rule".

2.1.4 As emphasised by the appellant, the patent in suit contains no explicit indications concerning the methods applicable for measuring or determining the numerical

values of the parameters characterising the particles used in the method of present claim 1.

2.1.5 The present case however differs from the case underlying decision T 0225/93 in that the contested patent provides sufficient guidance for the skilled person on how to obtain particles suitable for being used in the claimed method, see points 2.1 to 2.1.2 above. Hence, the considerations and conclusions in decision T 0225/93 (see Reasons 2.1.3, the first three sentences) are not applicable to the present case.

2.1.6 In particular, and although the burden of proof rests on its side, the appellant has not demonstrated that a skilled person following the guidance in the patent in suit, bearing in mind the "1/3 rule" and the other information given concerning particle and pore dimensioning, and measuring the mean particle and throughpore diameters by means of available methods appropriate in the particular technological context of the patent, would not be able to obtain suitable particles having the characteristics stated in claim 1, and hence would not be able to carry out the claimed method.

2.1.7 Therefore, under the present circumstances, the mere fact that the patent in suit contains no explicit indications concerning methods for measuring or determining the numerical values of the parameters characterising the particles used in the method of present claim 1 is not sufficient to establish insufficiency of the disclosure.

2.2 The appellant's further arguments do not convince the board that the amended patent lacks a sufficient disclosure:

2.2.1 The appellant has not shown that particles obtainable according to the guidance given in the patent would not be suitable for carrying out the claimed method due to an unsuitable total porosity. Hence the objection based on the alleged lack of information concerning the total porosity is disregarded.

2.2.2 Several methods exist (and existed before the priority date) for measuring the diameter of particles, as well as for determining a mean value, see e.g. R201 (post-published), page 63, Figure 3.6 and R202 (post-published), pages 18 to 21, section 1.2.1 and pages 30 to 32. This fact does not per se justify an objection under Article 100(b) EPC. It has not been shown that the mean diameter of the particles obtainable according to the indications in the patent would vary to a substantial degree depending on which measuring method was actually used, from amongst those methods a skilled person would consider appropriate in this particular technical context. Figure 4A of the patent shows scanning electron micrographs of two entire particles, which are stated to be of the PLRP-S 10µm 4000Å type (see caption of the figure and section [0069]. A sample of only two particles is not representative for the number of particles required for a chromatographic method. Measuring the size of only two particles by a method such as SEM is thus not a valid basis for statistically determining a mean diameter value. Hence, deviations of the diameters measurable on the two particles shown in Figure 4A from the indicated value

of 10µm do no amount to a contradiction with the description and do not hinder the determination of mean particle diameters, as alleged by the appellant.

2.2.3 Generally speaking, porous particles are usually characterised by average pore sizes, and methods for ascertaining their porosity were available before the priority date. As already mentioned above, the patent contains information on how to obtain particles that can be expected to lead to the pore geometry specified in present claim 1. A skilled person knowing that the particles must have relatively large throughpores and wanting to ascertain the mean diameters of the throughpores formed by the interstices between the packed particles (first throughpore set in claim 1) and of the intraparticle throughpores transecting each particle (second throughpores set in claim 1) had methods at its disposal, which methods can also be used in combination if expedient. In particular, it is plausible that when using mercury intrusion porosimetry, the portion of the intruded Hg volume versus pressure curve obtained corresponding to the largest pores, i.e. the pores formed by the interstices between the particles, can be distinguished from the curve portion corresponding to the smaller intraparticle throughpores, thereby permitting the determination of the mean diameter of the latter. At the oral proceedings, it was plausibly explained that the presence of intraparticle throughpores and an estimate of their mean diameter could be determined using SEM (pores visible at the surface of the particles), combined with TEM performed on series of thin slices of the particles to confirm the presence of throughpores and to determine their diameter. The sentence on page 13, lines 21-22 of R1

(post-published) does not mean that TEM does not permit measuring a diameter. This is apparent from the subsequent sentences in R1, which refer to the size of the throughpores. Additionally, the value of the mean diameter of the intraparticle throughpores could be evaluated using known measuring methods such as mercury intrusion porosimetry and size exclusion chromatography. The appellant also pointed out the presence of sub-pores in the preferred particles used according to the invention. However, as indicated in sections [0026] and [0074] of the patent in suit, such sub-pores, which comprise blind pores and loop pores, have a mean diameter in the vicinity of a few hundred Å and less than 700Å. Therefore, in measuring porosity, it would be possible to distinguish them from the throughpores belonging to the first and second throughpore sets. As can be inferred from the patent in suit in connection with some commercially available particles from PL (see section [0066], the mean diameter of the intraparticle throughpores will differ from the mean diameter of the totality of internal throughpores and sub-pores present in the particle. In the present case, the mere fact that the said measuring methods are not mentioned in the patent, that they may be considered as cumbersome and time-consuming and that their results are based on subjective interpretation of data does not as such mean that having to perform them represents an undue burden. To show the divergence of the results obtained when measuring pore diameters according to different methods, the appellant referred to two post-published references R301 and R302 for the first time during the oral proceedings. In R-301 pore-size distribution results obtained by six different methods, including mercury intrusion porosimetry and image analysis are compared

for a variety of woven and non-woven geotextiles. It appears from figures 1 to 3 of R-301 that the different measuring methods used lead to pore size distribution results differing to some extent. However, document R-301 belongs to a different technical field, the materials concerned are different from matrices made up by small porous particles (the minimum fibre diameter mentioned is 30µm, see Table 1 on page 303) and the smallest pore diameters measured are greater than 10µm, see e.g. figures 1 to 3. Only slides 7 and 10 of R-302 were referred to by the appellant. Slide 7 shows microscopic images of "porous monodisperse particles made through the swelling method by Ugelstad" and having a particle diameter of 30µm and a particle porosity of 59%. Slide 10 shows a graph with different pore size distribution curves measured according to the following methods, respectively: mercury intrusion, nitrogen ad- and desorption, image analysis and size exclusion. However, none of the two slides contains any apparent link to the other one. Slide 10 neither contains indications concerning the material(s) tested or the age and type of the machinery used, nor any comments or explanations concerning the different aspect of the curves. In view of the totally different product and field of application in the case of R301 and the lack of detail in the case of R302, this evidence does not conclusively establish that in the particular case of particles prepared according to the guidance given in the patent in suit, the variations in the pore diameter values measured would be so extensive that it would not be possible to ascertain whether the throughpores in a packing of the said particles included intraparticle throughpores (second throughpore set), dimensioned as required by present claim 1.

2.2.4 The relative expression "*substantially constant bandspreading*" used in claim 1 imposes no clear limitation on the degree of band spreading constancy that needs to be achieved in "*a range of liquid velocities above 1000 cm/hr*". The quoted features were already present in claim 1 as granted. Since the lack of clarity does not arise from a post-grant amendment and lack of clarity is not a ground of opposition, the claims cannot be refused on this ground. The latter phrase is quite broad in scope since it does not imply a minimum width of the said range of liquid velocities. On the other hand, as was pointed out by the appellant during the oral proceedings, a range of constant bandspreading can always be found by choosing a small enough range. Furthermore, as also mentioned during the oral proceedings, the slope of a plate height (equivalent to band spreading) versus flow rate curve of the type shown in Figures 14 and 15 of the patent depends on the chosen scales. In curve A of Figure 14, a flow rate range can be identified which extends from about 2.65 to about 3 ml/min, amounting to a range of from about 1000 to about 1130 cm/hr in view of the internal diameter of the column (4.5 mm), and wherein the curve is relatively flat, in particular in comparison to the remainder of the curve. As pointed out by the appellant at the oral proceedings, the curves in Figure 14 are smoothened curves, and a line of strictly constant plate height cannot be drawn between two neighbouring points of curve A. However, in view of the general considerations above, the board regards the plate height value H, and hence the band spreading, as remaining essentially unchanged or

constant within the flow rate range identified above, i.e. between 1000 and 1130 cm/hr.

2.2.5 Figures 15A and 15B and the corresponding text in section [0100] are silent about the internal diameter of the column and the size and/or pore size of the particles tested, and contain different indications concerning the linear flow velocity unit. Therefore it appears difficult to draw conclusions therefrom concerning the band spreading at liquid velocities above 1000 cm/hr.

2.2.6 The features in question are thus at least supported by Figure 14, curve A, in contrast to the appellant's allegations. The board sees no reason for which a skilled person trying to carry out the invention as claimed would not be able to know whether the features in question are achieved or not.

3. *Novelty*

3.1 R9 is a publication of a presentation given in March 1988 in the name of PL Ltd. R9 relates to the use of several rigid and macroporous polymeric packing particles in bio-HPLC. The particles specifically described differ in terms of their size (8µm and 10µm mentioned), chemical composition ("PLRP-S", "PL-SAX", "PL-AFC") and porosity (pore sizes from 100 to 4000 Å are mentioned), see the title, abstract, introduction, sheet 4 and summary.

3.1.1 On sheet 4 of R9, a graph entitled "MECHANICAL STABILITY" shows the interdependence of pressure and flow rate for several polymeric particles differing in

terms of particle size and pore size. The measurements represented in the graphs have been carried out in a 150 x 4.6 mm column. The curve for the particles designated "d. 4000Å 10µm" has been established at flow rates of up to more than 8.0 ml/min, this value undisputedly amounting to more than about 2880 cm/hr in view of the dimensions of the column. A "recommended maximum operating pressure" of 3000 psi is also indicated in the graph. For the particles "d", the flow rate corresponding to this pressure level is greater than 4.0 ml/min (about 1444 cm/hr).

3.1.2 The said graph is the result of experiments performed for the purpose of investigating the mechanical stability, i.e. the compressibility, of the porous polymer particles. Such measurements can be carried out independently of an actual chromatographic separation. Therefore, the mere fact that the specific liquid used in carrying out the measurements is referred to as "eluent" does not necessarily imply that chromatographic separations with previous loading of solutes have been performed. According to the respondent's statements at the oral proceedings, the specific liquid mentioned was a "normal eluent" and "a good mobile phase" to use in these tests, and was fed to the column via the eluent port. Hence, in the absence of any supporting evidence, the board does not accept the appellant's allegations that the terminology would have been "buffer" or that water would have been used instead of a mixture of ACN and water if there was nothing to elute. The graph and the accompanying text do thus not constitute a clear and unambiguous disclosure of a chromatography method according to claim 1 of the patent in suit.

3.1.3 On the other hand, the highest flow rate mentioned in other parts of R9 in connection with a chromatographic separation is 2 ml/min in 150 x 4.6 and 50 x 4.6 mm columns, corresponding to about 720 cm/hr, see page 8, the caption of the lower graph and page 10, the caption of the upper graph.

3.2 None of the other documents relied upon by the appellant discloses a chromatography method with all the features of present claim 1. Since this was not disputed by the appellant, a detailed reasoning needs not to be given. The method of claim 1 is thus novel.

4. *Inventive step*

4.1 Document R101 discloses chromatographic separations using a packing material comprising a powder of uniformly sized, porous microspheres having an average diameter of 0.5 to 20µm, preferably 1 to 10µm. The microspheres themselves consist essentially of a plurality of sintered uniform-sized colloidal particles, having a refractory metal oxide surface, arranged in an interconnected three-dimensional lattice defining internal pores. Interconnected pores having controlled dimensions and a uniform size distribution occupy more than 50 percent of the volume of the microspheres, which thus have a quite open structure, see in particular, column 2, "Summary"; column 3, line 66 to column 4, line 28; claims 9, 10, 14 and 16. In view of the described method of preparation, the board can accept at least for the sake of argument the appellant's view that the pores within the particles will essentially be throughpores. In column 6, lines 30

to 32, a pore size range of 50 to 2500 Å is mentioned. In view of this indication and of the text preceding the sentence in column 3, lines 40 to 60, the board can accept the appellant's argument that the size ranges mentioned in column 3, lines 61 to 64, i.e. 50 to 2500Å, preferably 75 to 1000Å, also relate to the **pore** diameters of the particles. Depending on the size and porosity of the packing particles, they may be used in gas or liquid chromatography, the latter including adsorptive liquid-solid and size exclusion chromatography (see column 5, lines 42 to 65 and examples 1 to 3).

4.1.1 However, R101 does not disclose particles having a mean diameter of more than 8µm and comprising, at the same time, internal throughpores having a mean diameter of more than 200nm (2000Å), let alone particles with a mean particle diameter to mean throughpore diameter ratio of less than 70 as required by present claim 1. In fact, a preferred range for the pore size according to R101 is 75 to 1000Å (see column 3, line 64), which is less than half of the lower limit required for the mean diameter of the throughpores according to present claim 1. In accordance with this teaching of R101, the largest mean pore size exemplified therein is 35nm (350Å), see examples 1 and 3), i.e. much less than the lower limit of 200nm required for the mean throughpore diameter according to present claim 1. Only example 2 of R101 relates to particles with a mean particle size greater then 8µm as required by present claims, but the pore size is about 75Å. The ratio of these two values is greater than 1066, i.e. much higher than the upper limit of 70 indicated in present claim 1. The molecules separated according to example 2 at very high carrier

liquid speeds of up to 34200 cm/hr (9,5 cm/sec) are relatively small, low molecular weight compounds, namely 3-phenylethanol and benzyhydrol. On the other hand, the separation of fractions of larger polystyrene molecules (molecular weights of 2030, 51000 and 411000) is only illustrated in example 3 which concerns size exclusion and not adsorption or affinity chromatography.

4.1.2 Starting from R101 as the closest prior art as suggested by the appellant, the technical problem can thus be seen in providing a further liquid chromatography method using a packed matrix of porous particles, which is suitable for separating biological molecules and wherein high peak resolution is achieved at high flow rates, see sections [0021] and [0023] of the patent in suit. In view of the information in the patent in suit, e.g. sections [0098], [0101], [0102] and in figures 13C, 16B, 16C, 17B, it is credible in the absence of evidence to the contrary that the technical problem has indeed been solved by the claimed process.

4.1.3 R101 does not address the issue of the ratio of particle size to throughpore size. Examples 2 and 3 of R101 differ substantially from each other in terms of the size of the compounds to be separated and of the corresponding packing materials and chromatography method applied. Hence, a skilled person could not, without applying ex-post facto considerations, gather from R101 that the separation of solutes comprising biological molecules would be possible with the desired speed and resolution by departing from the teaching of the examples of R101 and selecting particles as

specified in claim 1 within the broader ranges given in R101.

4.2 At the oral proceedings, the appellant presented approaches based on R9 and R6, respectively, as closest prior art. These references all relate to liquid bio-chromatography using macroporous particles having a diameter of 8µm or more. In view of these similarities, the board considers each of these prior art publications to be a more appropriate starting point for assessing inventive step than R101.

4.3 The board does not share the appellant's view that references R6 to R9 can be considered as one piece of prior art, similar to chapters in a book, merely because they have two authors in common. The references clearly relate to separate presentations given at different conferences or symposia and at different dates. What total information these two authors possibly "had in their minds" at a given point in time cannot be inferred from these documents. Therefore, these presentations have to be considered as separate pieces of prior art.

4.4 The particles mentioned in R6 to R9

4.4.1 References R6, R7, R8 and R9 all stem from PL Ltd. and all refer to porous polymeric packing particles, inter alia to particles bearing the trade names "PL-SAX" and "PLRP-S" and having pore sizes of 1000 or 4000Å and particle sizes of 8µm or 10µm.

4.4.2 Referring to sections [0066] to [0068] of the patent, where it is stated that such particles had throughpores

with diameters exceeding 2000 or 6000Å, respectively, the appellant considered that these features were also disclosed in the above references as far as they related to "PL-SAX" and "PLRP-S" particles with a pore size of 1000 or 4000Å.

4.4.3 Reference R19 however shows that as late as in October 1988, i.e. several months before the priority date of the patent in suit, the properties of PL-SAX particles, and more particularly their protein capacity, still varied between batches due to the fact that the production methods were still under development (see page 1 and compare the values given for "8-SAX…" and "10-SAX…" particles respectively). This was not contested by the appellant.

4.4.4 R6, R7, R8 and R9 concern presentations made in September 1987, November 1987, in February 1988 and in March 1988, respectively, i.e. at least six months up to more than one year before the date appearing on R19.

4.4.5 Considering

- that none of these references mentions intraparticle throughpores, let alone a mean diameter thereof or convective flow therethrough during chromatography,

- that the appellant has provided no evidence showing more precisely the internal pore structure, and in particular the mean diameter of any throughpores possibly present, of particles available as PL-SAX or PLRP-S with a pore size of 1000Å or 4000Å **at the time of the presentations** referred to in R6 to R9,

- that, however, at least the production of PL-SAX 1000 and 4000Å particles was still under development and undergoing changes several months **after** the contents of references R6 to R9 were presented to the public (see R19),

the board is not convinced that any of R6 to R9 implicitly disclose in a clear and unambiguous manner the use of particles having internal throughpores with a mean diameter as specified in present claim 1, merely because they refer to particles bearing the trade names "PL-SAX" and "PLRP-S".

The fact that the present inventors realised that products commercialised under the said trade names had, at some later point in time but before the priority date of the patent in suit, the pore structure required by present claim 1 cannot modify the nature of the subject-matter disclosed in R6 to R9.

4.5 R9 as closest prior art

4.5.1 R9 emphasises that the PL-SAX, PLRP, and PL-APC packings described therein are mechanically stable up to pressures of 5000psi and resistant to extreme changes of flow rate and pressure, and that they can provide "high-efficiency separations of biomolecules", i.e. "no-compromise" separations with stability and speed (see abstract, introduction and summary on pages 2, 3 and 11). However, none of the separations exemplified is carried out at more than about 720 cm/hr. Even the only separation involving 10µm 1000Å particles in a relatively short 50 x 4.6mm column is carried out at about 720 cm/hr (upper half of page 10), i.e. at

about half the velocity required by present claim 1. A 10µm 4000Å particle not further specified is only referred to in form of an electron micrograph on page 3, no application test data are provided.

4.5.2 As it appears from the above points 3.1.2, 3.1.3 , 4.4.5 and 4.5.1, R9 does not disclose a chromatographic separation method involving a "velocity greater than 1500 cm/hr" and particles having a mean diameter of greater than 8µm and comprising throughpores meeting the dimensional criteria specified in present claim 1.

4.5.3 Starting from R9, the technical problem to be solved can thus be seen in providing a liquid chromatography method for separating biological molecules, which permits operating at higher fluid flow rates while still achieving high peak resolution, see sections [0021] and [0023] of the patent in suit. In view of the results reported in Figures 13C, 16B, 16C and 17B and the corresponding text in sections [0098], [0101] and [0102], it is credible that the problem can be solved by the use of particles as specified in claim 1 in conjunction with a velocity of greater than 1500 cm/hr.

4.5.4 In the mechanical stability tests described on page 4 of R9 different particles are compared. The results inter alia show that operation of a packed 150 x 4.6mm column at higher pressures and flow rates does not lead to the compression of some 4000Å 10µm particles not further specified, and that 3000psi are recommended as the maximum operating pressure. The mere fact that the liquid used in the stability tests was a solvent mixture and was referred to as eluent does not, as such,

suggest carrying out a particular chromatographic separation at a particular pressure or flow rate.

4.5.5 The examples of R9 illustrate what the authors understood by "high efficiency separations" and "with speed" in connection with some specific separations. It can be assumed that the chromatograms presented were recorded under conditions leading to optimum results. Reference R9 relates essentially to applications of 8µm particles (see summary on page 11). As far as actual bio-chromatographic separations are concerned, the highest flow rate disclosed for both 8 and 10µm particles is 2 ml/min in a 50 x 4.6mm column, corresponding to about 720 cm/hr (see lower graph on page 8 and upper graph on page 10).

4.5.6 When performing liquid chromatography with conventional porous particulate media based on diffusive processes, the skilled person would expect a loss of resolution with increasing liquid velocity. This was not disputed. R9 is silent about the presence of intraparticle throughpores as defined in claim 1, let alone any convective flow therethrough. Hence, the skilled person not knowing the present patent would not be prompted by R9 and the stability data contained therein to further increase the liquid velocity in any of the specific separations referred to in the examples. Moreover, R9 contains nothing that could suggest the use of particles having internal throughpores meeting the three dimensional criteria indicated in present claim 1.

4.6 R6 as closest prior art

4.6.1 R6 relates to macroporous polymeric packing particles of 8µm diameter for bio-HPLC, referred to as "PLPR-S", "PL-SAX" and "PL-AFC Prot A". The introduction and summary (pages 2 and 12) are identical to those in R9 (pages 3 and 11) except for the pore sizes mentioned in the respective summaries (300 to 1000Å in R6 instead of 100 to 4000Å in R9). A particle diameter of 10µm is only mentioned on page 2 of R6 in the form of an electron micrograph with the caption "4000Å 10µm particle".

4.6.2 In the upper half of page 6, under the heading "Mechanical Stability", test results are reported in the form of a graph of pressure versus flow rate, the eluent used being water. The particles tested were of the PL-SAX 1000Å 8µm type in a 150 x 4.6mm column. The graph and the corresponding text disclose that the particles are stable up to pressures of 4000psi, that the maximum recommended operating pressure is 3000psi, and that "this enables flow rates of 4 ml/min to be used with a 150 x 4.6mm column and 10 ml/min with a 50 x 4.6mm column packed with 8µm analytical material", i.e. about 1444 and 3610 cm/hr, respectively. For reasons analogous to those given in point 3.1.2, the test results concerning the stability of the particles do not disclose any actual chromatographic separation.

4.6.3 Page 6 of R6 also shows three chromatograms which were obtained by separating OVA (ovalbumin) and STI (soya bean trypsin inhibitor) at varying flow rates. The type of particles actually used in these runs is not indicated in the corresponding caption and text. Purely for the sake of argument and in favour of the appellant,

the board bases its following assessment on the assumption that in view of the similarity of the information and data presented on page 6 of R6 and on page 3 of R8 both the stability tests and the three chromatograms shown R6 were indeed carried out with PL-SAX 1000Å 8µm particles as additionally indicated in R8. The three chromatograms obtained using a 50 x 4.6mm column and flow rates of 0.5, 1.5 and 4 ml/min, the latter value amounting to 1444 cm/hr. Six numerical resolution values ("Rs") obtained for six flow rates from 0.5 to 4.0 ml/min are presented in a table. The authors of R6 conclude that "the optimum flow rate was determined at 1.0 - 1.5 ml/min but with adequate resolution at 4.0 ml/min". No higher bed velocity is mentioned in the remainder of R6.

4.6.4 As it appears from the above points 4.6.1 to 4.6.3, R6 does not disclose a chromatography method involving a "velocity greater than 1500 cm/hr" with particles having a mean diameter of greater than 8µm, let alone wherein these particles have internal throughpores meeting the dimensional criteria specified in present claim 1 (see point 4.4.5).

4.6.5 The technical problem can thus be considered to be the same as in the case of R9 as closest prior art, see point 4.5.3.

4.6.6 As in the case of R9, the mechanical stability data reported in R6 merely characterise the tested particles and define potential operating ranges. They do not, as such, suggest running a particular separation at a pressure or flow rate close to the upper limits thereof. R6 is concerned with media permitting efficient

chromatographic separations, see Introduction, the first two paragraphs, "no-compromise separations with stability and speed"; Summary, "high efficiency separations" and "extreme changes of flow rate / pressure". The examples of R6, carried out at flow rates ranging from 0.5 to 4.0 ml/min in 4.6mm columns, i.e. at about 180 to 1444 cm/hr, illustrate more precisely what the authors understood by these expressions in connection with some specific separations. R6 relates essentially to applications of 8µm particles (see Summary on page 11). On page 6 of R6, the resolution corresponding to the flow rate of 1444 cm/hr is significantly lower (more than 30%) than at the optimum flow rate of 1.5 ml/min (about 540 cm/hr), but is still rated "adequate".

4.6.7 Like R9, R6 is silent about the presence of intraparticle throughpores as defined in claim 1 let alone convective flow therethrough. As already indicated above, when using conventional porous media, the skilled person would expect a loss of resolution with increasing liquid velocity. The board can accept that under certain circumstances the speed of the separation might be more important than a maximal resolution, as it appears to be the case e.g. in Figures 20D and 20F pointed out by the appellant during the oral proceedings. It can nevertheless be assumed that the chromatograms shown in R6 were recorded under conditions leading to optimum results in terms of speed and resolution. In this connection, the board notes that the chromatograms on page 6 were recorded using a relatively short column (length 50mm) at a maximum flow rate of 1444 cm/hr, although according to the mechanical stability tests reported on the same page

such a column would tolerate much higher flow rates of up to 3610 cm/hr (10ml/hr). It can thus be concluded that the authors of R6, although aware of the mechanical stability of PL-SAX 1000Å 8µm at higher flow rates, did not envisage achieving an adequate balance of speed and resolution at flow rates of more than 1444 cm/hr in a particular chromatographic separation, let alone at flow rates of up to 10 ml/hr corresponding to the "maximum recommended pressure" for a column of 50mm length.

4.6.8 Hence, even assuming that the three chromatograms and the data in the table on page 6 of R6 were actually recorded using PL SAX 8µm 1000Å particles as in R8, the skilled person not knowing the present patent would not be prompted by R6 to further increase the liquid velocity beyond 1444 cm/hr, i.e. to more than 1500 cm/hr, in a separation as described therein. Moreover, like R9, R6 contains nothing that could suggest the use of particles with a mean diameter greater than 8µm and having internal throughpores meeting the three dimensional criteria indicated in present claim 1.

4.6.9 As pointed out by the appellant, high-speed liquid-solid chromatographic separations are known from e.g. R101. However, considering the different media and separations addressed in R101, the skilled person starting from R6 would not consider this document, or at least would not be induced by it to increase the flow rate in the separations described in R6, without at the same time replacing the particles.

4.7 Combination of R9 and R6

Since, as mentioned above, none of R9 and R6 suggests a liquid chromatography separation at liquid velocities of more than 1500 cm/hr using a packed bed of particles with internal throughpores meeting the dimensional criteria indicated in present claim 1, the combination of these two references cannot lead to the subject-matter of claim 1 in an obvious manner.

4.8 Combination of R9 with R18b

4.8.1 The brochure R18b contains technical and commercial information but bears no publication date. According to R102 a copy of R18b had been handed out together with the price list R18a to an employee of the respondent by an employee of PL Ltd. in June 1989, i.e. before the priority date of the patent in suit (see 2nd paragraph on page 13). Since the respondent did not contest this fact and did not invoke any kind of confidentiality agreement in connection with the handing out of the said copy, the board accepts that the content of R18b belongs to the prior art pursuant to Article 54(2) EPC.

4.8.2 In R18b, PL describes a range of rigid polymeric chromatography media suitable for "fast biomolecular separations" and "ultra-fast sub-minute analyses" with high resolution and physical and chemical stability. R18b inter alia mentions "High resolution 8µm and 10µm particles for analytical and preparative separations" and "15-25µm particle size" media suitable for "low and medium pressure liquid chromatography". The controlled pore size of the particles may range from 100 to 4000Å. According to R18b, "increasing the pore size from 100Å to 4000Å improves the permeability for larger

macromolecules. This improves peak shape and efficiency by improving the rate of transfer between the eluent and the particle pore volume". Moreover, it is indicated that all media are "stable at high flow rates and pressures up to 3000psi", see page 1, left-hand column, page 9, first sentence and table "Ordering information". R18b also contains product specifications for analytical columns and media, as well as examples of separations using the media, see pages 2 to 8. The highest bed velocities are disclosed on page 8 of R18b in connection with examples of "high speed/high resolution separations" using packings of either "PLRP-S 4000Å 8µm" or "PL-SAX 4000Å 8µm" media. The chromatograms were recorded using a 50 x 4.6mm column at a flow rate of 4.0 ml/min, corresponding to a value of about 1444 cm/hr. On the same page, a "Maximum Pressure" of 3000 psi and the following "Maximum Permissible" flow rates are indicated: 4 ml/min for a 4.6 I.D. column, i.e. about 1444 cm/hr, and 10 ml/min for a 7.5 mm I.D. column, i.e. about 1358 cm/hr. The other chromatograms shown in R18b were recorded at substantially lower flow rates.

4.8.3 Although on page 8 "unique mass transfer characteristics" are mentioned, R18b is silent about intraparticle throughpores meeting the three size criteria of present claim 1 or convective flow therethrough. In the absence of any further evidence, the mere reference to the trade names "PLRP-S 4000Å 15-25µm" and "PL-SAX 4000Å 15-25µm" (see table "Ordering information" on page 9) is not sufficient to represent a disclosure of the throughpore related features. Moreover, despite all the references to "high speed", "high flow rates" and stability up to 3000 psi, and

although the skilled person could infer from the mechanical stability graph in R9 that the pressure required for achieving a given flow rate decreases with increased particle size, the highest bed velocity actually mentioned in R18b in connection with a particular chromatographic separation is less than the one required by present claim 1. Even assuming for the sake of argument in the appellant's favour that the indications on page 8 of R18b concerning the maximum permissible flow rates related only to the 8µm particles, there is no suggestion in R18b to use higher flow rates in a particular chromatographic separation of specific biological molecules involving the "15-25µm" particles, let alone with particles having internal throughpores meeting the dimensional criteria indicated in claims 1 and 12.

4.8.4 Since neither R9 nor R18b suggests applying a liquid velocity of more than 1500 cm/hr, their combination does not lead to the claimed subject-matter in an obvious manner.

4.9 The other prior art documents cited by the appellant, but not relied upon at the oral proceedings, i.e. R5 and R7, do not contain additional information which, in combination with the preceding documents, would point towards the process of claim 1. In particular, although the lecture notes R5 refer to "pores that extend through the entire particle", they contain no data which would permit to infer what "fast runs" means, i.e. what flow velocities (in cm/hr) were used. They do not describe chromatographic separations in a manner sufficiently clear and detailed to justify an inventive

step objection when read without the knowledge of the invention.

4.10 References R13 and A1 stem from US court cases. Like post-published R15, they were only superficially referred to by the appellant in writing, but not discussed at the oral proceedings. Since they have no apparent immediate bearing on the present decision, they need not be dealt with. Even if it was to be considered as evidence, R15 (page 114) does not unambiguously establish that the disclosure made during the lecture referred to in R5 went beyond what is indicated in the latter.

4.11 The method of claim 1 is thus based on an inventive step.

5. Independent claim 12 relates to the use of particles in the novel and inventive chromatography method of claim 1. Independent claim 23 relates to the use of a matrix for conducting adsorptive liquid chromatography comprising use of particles according to claim 12, and independent claim 24 relates to the use of a chromatography system including use of a matrix according to claim 23. Consequently, the subject-matter of these claims, as well as of dependent claims 2 to 11, 13 to 22, 25 and 26, is also novel and inventive.

Auxiliary requests

6. Since the respondent's main request is allowed, there is no need to deal with its three auxiliary requests.

Order

For these reasons it is decided that:

The appeal is dismissed.

The Registrar The Chairman

C. Vodz M. Eberhard