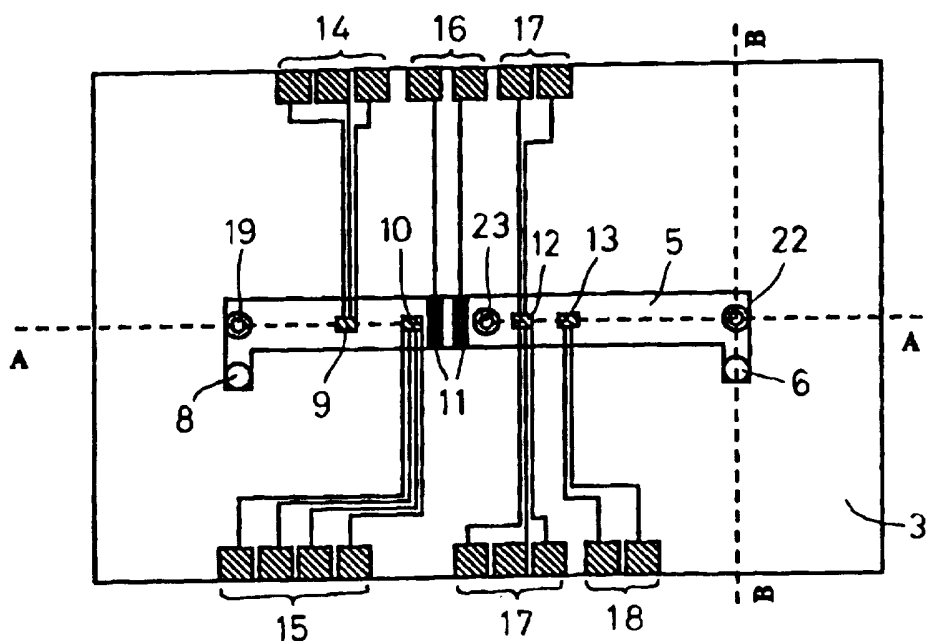




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : G01N 30/78, 21/05	A1	(11) International Publication Number: WO 96/34282 (43) International Publication Date: 31 October 1996 (31.10.96)
(21) International Application Number: PCT/SE96/00548 (22) International Filing Date: 25 April 1996 (25.04.96) (30) Priority Data: 9501592-1 27 April 1995 (27.04.95) SE (71) Applicant (for all designated States except US): PHARMACIA BIOTECH AB [SE/SE]; S-751 82 Uppsala (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): ÖHMAN, Ove [SE/SE]; Asplunda, Uppsala-Näs, S-755 91 Uppsala (SE). EK-STRÖM, Björn [SE/SE]; Järpvägen 27, S-756 53 Uppsala (SE). NORLIN, Peter [SE/SE]; Svartbäcksgatan 101C, S-753 35 Uppsala (SE). (74) Agents: WIDÉN, Björn et al.; Pharmacia AB, Patent Dept., S-751 82 Uppsala (SE).		(81) Designated States: JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published With international search report.

(54) Title: APPARATUS FOR CONTINUOUSLY MEASURING PHYSICAL AND CHEMICAL PARAMETERS IN A FLUID FLOW



(57) Abstract

An apparatus for continuously measuring physical and chemical parameters in a fluid flow comprises a single flow cell having a fluid interface (6, 8) for conducting the fluid through the flow cell, an electrical interface (14 - 18) connected to at least one first means (9 - 13) provided in the flow cell wall for measuring at least one first parameter of the fluid in the flow cell, and an optical interface (3, 19, 22, 23) for transmitting light into the flow cell and for receiving light from the flow cell to measure at least one second parameter of the fluid in the flow cell.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

APPARATUS FOR CONTINUOUSLY MEASURING PHYSICAL AND CHEMICAL PARAMETERS IN A FLUID FLOW

TECHNICAL FIELD

5 The invention relates to an apparatus for continuously measuring physical and chemical parameters in a fluid flow.

BACKGROUND OF THE INVENTION

10 Liquid chromatography is a widely used technique for separation and analysis of chemical compounds. The basic principle of the chromatography technique is to let a test sample travel through a column containing a supporting medium, and there interact with substances of two different phases, namely a mobile phase and a stationary phase.
15 Different sample components will interact to different degrees with the two phases, and those interacting more strongly with the stationary phase will eventually be lagging those preferring the mobile phase. The result is a separation of the test sample components during the passage
20 of the column.

 To detect the sample components as they leave the separation column, several detector techniques are employed.

 So far, separate detectors have been used to detect
25 different parameters of the sample components as they leave the separation column. If more when one parameter are to be measured, different detectors would have to be interconnected by means of e.g. a flexible tubing in order to carry out the measurement.

30

BRIEF DESCRIPTION OF THE INVENTION

 The object of the invention is to bring about an apparatus which makes it possible to measure more than one parameter in one and the same volume of a fluid flow.

35 This is attained by the apparatus according to the invention in that it comprises a single flow cell having a fluid interface for conducting the fluid through the flow cell, an electrical interface connected to at least one

first means provided in the flow cell wall for measuring at least one first parameter of the fluid in the flow cell, and an optical interface for transmitting light into the flow cell and for receiving light from the flow cell to
5 measure at least one second parameter of the fluid in the flow cell.

BRIEF DESCRIPTION OF THE DRAWING

The invention will be described more in detail below
10 with reference to the appended drawing, on which

Fig. 1 is a plan view of an embodiment of a sensor chip for a flow cell according to the invention,

Fig. 2 is a schematic longitudinal sectional view of an embodiment of a flow cell according to the invention along
15 line A-A as indicated on the sensor chip in Fig. 1, and

Fig. 3 is a schematic cross-sectional view along of the flow cell in Fig. 2 along line B-B as indicated on the sensor chip in Fig. 1.

PREFERRED EMBODIMENTS OF THE INVENTION

20 Fig. 2 is a schematic longitudinal sectional view of an embodiment of a flow cell 1 according to the invention along line A-A as indicated in Fig. 1.

In the flow cell 1, a flow channel 2 is defined between
25 a sensor chip 3, a plan view of which is shown in Fig. 1, and a silicon wafer 4, which has been anisotropically etched with a KOH solution to produce the flow channel 2. As is well known per se, this etching process will produce a flow channel with sloped or inclined side-walls as
30 apparent from Figs. 2 and 3 if mask/substrate orientation and etchant are choosen correctly.

It is to be understood that the wafer 4 does not necessarily have to be a silicon wafer but that other materials are possible.

35 In the embodiment shown, the sensor chip 3 is transparent and made of quartz. However, the sensor chip 3 does not have to be made of quartz but other materials are also possible.

On the sensor chip 3 in Fig. 1, the bottom area of the flow channel 2 is denoted 5. That bottom area 5 of the flow channel 2 can also be termed the measurement area of the flow cell 1.

5 Fig. 3 is a schematic cross-sectional view of the flow cell 1 according to the invention along line B-B as indicated on the sensor chip 3 in Fig. 1.

With reference to Fig. 3, fluid is introduced into the flow channel 2 through an inlet opening 6 in the sensor
10 chip 3 by means of an inlet capillary tube 7 or other means. An outlet opening 8 for the fluid is indicated on the sensor chip 3 in Fig. 1, which outlet opening 8 is connected to an outlet capillary tube (not shown).

The inlet tube 7 and the outlet tube (not shown) for
15 the fluid may be located in a fixture (not shown) on which the flow cell 1 is docked upon measurement, in which case the inlet opening 6 and the outlet opening 8 in the sensor chip 3 constitute the fluid interface of the flow cell 1.

The measurement area 5 of the sensor chip 3 is provided
20 with sensor elements 9 - 13 for measuring different parameters of the fluid in the flow channel 2.

The sensor elements 9 - 13 which may be produced by thin film technique, are all electrically connected to contact pads 14, 15, 16, 17 and 18, respectively, on the
25 sensor chip 3, which contact pads 14 - 18 in their turn are connected, in a manner not shown, by gold wires to connecting pins forming the electrical interface to the flow cell 1.

In the embodiment of the sensor chip 3 shown in Fig. 1,
30 the sensor element 9 measures pH, and may comprise a known pH sensor in the form of an ion sensitive field effect transistor (ISFET) or a light-addressable potentiometric sensor (LAPS).

The sensor element 10 is adapted to measure the flow
35 rate of the fluid in the flow channel 2, and may comprise in a manner not shown a known thermocouple and a known heating resistor.

The sensor elements 11 are adapted to measure the

conductivity of the fluid. According to an alternative embodiment, one of the sensor elements 11 may be located in the silicon wafer 4 above the sensor element 11 located on the sensor chip 3.

5 The sensor element 12 is in the embodiment shown in Fig. 1, adapted to measure the pressure of the fluid, while sensor element 13 is adapted, in a manner known per se, to measure the temperature of the fluid.

10 According to the invention, the flow cell 1 is adapted to measure light absorption of the fluid in the flow cell as well as fluorescence and turbidity of the fluid in the flow cell.

15 To accomplish this in the embodiment shown on the drawing, an optical fibre 19 (Fig. 2) is provided to transmit light into the flow channel 2 through the transparent sensor chip 3. It should be pointed out that "fibre 19" actually can comprise more than one fibre. Also, it is to be understood that the fibre 19 may be replaced by a light source, e.g. a laser diode.

20 To measure light absorption of the fluid in the flow cell, UV, IR or visible light is transmitted into the flow cell through the optical fibre 19. The light transmitted into the flow cell is reflected by the inclined end wall 20 of the flow channel 2 towards the other end wall 21 of the flow channel 2, which end wall 21 is also inclined to
25 reflect the light towards an optical fibre 22 which in a manner not shown is connected to a device (not shown) for measuring light absorption of the fluid. Also "fibre 22" may comprise more than one fibre. The fibre 22 can be
30 replaced by a light receiver, e.g. a photodiode. It is actually to be understood that combinations of optical fibres, laser diodes, and photodetectors may be used in any desired combination and manner.

35 To measure the fluorescence of the fluid, excitation light from e.g. a laser (not shown) may be conducted into the flow channel 2 through the optical fibre 19, and fluorescence may be picked up by an optical fibre 23 and conducted to a fluorescence detector (not shown).

As an alternative to a single fibre 23 located between the fibres 19 and 22, a bundle of fibres can be provided. One fibre may then be adapted to transmit excitation light into the fluid while the other fibres are adapted to
5 receive the fluorescence from the fluid. Also in this case, the optical fibres may be replaced by a laser diode and a photodetector.

To measure the turbidity of the fluid in the flow cell, light, UV, IR or visible, is transmitted into the flow
10 channel 2 by means of the optical fibre 19 and received by the optical fibre 23 which is then connected to a device for measuring the turbidity.

In the embodiment shown, the transparent sensor chip 3 constitutes the optical interface to the flow cell 1. The
15 optical fibres 19, 22 and 23 may also be fixed in the above-mentioned fixture (not shown) on which the flow cell 1 is docked upon measurement together with the inlet and outlet capillary tubes for the fluid.

In that case, an integrated fluid and optical interface
20 would be obtained. To secure the optical fibres and the capillary tubes in the fixture, the latter could be provided with V-grooves, and the optical fibres and the capillary tubes could be embedded by a polymer material in the V-grooves.

25 In case the optical fibres are replaced by combinations of laser diodes and photodetectors, the latter components may also be fixed in said fixture.

It is also possible to make the sensor chip 3 of a non-transparent material. In that case, the sensor chip 3 has
30 to be provided with openings or transparent windows (not shown) for the optical fibres 19, 22 and 23. Those openings or transparent windows would then constitute the optical interface of the flow cell.

According to one embodiment of the invention and as
35 apparent from Figs. 2 and 3, both the cross-section of the flow channel 2 and the longitudinal section of the flow channel 2 has the form of a truncated, equally sided triangle.

When the flow channel, according to the described embodiment of the invention, is anisotropically etched in silicon, as apparent from the embodiment shown in Fig. 2, the optical fibres 19 and 22 have to be slightly inclined
5 in order for the light transmitted into the flow cell through the fibre 19 to be reflected straight through the flow cell by the end wall 20 thereof and for the light reflected by the end wall 21 to be received by the optical fibre 22.

10 To increase the light transmission of the flow cell according to the invention, the surfaces of the flow channel may be coated with a layer of a reflective material, e.g. a thin aluminium film which may be deposited by means of sputtering or PVD.

15 If the chemical resistance of the reflective layer is poor, as is the case with an aluminium layer, this can be remedied by depositing a further layer, e.g. of SiO_2 , on the aluminium layer.

It should also be pointed out that instead of producing
20 a flow channel by etching, a flow channel can be defined between two flat surfaces which are kept separated by means of intermediate spacers.

As apparent from the above description, the flow cell according to the invention constitutes a multisensor for
25 measuring any parameter associated with liquid chromatography measurements in one and the same very small volume of the fluid.

However, it is to be understood that the use of the flow cell according to the invention is not limited to the
30 field of liquid chromatography, but it can equally well be used in connection with capillary electrophoresis, flow injection analysis (FIA) for general biotechnical processes or applications of similar nature, chemical/biochemical reactors etc.

CLAIMS

1. An apparatus for continuously measuring physical and chemical parameters in a fluid flow, **characterized in** that
5 it comprises a single flow cell (1) having a fluid interface (6, 8) for conducting the fluid through the flow cell (1), an electrical interface (14 - 18) connected to at least one first means (9 - 13) provided in the flow cell wall for measuring at least one first parameter of the
10 fluid in the flow cell (1), and an optical interface (3, 19, 22, 23) for transmitting light into the flow cell (1) and for receiving light from the flow cell (1) to measure at least one second parameter of the fluid in the flow cell (1).
- 15 2. The apparatus according to claim 1, **characterized in** that said electrical interface (14) is connected to a pH measuring means (9) in the flow cell wall, and that said optical interface (3, 19, 22) is adapted to transmit UV, IR
20 or visible light into the flow cell (1) and to receive that light for measuring light absorption of the fluid in the flow cell.
3. The apparatus according to claim 1 or 2, **characterized in** that said electrical interface (16, 18) is connected to
25 a means (11, 13) in the flow cell wall for measuring conductivity and temperature of the fluid in the flow cell.
4. The apparatus according to claims 1, 2 or 3,
30 **characterized in** that said optical interface (3) is provided with means (23) for measuring fluorescence of the fluid in the flow cell.
5. The apparatus according to claim 4, **characterized in**
35 that said optical interface (3, 19) is adapted to transmit fluorescence excitation light into the fluid in the flow cell.

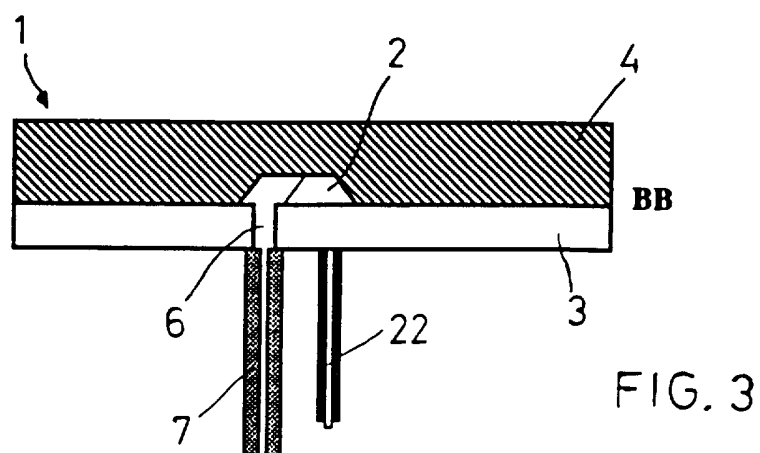
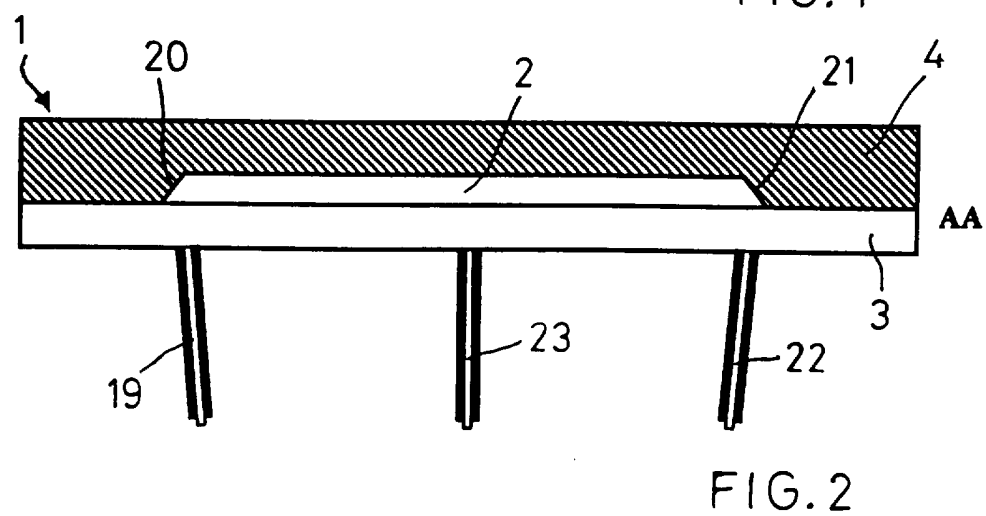
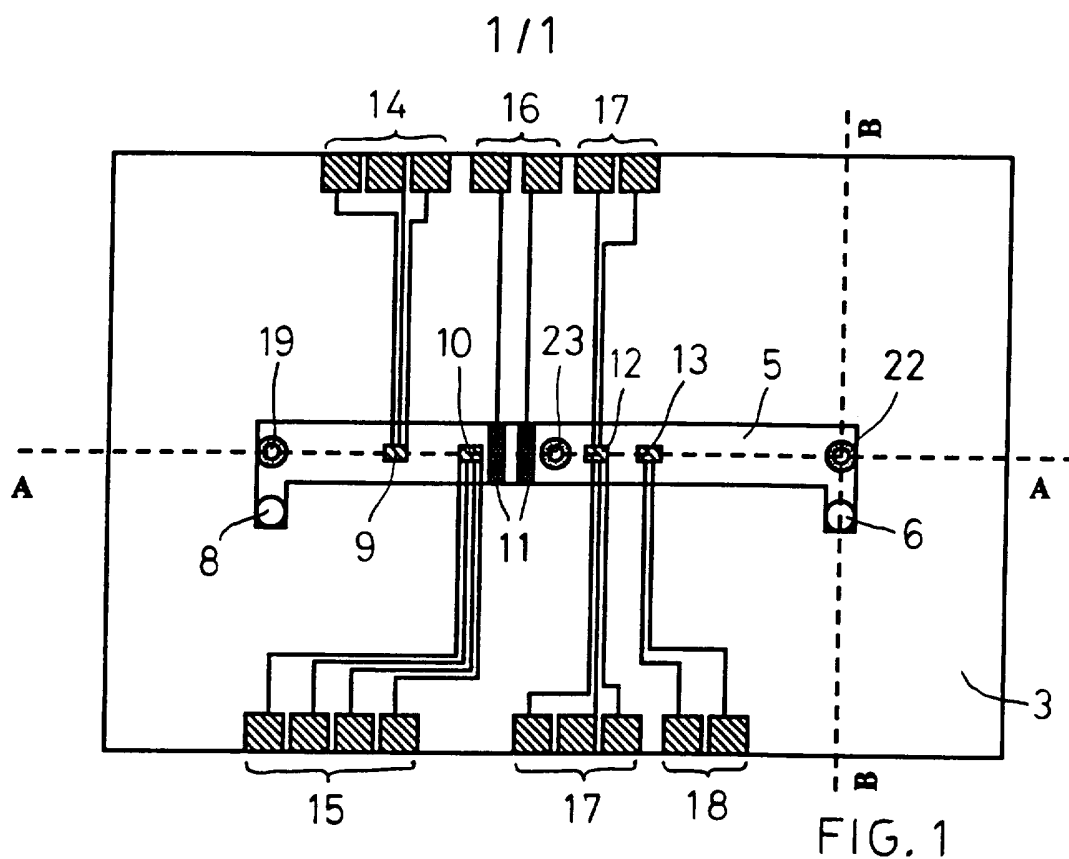
6. The apparatus according to any of claims 1 - 5,
characterized in that said electrical interface (15, 17) is
connected to means (10, 12) in the flow cell wall for
measuring pressure and flow rate of the fluid in the flow
5 cell.

7. The apparatus according to any of claims 4 - 6,
characterized in that said optical interface (3, 19, 23) is
adapted to transmit light into the flow cell (1) and to
10 receive that light to measure turbidity of the fluid in the
flow cell.

8. The apparatus according to any of claims 1 - 7,
characterized in that the cross-section of the flow channel
15 (2) of the flow cell is a truncated, equally sided
triangle.

9. The apparatus according to any of claims 1 - 8,
characterized in that the longitudinal section of the flow
20 channel (2) of the flow cell is essentially a truncated,
equally sided triangle.

10. The apparatus according to claim 9, **characterized in**
that inclined end walls (20, 21) of the flow cell are
25 adapted to reflect said light transmitted through the flow
cell to measure light absorption of the fluid in the flow
cell.



INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 96/00548

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: G01N 30/78, G01N 21/05

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPIL, IFIPAT, EPODOC

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0333939 A2 (BEUKEVELD G.J.J. DRS.), 27 Sept 1989 (27.09.89), page 5, column 8, line 28 - column 8, line 41; page 6, column 9, line 49 - column 9, line 57; page 2, column 1, line 29 - column 1, line 34 --	1-10
A	US 5408313 A (M.J. PONSTINGL ET AL), 18 April 1995 (18.04.95), column 5, line 9 - line 13, abstract -- -----	1-10

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 July 1996

Date of mailing of the international search report

06.08.1996

Name and mailing address of the ISA/

Swedish Patent Office

Box 5055, S-102 42 STOCKHOLM

Facsimile No. +46 8 666 02 86

Authorized officer

Jan Carlerud

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT
Information on patent family members

01/07/96

International application No.
PCT/SE 96/00548

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A2- 0333939	27/09/89	NL-A- 8703139	17/07/89
US-A- 5408313	18/04/95	US-A- 5241368	31/08/93