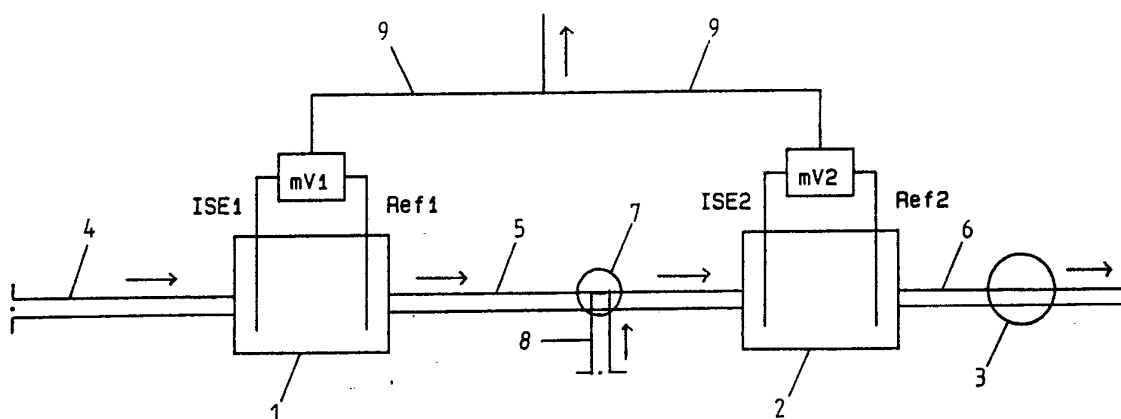




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification<sup>4</sup> : <b>G01N 27/56, 33/84, 33/48</b></p>	<p><b>A1</b></p>	<p>(11) International Publication Number: <b>WO 88/ 02487</b> (43) International Publication Date: 7 April 1988 (07.04.88)</p>
<p>(21) International Application Number: PCT/FI87/00134 (22) International Filing Date: 2 October 1987 (02.10.87) (31) Priority Application Number: 864006 (32) Priority Date: 3 October 1986 (03.10.86) (33) Priority Country: FI (71) Applicant (for all designated States except US): KONE OY [FI/FI]; Munkkiniemen puistotie 25, SF-00330 Helsinki 33 (FI). (72) Inventors; and (75) Inventors/Applicants (for US only) : IVASKA, Ari [FI/FI]; Vanha hämeentie 63 B, SF-20540 Turku (FI). LEWENSTAM, Andrzej [FI/FI]; Parivaljakonkuja 2 D 22, SF-00410 Helsinki (FI). WÄNNINEN, Erkki [FI/FI]; Linnankatu 5 B, SF-20110 Turku (FI).</p>		<p>(74) Agent: PATENTTITOIMISTO OY HEINÄNEN AB PATENTBYRÅ; Annankatu 31-33 C, SF-00100 Helsinki 10 (FI). (81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report. With amended claims. In English translation (filed in Finnish).</i></p>
<p>(54) Title: METHOD FOR DETERMINATION OF TOTAL CARBONATE CONTENT ESPECIALLY IN BIOLOGICAL FLUIDS</p>		



## (57) Abstract

Electrochemical method for determining the total carbonate content especially in biological fluids. According to the invention, using an ion-selective electrode, the concentration of certain ions dissociable from carbon acid or affecting the dissociation balance is first measured for the fluid. The fluid is then mixed with another fluid containing a reactive component that changes the ionic concentration just measured, whereupon the said concentration is again measured by means of an ion-selective electrode, thus producing two different measurement results, from which the total carbonate content can be determined. The measurement preferably relates to the pH of the fluid, while metallic ions such as  $\text{Cu}^{2+}$ , forming a complex with hydrocarbonate ions, are used as the component to be added to alter the pH.

**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.

<b>AT</b> Austria	<b>FR</b> France	<b>ML</b> Mali
<b>AU</b> Australia	<b>GA</b> Gabon	<b>MR</b> Mauritania
<b>BB</b> Barbados	<b>GB</b> United Kingdom	<b>MW</b> Malawi
<b>BE</b> Belgium	<b>HU</b> Hungary	<b>NL</b> Netherlands
<b>BG</b> Bulgaria	<b>IT</b> Italy	<b>NO</b> Norway
<b>BJ</b> Benin	<b>JP</b> Japan	<b>RO</b> Romania
<b>BR</b> Brazil	<b>KP</b> Democratic People's Republic of Korea	<b>SD</b> Sudan
<b>CF</b> Central African Republic	<b>KR</b> Republic of Korea	<b>SE</b> Sweden
<b>CG</b> Congo	<b>LI</b> Liechtenstein	<b>SN</b> Senegal
<b>CH</b> Switzerland	<b>LK</b> Sri Lanka	<b>SU</b> Soviet Union
<b>CM</b> Cameroon	<b>LU</b> Luxembourg	<b>TD</b> Chad
<b>DE</b> Germany, Federal Republic of	<b>MC</b> Monaco	<b>TG</b> Togo
<b>DK</b> Denmark	<b>MG</b> Madagascar	<b>US</b> United States of America
<b>FI</b> Finland		

## METHOD FOR DETERMINATION OF TOTAL CARBONATE CONTENT ESPECIALLY IN BIOLOGICAL FLUIDS

The present invention concerns a method for determining the total carbonate content especially in biological fluids, using an ion-selective electrode for the measurement.

The biological fluids referred to are, in particular, human blood and urine, which are analyzed to determine their carbonate content for diagnostic purposes.

The methods currently used for electrochemical determination of the carbonate content in fluids are based on two fundamental solutions. One of these uses an electrode designed for sensing carbon dioxide (CO<sub>2</sub>), while the other uses an electrode sensitive to carbonate ions.

The first solution is represented by US patent 4 490 234, which proposes a method for determination of the total carbonate content of a fluid involving measurement of the amount of carbon dioxide in the fluid by means of an element consisting of a renewable electrolyte disposed between a gas-permeable membrane and an ion-selective electrode. In this method, the sample fluid is premixed with an acid to release carbon dioxide. The gassed sample is then pumped toward the said element, while at the same time the electrolyte is also circulated by pumping. When the sample reaches the element, the pumping is stopped. The gas then diffuses through the membrane into the electrolyte solution, and the change occurring in the latter is measured by the ion-selective electrode and translated into a total carbonate content value. The disadvantages of this method are the need for a two-channel fluid pumping system and the use of a gas-permeable membrane, which may be blocked with time by constituents in the sample, such as proteins.

The second solution is represented by US patent 4 196 056, which involves the use of an ion-selective carbonate electrode. As described in the patent publication, the sample is premixed with a pH buffer solution containing a mercury/EDTA complexone. This has the main effect of producing carbonate ions, which are then sensed by the said electrode. The drawbacks of this method are related in the first place to the imperfections of the ion-selective electrode, such as instability of the signal, long response time, short durability and sensitivity to other ions and substances.

The object of the present invention is to provide a new method for determining the total carbonate content of a fluid in a simple, fast and accurate manner without the above-mentioned disadvantages of existing methods. The invention is characterized in that the fluid is first subjected to a measurement, using an ion-selective electrode, of the concentration of certain ions dissociable from carbon acid or affecting the dissociation balance, that the fluid is then mixed with another fluid containing a reactive component that changes the concentration of said ions thus measured, and that after the mixing the said ionic concentration is again measured by means of an ion-selective electrode, thus producing two different measurement results, from which the total carbonate content can be determined.

The method of the invention allows concentration measurements without generation of gases. The advantage of this is that no accurate pressure control and no dialysis membrane is necessary. This obviates the danger of membrane blockages, which present the worst problems with the existing methods.

Further, an essential feature in the method of the invention is that the determination of the carbonate content is based on the chemical laws governing the balance of different ions in a fluid, i.e. the method has an exact scientific foundation ensuring reliable results.

An embodiment of the method of the invention is characterized in that it uses a fluid containing ions of a metal, e.g.  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  or  $\text{Zn}^{2+}$ , which form a complex with carbonate or hydrocarbonate ions. Copper ions are particularly suitable, since reagents containing copper ions are stable and inexpensive. A copper ion reacts with a hydrocarbonate ion as follows:



A similar reaction also occurs with the other metallic ions mentioned above. Thus in all cases the reaction involves ions only and is very fast.

Another embodiment of the method of the invention is characterized in that the electrochemical concentration measurement relates to hydrogen ions, in other words, that the pH of the fluid containing carbonate is measured. The measurement can be performed using a glass electrode, which, unlike the electrodes normally used for testing biological fluids, is fast, accurate and free of interference in operation.

In an advantageous embodiment of the method of the invention, the carbonate content is determined by using a device consisting of two cascade-connected measuring cells, the fluid containing carbonate being stopped in the first cell, where the concentration of given ions, such as hydrogen ions, is measured with an ion-selective electrode incorporated in the cell, from where the fluid is then pumped into the second cell, where it is stopped and the said ionic concentration is again measured with an ion-selective electrode incorporated in the cell, the fluid being mixed between the two cells with another fluid containing a component, e.g. metallic ions forming a complex with carbonate or hydrocarbonate ions, altering the said ionic concentration. The necessary fluid motion can be achieved with a single one-channel peristaltic pump, and in general the required equipment is simple in construction.

- 4 -

The second fluid to be added to the fluid under carbonate measurement preferably contains metallic ions such as copper, lead, cadmium, manganese or zinc ions in the form of a dissolved salt, e.g. a nitrate with a concentration in the range of  $1 \times 10^{-3} \text{M}$  -  $2.5 \times 10^{-2} \text{M}$ , said ions forming a complex with the carrier electrolyte, e.g. potassium nitrate, which may have a concentration in the range of  $0.15 \text{M}$  -  $1 \text{M}$ , and a substance inhibiting secondary reactions of the metallic ions, e.g. hydrogen peroxide or  $\text{Fe}^{3+}$  ions, preferably in a concentration of  $1 \times 10^{-4}$  -  $1 \times 10^{-1} \text{M}$ .

In the following, the invention is described in greater detail by the aid of an example, reference being made to the drawing attached, showing a diagram of set of equipment designed for applying the method of the invention.

The equipment shown in the drawing comprises a measuring cell 1, which is provided with an ion-selective electrode ISE1 and a reference electrode REF1. the potential difference between these being measured with a potentiometer mV1. a second measuring cell 2, which is provided with an ion-selective electrode ISE2 and a reference electrode REF2, the potential difference between these being measured with a potentiometer mV2, and tubes 4, 5, 6, connected to a pump 3 and serving to pass the fluid sample through the measuring cells. The tube 5 between the two cells is provided with a valve 7 communicating with a branch tube 8, through which a second fluid can be added to the fluid flowing in the tube 5 between the cells. The potential differences measured in the two cells are transmitted to a computer or a similar calculating device via the potentiometer output conductors, identified with reference number 9 in the drawing.

In the most advantageous application of the invention, the fluid sample, e.g. a blood sample, for which the total carbonate content is to be found out, is passed via the tubes 4, 5, 6 through the measuring cells 1 and 2, and a potentiometric pH measurement is performed in both cells, using glass electrodes sensitive to

- 5 -

hydrogen ions. For each measurement, the sample is stopped in the cell. Between the cells, a second fluid, introduced via the tube 8, is added to the sample. This second fluid is preferably a reagent, e.g. a mild copper nitrate solution, containing divalent copper ions, potassium nitrate as a carrier electrolyte and hydrogen peroxide to inhibit secondary reactions of the copper ions. The copper ions react with hydrocarbonate ions contained in the sample in the manner expressed by the equation on page 3, thus consuming hydrocarbonate ions and producing carbon acid, thereby altering the pH of the sample. Therefore, the pH measurement in the second cell 2 yields a result different from that obtained in the first cell 1, and the total carbonate content of the sample can be calculated from these two results.

The total carbonate content CL is calculated from the formula:

$$CL = Q \left( \frac{1 + 2[H^+]_2 K_{II}}{\frac{[H^+]_2 K_{II}}{\alpha_{HL(H)}_1} - \frac{1}{\alpha_{H_2L(H)}_1}} \right)$$

where Q is a constant determined by the size of the sample and the quantity and concentration of the reagent employed, and where

$$\alpha_{HL(H)}_1 = \frac{1}{K_I [H^+]_1} + 1 + K_{II} [H^+]_1$$

$$\alpha_{H_2L(H)}_1 = \frac{1}{K_I K_{II} [H^+]_1^2} + \frac{1}{K_{II} [H^+]_1} + 1$$

- 6 -

$$K_I = \frac{[\text{HCO}_3^-]}{[\text{CO}_3^{2-}][\text{H}^+]}$$

$$K_{II} = \frac{[\text{H}_2\text{CO}_3]}{[\text{HCO}_3^-][\text{H}^+]}$$

The subscripts "1" and "2" in the formulas above refer to the pH measurements in cells 1 and 2 respectively. The constants Q and  $K_I$  are calculated beforehand from the formula (I) and using two buffer solutions with a known  $C_L$  value. Once the constants have been determined, the formula can be used to calculate the total carbonate content  $C_L$ .

It is obvious to a person skilled in the art that the embodiments of the invention are not restricted to the example described above, but that they may instead be varied in the scope of the claims presented below. Thus it is possible that instead of copper ions, other ions forming a complex with carbonate or hydrocarbonate ions are used, e.g.  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  or  $\text{Zn}^{2+}$  ions, or that instead of the pH, the concentration of hydrocarbonate or carbonate ions is measured in the cells. Further, it is possible to interchange the sample containing carbonate and the reagent, such as the mentioned reagent containing copper ions, so that the reagent is first brought into the measuring cell 1, where its copper content is measured with a suitable copper-selective electrode, whereupon the sample containing carbonate is added to the reagent via the tube 8 and the copper content of the mixture thus obtained is measured in the second cell 2 with a copper-selective electrode. The total carbonate content of the sample can then be calculated from the measurement results obtained.



**CLAIMS**

1. Method for determining the total carbonate content especially in biological fluids, using an ion-selective electrode for the measurement, characterized in that the concentration of certain ions dissociable from carbon acid or affecting the dissociation balance is first measured for the fluid by means of an ion-selective electrode, that the fluid is then mixed with another fluid containing a reactive component that changes the concentration of said ions just measured, and that after the mixing the said ionic concentration is again measured by means of an ion-selective electrode, thus producing two different measurement results, from which the total carbonate content can be determined.
2. Method according to claim 1, characterized in that it uses a fluid containing metallic ions, e.g.  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  or  $\text{Zn}^{2+}$ , which form a complex with carbonate or hydrocarbonate ions.
3. Method according to claim 1 or 2, characterized in that the pH of the fluid containing carbonate is measured.
4. Method according to one of the claims above, characterized in that the carbonate content is determined by using a device comprising two cascade-connected measuring cells, whereby the fluid containing carbonate is stopped in the first cell, where the concentration of given ions, such as hydrogen ions, is measured with an ion-selective electrode incorporated in the cell, from where the fluid is then pumped into the second cell, where it is stopped and the said ionic concentration is again measured with an ion-selective electrode incorporated in the cell, the fluid being mixed between the two cells with another fluid containing a component, e.g. metallic ions forming a complex with carbonate or hydrocarbonate ions, altering the said ionic concentration.

- 8 -

5. Method according to claim 2 or 4, characterized in that the said second fluid contains metallic ions such as copper, lead, cadmium, manganese or zinc ions in the form of a dissolved salt, e.g. a nitrate, which form a complex with the carrier electrolyte, e.g. potassium nitrate, and a substance, e.g. hydrogen peroxide or  $\text{Fe}^{3+}$  ions, inhibiting secondary reactions of said metallic ions.

## AMENDED CLAIMS

[received by the International Bureau on 29 February 1988 (29.02.88)  
original claims 1-5 replaced by amended claims 1-4 (2 pages)]

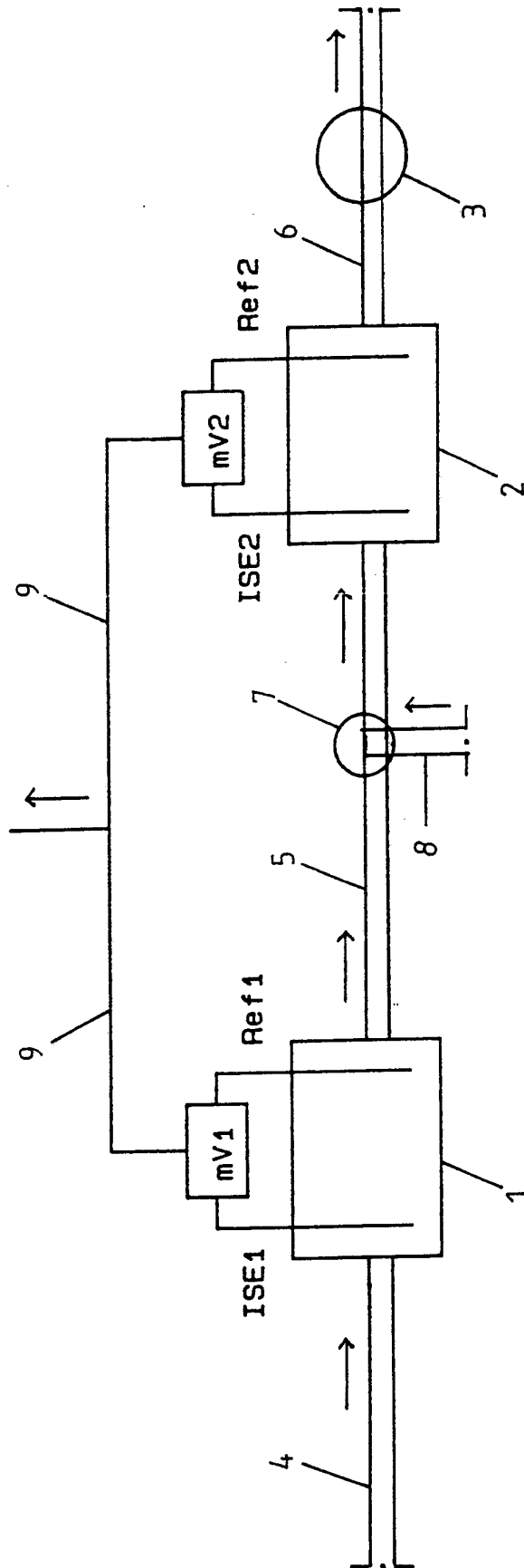
1. Method for determining the total carbonate content especially in biological fluids, the method comprising the steps of measuring the concentration of an ion contained in a fluid, said ion being dissociable from carbon acid or affecting the dissociation balance and said measurement being carried out by means of an ion-selective electrode, then at a second step mixing the fluid with another fluid containing a reactive component that changes the concentration of said ion measured at the first step and after the mixing measuring the said ionic concentration anew by means of an ion-selective electrode, thus producing two different measurement results from which the total carbonate content can be determined, characterized in that the method uses a fluid containing metallic ions such as  $\text{Cu}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Mn}^{2+}$  or  $\text{Zn}^{2+}$ , which form a complex with carbonate or hydrocarbonate ions.

2. Method according to claim 1, characterized in that the pH of the fluid containing carbonate is measured.

3. Method according to claim 1 or 2, characterized in that the carbonate content is determined by using a device comprising two cascade-connected measuring cells, whereby the fluid containing carbonate is stopped in the first cell, where the concentration of given ions, such as hydrogen ions, is measured with an ion-selective electrode incorporated in the cell, from where the fluid is then pumped into the second cell, where it is stopped and the said ionic concentration is again measured with an ion-selective electrode incorporated in the cell, the fluid being mixed between the two cells with another fluid containing metallic ions forming a complex with carbonate or hydrocarbonate ions, altering the said ionic concentration.

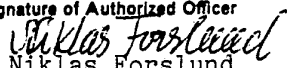
4. Method according to claim 1 or 3, characterized in that the said second fluid contains metallic ions such as copper,

lead, cadmium, manganese or zinc ions in the form of a dissolved salt, e.g. a nitrate, which form a complex with the carrier electrolyte, e.g. potassium nitrate, and a substance, e.g. hydrogen peroxide or  $\text{Fe}^{3+}$  ions, inhibiting secondary reactions of said metallic ions.



# INTERNATIONAL SEARCH REPORT

International Application No PCT/FI87/00134

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup>		
According to International Patent Classification (IPC) or to both National Classification and IPC <sup>4</sup>		
G 01 N 27/56, 33/84, 33/48		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
<b>Classification System</b>	<b>Classification Symbols</b>	
IPC 4 US C1	G 01 N 33/84, /48, /49, /00, 27/26, /30, /36, /46, /56 436: 19, 68, 100, 133, 145-146; 422: 68; 23: 230B,R; 204: IT, 196B,G,M,P, 409, 416-320, 433	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched <sup>8</sup>		
SE, NO, DK, FI classes as above		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT <sup>9</sup></b>		
<b>Category <sup>10</sup></b>	<b>Citation of Document, <sup>11</sup> with indication, where appropriate, of the relevant passages <sup>12</sup></b>	<b>Relevant to Claim No. <sup>13</sup></b>
A	US, A, 4 264 328 (MARSONER) 28 April 1981	1-5
A	US, A, 4 196 056 (KUMAR) 1 April 1980	1-5
A	US, A, 4 490 234 (BUZZA) 25 December 1984	1-5
A	EP, A2, 0 155 162 (TECHNICON INSTRUMENTS CORPORATION) 18 September 1985	1-5
A	US, A, 4 149 949 (BUZZA) 17 April 1979	1-5
A	US, A, 4 003 705 (BUZZA ET AL) 18 January 1977	1-5
A	US, A, 4 490 235 (CALZI) 25 December 1984	1-5
	.../...	
<p><sup>10</sup> Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"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</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"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.</p> <p>"&amp;" document member of the same patent family</p>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
1988-01-04	1988 -01- 1 3	
International Searching Authority	Signature of Authorized Officer	
Swedish Patent Office	 Niklas Forslund	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	US, A, 4 353 867 (LUZZANA) 12 October 1982 & EP, 0052718 JP, 57131045 CA, 1174731 AU, 550744	1
Y	Patent Abstract of Japan, Vol 7, No 57, (P-181) (1202), abstract of JP 57-203943, publ 1982-12-14	1