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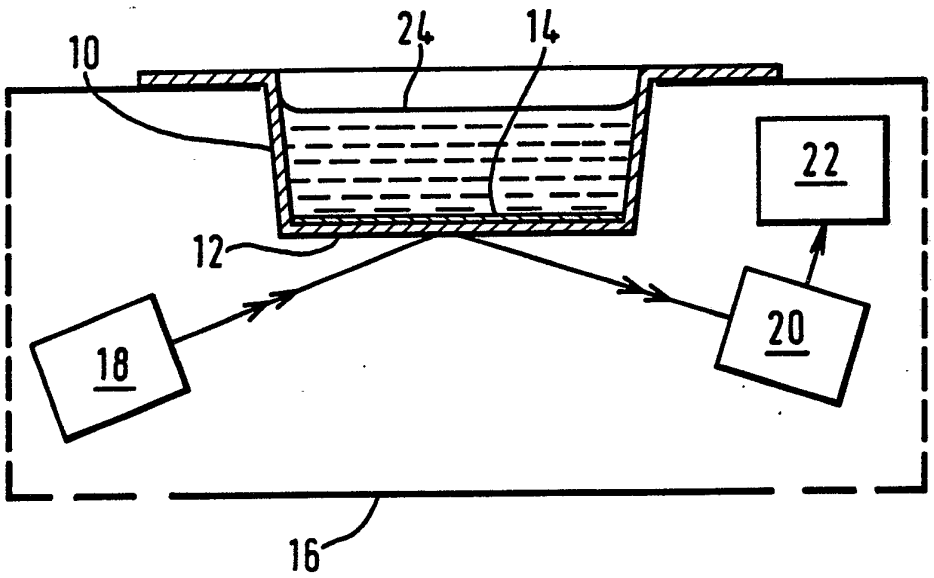

(57) Abstract

A method of assaying for a ligand in a sample which comprises incubating the sample in contact with one surface of an optical structure capable of exhibiting surface plasmon resonance, the said surface having adsorbed thereon or bound thereto, either directly or indirectly, a specific binding partner for the ligand it is desired to detect; irradiating the other surface of the optical structure with radiation of an appropriate wavelength; and analysing the reflected radiation in order to determine whether, and if desired the extent to which and/or rate at which, the surface plasmon resonance characteristics of the optical structure are altered by formation of a complex between the ligand and the specific binding partner. An apparatus for detecting one or more ligands in a sample suitable for use in the method of the invention is also provided.

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Improved Assay Technique and Apparatus therefor

Field of the invention

This invention concerns assay techniques by which qualitative and/or quantitative detection of chemical, biochemical or biological analytes in a sample can be determined and also concerns apparatus by which such techniques can be performed.

Background to the invention

Assay techniques referred to are based on the affinity between the analyte which is to be assayed and a receptive material for example a ligand or a specific binding partner, which is coated onto a particular type of surface. Reference is made to International Patent Publications WO84/02578 and WO86/01901 for descriptions of assay techniques comprising (a) coating at least a predetermined part of a surface having a preformed relief profile on a substrate with a thin film of a material capable of binding the species to be assayed, the surface part being optically active with respect to radiation at least over a predetermined band of wavelengths, and (b) contacting the coated surface with the sample and observing the optical properties of the surface part in order to determine a qualitative and/or quantitative change in optical properties as a result of the binding of the species onto the thin film of material on the surface.

As described in those publications, the preformed relief profile is typically in the form of an optical grating which may be a simple single grating of two or more crossed gratings the ridges of which may have square, sinusoidal or triangular cross-sectional shape, and as employed herein, references to a grating are intended to encompass all such gratings.

In publication WO84/02578, the change in optical properties of the grating as a result of the binding of an analyte to be assayed (such as a specific antigen in a blood serum) is brought about essentially as a result of (1) the mass or bulk of the bound analyte and (2) its dielectric properties.

In publication WO86/01901 the binding events are monitored by changes in the fluorescent properties of a dye-tagged binding partner attached to the sensor surface.

In each of the described techniques the grating surface is essentially opaque, at least at the wavelength of the radiation used for illumination, and the grating can therefore be considered to be a reflective diffraction grating. Changes in the properties of the grating which occur as a result of the deposition thereon of analytes or other material as a result of the assay technique appear as changes in the reflective characteristics of the grating (for WO84/02578) and fluorescent emission characteristics (WO86/01901) in the manner described in the aforementioned specifications. However, the successful operation of such assay techniques relies on the ability to illuminate the grating surface and therefore any material other than that bound to the surface through a specific binding reaction will interrupt the passage of light and therefore it has been difficult to conceive how such a test could be carried out "in the wet". "In the wet" means with a liquid in contact with the grating surface and, using conventional technology, an assay of this type is particularly difficult if the liquid absorbs or scatters light at the wavelength of excitation or observation.

It is therefore an object of the present invention to provide an alternative method and apparatus by which an assay technique can be performed "in the wet" and even in the presence of absorption or scattering by particles in suspension in the liquid.

Summary of the invention

Thus, in its broadest aspect, the invention provides a method of assaying for a ligand in a sample which comprises incubating the sample in contact with one surface of an optical structure capable of exhibiting surface plasmon resonance, the said surface having adsorbed thereon or bound thereto, either directly or indirectly, a specific binding partner for the ligand it is desired to detect; irradiating the other surface of the optical structure with radiation of an appropriate wavelength; and analysing the reflected radiation in order to determine whether, and if desired the extent to which and/or rate at which, the surface plasmon resonance characteristics of the optical structure are altered by formation of a complex between the ligand and the specific binding partner.

Preferably the optical structure is a diffraction grating of a clear plastics or glass material and the grating is coated with a thin metal or metal-like layer which is partially reflective and partially transmissive, at least at the wavelength of radiation which is to be used to illuminate and observe the grating for investigative purposes.

Preferably the ligand to be assayed for will be an antibody or an antigen and the specific binding partner will then be the complementary antigen or antibody.

The invention further provides an apparatus for detecting one or more ligands in a sample which apparatus comprises a reservoir for holding the sample to be tested, at least part of an internal surface of said reservoir comprising an optical structure capable of exhibiting surface plasmon resonance, that surface of the said structure which in use will contact the sample having adsorbed thereon or bound thereto, either directly or indirectly, a specific binding partner for the ligand it

is desired to detect.

One embodiment of the apparatus of the invention further comprises means for irradiating from outside the reservoir that surface of the optical structure which in use will be remote from the sample; and means for analysing the reflected radiation in order to determine whether, and if desired the extent to which and/or rate at which, the surface plasmon resonance characteristics of the said optical structure are altered by formation of a complex between the ligand and the specific binding partner.

Typically the reservoir comprises a shallow well having a flat bottom having a diffraction grating formed on its upper face within the well and a partially reflecting, partially transmitting thin metal or metal-like film applied to the grating surface.

Where it is important to protect the metal from the liquid or other materials to be put in the reservoir, the metal film is preferably coated by a further film of an inert material which, whilst enabling the diffraction grating to exhibit surface plasmon resonance when activated by appropriate wavelength light, nevertheless prevents chemical interaction between the metal and the reactants within the reservoir.

The advantage of the invention is that the assay technique can be employed when using samples "in the wet" and in the presence of scattering particles in the liquid sample.

A further advantage is that reactions can be monitored as they occur and therefore the end point of the assay can be predicted, thereby reducing the length of time taken to perform the assay.

An additional advantage is that no separation of the sample from the sensor is needed before measurement.

The invention is thus suitable for use with whole blood samples and other biological samples containing

light scattering compounds or particulate material.

The invention also enables a competition assay to be performed in the wet or dry where a reagent antigen is fluorophor-labelled, or a sandwich assay to be performed in which a second antibody is fluorophor-labelled.

The use of fluorophor-labelled material is possible provided the detection means is capable of discriminating between different wavelengths of light received thereby and in particular determining whether or not light of a wavelength corresponding to that produced by the fluorophor label is present in the light re-radiated from the grating.

In a typical apparatus, a monochromatic or quasi-monochromatic light source is used as the primary source for illuminating the grating and typically a laser is used for this purpose, although it will be understood that this by no means restricts the choice of light source to a laser, and the angle of incidence of the light is altered. Alternatively, a polychromatic e.g. white light source may be used, the angle of incidence held constant and the wavelength characteristics of the reflected light analysed in order to detect surface plasmon resonance effects.

The invention will now briefly be described with reference to the accompanying drawings in which:

Figure 1 shows the effect of surface plasmon resonance via back illumination of a diffraction grating; and

Figure 2 illustrates diagrammatically apparatus by which an assay may be performed in accordance with the invention and which itself embodies the preferred constructional features of the invention.

Figure 1 shows the results obtained from an injection moulded diffraction grating which was coated with 50 nm of silver by vacuum evaporation and interrogated from the underside of the grating with a helium-neon laser (λ=633 nm). The reflected light intensity was monitored

with changing angle of incidence (θ). Over a specific range of angles the dip in reflectivity characteristic of surface plasmon resonance was observed. Interrogating an area of the metallised test device without the grating gave no change in intensity of reflected light over the angle range covered.

In Figure 2 a shallow well 10 is formed in a clear plastics material having a flat base 12 on the upper internal face of which is formed a diffraction grating 14 by any appropriate process such as impression moulding or machining or otherwise.

The upper surface of the grating 14 is coated with a semi-reflective metal or metal-like film for example silver or aluminium or copper or gold, and if an intermediate inert layer is required between the metallic film and the reagents to be placed in the well, the surface is itself coated with a layer of an appropriate buffer material such as an oxide of silicon.

The grating is coated with a thin film of material comprising specific antigens, antibodies or other binding partners which may be tagged with a fluoroescent compound and the well is now ready to receive a liquid containing the specific antibody or antigen or complementary binding partners to be tested.

The article containing the well is inserted into apparatus shown diagrammatically at 16 which contains a laser light source 18, light from which is projected, using suitable optical means (not shown), to impinge on the underside of the well at an appropriate angle of incidence to set up surface plasmon resonance in the diffraction grating. A wavelength sensitive light detector 20 is also located within the housing 16 and the output from the detector 20 is supplied to electrical processing and display apparatus such as 22 which may or may not be included within the housing.

Adjustments are made to the detector 20 and

processing and display circuits 22 to produce a first reading when the well is illuminated in position and contains a non-ligand containing liquid.

A sample which may or may not contain a ligand is then added to the well in any convenient manner and the output of the detector 20 as displayed by the apparatus 22 is monitored to determine any change.

If a fluorescent assay technique is employed, the detector can be set to determine whether or not any light of the wavelength of the fluorescent label in the assay can be detected.

It will be seen that if the liquid sample denoted by reference numeral 24 in the drawing contains light scattering particles, these would interfere with the transmission of light through the liquid, and thus to and from the diffraction grating, and would render impractical any observation of the diffraction grating through the liquid. However, using the illumination method illustrated the surface plasmon resonance effect as determined by the detector 20 will not be affected by the presence of light scattering particles in the liquid, and the assay technique can be performed "in the wet".

Although the illustrated example shows only one well, the light source 18 may of course be used to illuminate a plurality of wells simultaneously and either a corresponding plurality of detectors may be employed or a detector set to scan each well in succession may be employed so that an assay of a large number of different samples can be carried out relatively quickly.

Alternatively, a number of defined regions on the surface of a single well can each have a different binding partner thereon and these can be employed either to measure a number of different analytes simultaneously, or to provide a measure of the non-specific binding, by comparison with "control" regions, which may carry for example a binding partner which is not itself specific for any ligand which may be present in the sample to be tested.

Claims

1. A method of assaying for a ligand in a sample which comprises incubating the sample in contact with one surface of an optical structure capable of exhibiting surface plasmon resonance, the said surface having adsorbed thereon or bound thereto, either directly or indirectly, a specific binding partner for the ligand it is desired to detect; irradiating the other surface of the optical structure with radiation of an appropriate wavelength; and analysing the reflected radiation in order to determine whether, and if desired the extent to which and/or rate at which, the surface plasmon resonance characteristics of the optical structure are altered by formation of a complex between the ligand and the specific binding partner.

2. A method as claimed in claim 1 wherein the optical structure is a diffraction grating.

3. A method as claimed in claim 1 or claim 2 wherein the specific binding partner is an antigen or an antibody.

4. A method as claimed in any one of the preceding claims wherein the optical structure is coated with a thin metal or metal-like layer which is partially reflective and partially transmissive at the wavelength of radiation used.

5. An apparatus for detecting one or more ligands in a sample which apparatus comprises a reservoir for holding the sample to be tested, at least part of an internal surface of said reservoir comprising an optical structure capable of exhibiting surface plasmon resonance, that surface of the said structure which in use will contact the sample having adsorbed thereon or bound thereto,

either directly or indirectly, a specific binding partner for the ligand it is desired to detect.

6. An apparatus as claimed in claim 5 further comprising means for irradiating from outside the reservoir that surface of the optical structure which in use will be remote from the sample and means for analysing the reflected radiation in order to determine whether, and if desired the extent to which and/or rate at which, the surface plasmon resonance characteristics of the said optical structure are altered by formation of a complex between the ligand and the specific binding partner.

7. An apparatus as claimed in claim 5 for detecting a plurality of ligands in a sample which comprises a reservoir having a plurality of discrete regions, each discrete region comprising an optical structure as defined in claim 5, that surface of each optical structure which in use will contact the sample having a different specific binding partner adsorbed thereon or bound thereto.

8. An apparatus as claimed in claim 5 which comprises a plurality of reservoirs as defined in claim 5.

9. An apparatus as claimed in claim 7 or claim 8 further comprising means for irradiating from outside the reservoir that surface of the optical structure which in use will be remote from the sample and a plurality of means for analysing the reflected radiation or a single means for analysing the reflected radiation capable of scanning each discrete region or reservoir in succession in order to determine whether, and if desired the extent to which and/or rate at which, the surface plasmon resonance characteristics of the said optical structure are altered by formation of a complex between the ligand

and the specific binding partner.

10. An apparatus as claimed in any one of claims 5 to 9 wherein the optical structure is a diffraction grating.

11. An apparatus as claimed in any one of claims 5 to 10 wherein the specific binding partner is an antigen or an antibody.

12. An apparatus as claimed in any one of claims 5 to 11 wherein the optical structure is coated with a thin metal or metal-like layer which is partially reflective and partially transmissive at the wavelength of radiation used.

13. An apparatus as claimed in any one of claims 5 to 12 wherein the irradiation means is a monochromatic or quasi-monochromatic light source.

14. An apparatus as claimed in claim 13 wherein the light source is a laser.

FIG. 1.

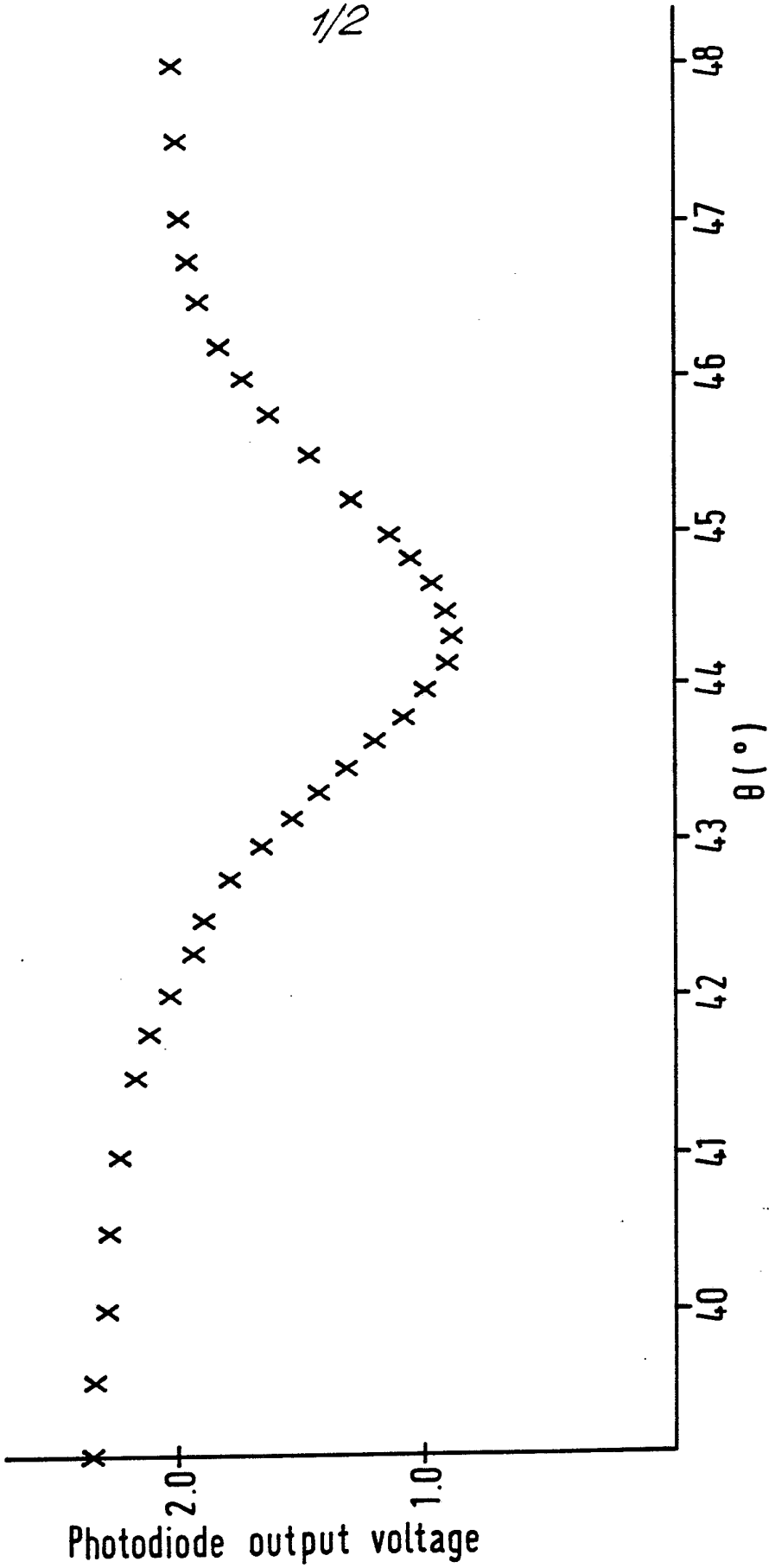
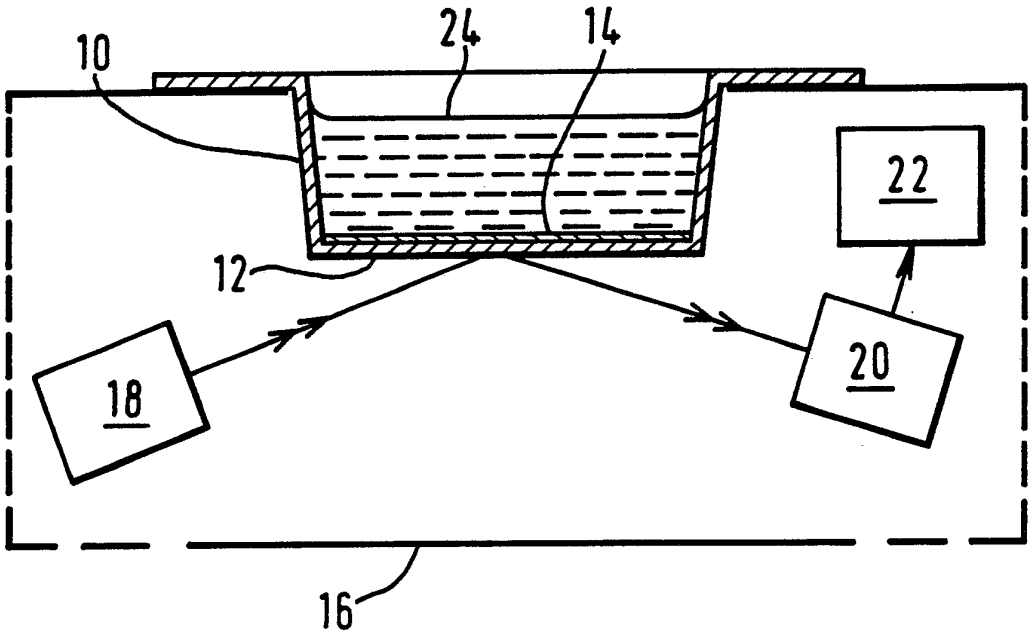


FIG. 2.



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/00176

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: G 01 N 33/553; G 01 N 21/47; G 01 N 21/64

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X,P, L	EP, A, 0254575 (ARES-SERONO RESEARCH AND DEVELOPMENT LTD) 27 January 1988, see the whole document	1-4,10-13
X	WO, A, 84/02578 (COMTECH RESEARCH UNIT LTD) 5 July 1984, see page 2, line 21 - page 3, line 7; page 6, line 32 - page 8, line 9; page 12, line 2 - page 18, line 18; figures 1-3 (cited in the application)	1-13
X	WO, A, 86/01901 (J.R. NORTH et al.) 27 March 1986, see page 2, line 15 - page 4, line 17; page 5, lines 7-22; page 16, line 2 - page 18, line 21 (cited in the application)	1-13
X	EP, A, 0073980 (MERCK PATENT GMBH) 16 March 1983, see page 2, lines 13-20; page 3, lines 11-28; page 4, line 14 - page 5, line 15; page 7, line 9 - page 8, line 28; claims 1,3,5-8	1-13

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
20th June 1988	15 JUIL 1988
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

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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
X	EP, A, 0171148 (UNILEVER PLC) 12 February 1986, see page 3, lines 19-30; page 10, lines 5-36; page 11, lines 16-21; page 17, lines 1-21; page 17, example; page 26, line 3 - page 29, line 10 --	1-13
X	EP, A, 0167335 (D.F.NICOLI & V.B. ELINGS) 8 January 1986, see page 12, lines 16-22; page 13, lines 24-30; page 32, line 8 - page 33, line 4; page 34, lines 11-17; page 49, line 15 - page 50, line 4; page 53, line 1 - page 55, line 7; figures 1-4 --	1-14
X	US, A, 3979184 (I. GIAVER) 7 September 1976, see column 2, lines 17-34; column 4, lines 38-54; column 4, example 1; column 5, example 2; column 5, line 46 - column 6, line 47 cited in the application	1,3,11

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8800176 SA 21046

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 04/07/88
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A- 0254575	27-01-88	AU-A-	7600787	28-01-88
		JP-A-	63100355	02-05-88
WO-A- 8402578	05-07-84	EP-A,B	0112721	04-07-84
		AU-A-	2410684	17-07-84
		JP-T-	60500309	07-03-85
		AU-B-	570425	17-03-88
WO-A- 8601901	27-03-86	EP-A-	0178083	16-04-86
		AU-A-	4806285	08-04-86
		JP-T-	62500736	26-03-87
EP-A- 0073980	16-03-83	DE-A-	3135196	17-03-83
		JP-A-	58055758	02-04-83
		US-A-	4521522	04-06-85
EP-A- 0171148	12-02-86	WO-A-	8600135	03-01-86
		WO-A-	8600138	03-01-86
		WO-A-	8600141	03-01-86
		EP-A-	0170375	05-02-86
		EP-A-	0170376	05-02-86
		AU-A-	4491085	10-01-86
		AU-A-	4491185	10-01-86
		AU-A-	4491385	10-01-86
		JP-T-	61502418	23-10-86
		JP-T-	61502419	23-10-86
		JP-T-	61502420	23-10-86
		CA-A-	1231136	05-01-88
EP-A- 0167335	08-01-86	US-A-	4647544	03-03-87
US-A- 3979184	07-09-76	FR-A,B	2312224	24-12-76
		DE-A,C	2623100	09-12-76
		GB-A-	1533286	22-11-78
		JP-A-	51148015	18-12-76