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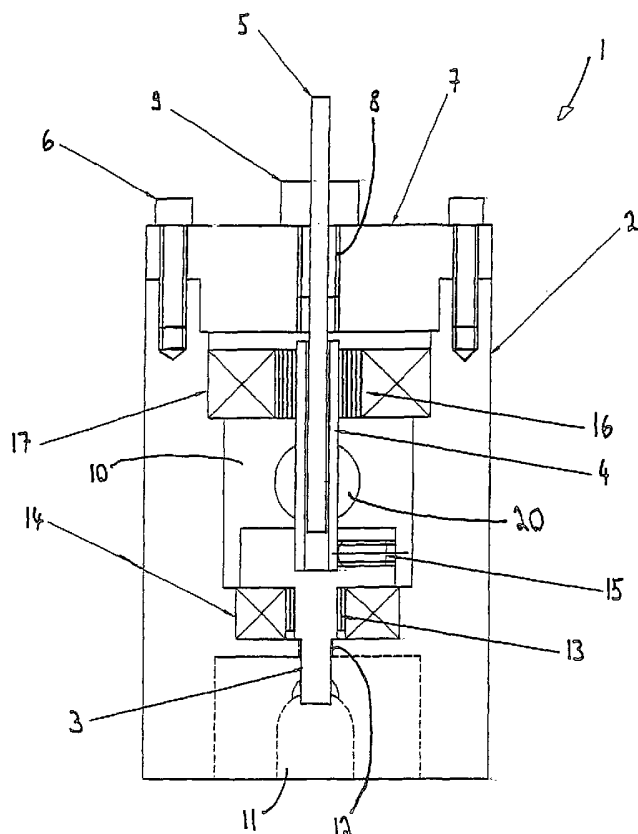
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[Continued on next page]

(54) Title: A SPECTROPHOTOMETRY DEVICE



(57) Abstract: A device primarily for use in spectrophotometry, comprises one or more housings; one or more windows appropriately located in one or more walls of the or each housing; one or more tubes releasably or permanently mounted to a drive means; and one or more rods located partly or entirely within the or each corresponding tube when the device is in use, wherein said drive means are adapted to rotate the or each tube around its corresponding rod so that one or more measurements can be obtained for the or each sample inserted into the device.



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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii)) for the following designations AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU,*

SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, UZ, VC, VN, YU, ZA, ZM, ZW, ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG)

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A SPECTROPHOTOMETRY DEVICE

Field of the Invention

The invention relates to the general field of devices used in the study of macromolecular structures.

Studies of, for example, membrane transport and membrane protein function call for a thorough knowledge of molecular interactions within the membrane, between the lipids themselves and between lipids and other species such as proteins, drugs and ions. To this end, the locations and orientations of molecules bound to the membrane can give important information. Flow linear dichroism (LD) of molecules bound to the bilayer of shear-deformed liposomes is one of the few direct methods potentially available for the study of the orientation of membrane guest molecules, provided that the molecules of interest have significant absorption in the visible, infra-red or UV regions of the spectrum.

LD is also an ideal technique for the study of polymeric materials such as DNA, protein, polysaccharides that can be oriented by shear forces. Application to long species such as carbon nanotubes (Bucky Tubes) is also possible.

As is well known in this field, linear dichroism is the difference in absorption of light polarised parallel to an orientation direction and light polarised perpendicular to that

direction. A successful study of this type is dependent on the actual device used to obtain the required orientation and the required spectroscopic measurements.

Amongst the prior art known to the applicant are devices used in conjunction with the method which is commonly known in this field as Couette flow. In these devices, two concentric cylinders are habitually used with an annular gap of approximately 500 μm . The outer cylinder is arranged to be stationary and incorporates two windows to allow the light to be incident radially on the cell. The inner cylinder is adapted to rotate and is required to be transparent to the intended radiation. Linear flow arrangements have also been implemented.

One of the drawbacks of traditional LD cells with some samples is the excessive bubble formation and turbulence which reduces the signal to noise ratio. Another relates to the comparatively large pathlength which reduces the average shear-flow orientation of the molecules.

One of the objects of the present invention is to provide a device which operates with an improved signal to noise ratio due to a reduction of bubble formation and improved orientation.

It is a further object of this invention to provide a device which radically reduces the sample size needed per test without compromising the sensitivity of the analytical procedure.

Another objective of this invention is to provide cell components with improved material properties and mechanical behaviour in order to further improve the quality of the measurements.

In this current patent specification, the applicant presents a device primarily for use in spectrophotometry, comprising one or more housings; one or more windows appropriately located in one or more walls of the or each housing; one or more tubes releasably or permanently mounted to a drive means; and one or more rods located partly or entirely within the or each corresponding tube or capillary when the device is in use, wherein said

drive means are adapted to rotate the or each tube around its corresponding rod so that one or more measurements can be obtained for the or each sample inserted into the device.

Using a tube or capillary as the outer part of the Couette sample holder and the inner part
5 being a corresponding rod, the sample size needed per test can be radically reduced while the sensitivity of the analytical procedure is not compromised.

In addition the cost of the optically transparent material is significantly reduced.

10 Since the inner rod is not driven by the drive means, the disassembly of the rod can be carried out with ease. This increases the flexibility of the device particularly when a larger number of samples are to be successively studied.

In a subsidiary aspect in accordance with the first broad encapsulation of the present
15 disclosure, the or each tube is releasably mounted on the spindle.

This feature is particularly advantageous because it improves the accessibility to the tube in order to even further improve its ability of being cleaned or even replaced. Due to the tube's generally elongated shape, the tube may be susceptible of breakage and therefore
20 the ability of releasing the tube from the spindle will have the additional advantage of being able to replace the tube independently from the spindle.

In an additional subsidiary aspect in accordance with the first broad aspect, the or each rod is releasable without having to remove the housing top cover.

25

The ability of removing the rod without having to remove the housing top cover is advantageous because it permits the rapid accessibility to the inner sections of the device for cleaning purposes and for the insertion of a particular sample.

30 In a further subsidiary aspect, the or each rod is immobilised when the device is in use.

In an additional subsidiary aspect, the drive means incorporates a spindle which is supported by at least one essentially horizontal surface and at least one essentially vertical surface of a corresponding bearing means.

- 5 This arrangement is advantageous because it permits a more precise control of the rotation of the tube relative to the rod and by so doing enables the accuracy of the measurements to be maintained during the use of the device.

In a further subsidiary aspect, the device incorporates respective upper and lower rotating
10 bearings which remain undisturbed in the housing if and when the housing top cover is removed.

This feature is particularly advantageous because it avoids having to move the bearings since the removal of these components may affect the accuracy of the measurements if
15 these were incorrectly replaced within the housing.

According to a second broad independent aspect, the applicant provides a device primarily for use in spectrophotometry, comprising one or more housings, one or more windows appropriately located in one or more walls of the or each housing; one or more
20 cylinders releasably or permanently mounted to a driving means; and one or more sleeves located partly or entirely around the or each cylinder when the device is in use, wherein at least the cylinder is a crystal of calcium fluoride (CaF_2), quartz, or other optically transparent, as required, material and said driving means is adapted to rotate the or each cylinder within the or each sleeve so that one or more measurements can be obtained for
25 the or each sample inserted into the device.

Alternatively the design could have an outer rotating sleeve and inner stationary unit with a light path through the middle.

- 30 The choice of CaF_2 as the material is particularly advantageous as it permits the extension of the measurement range into the infra-red region.

This choice of material is particularly advantageous as it permits the extension of the measurement range into the infra-red region.

In a subsidiary aspect of this second broad independent aspect, the device comprises an annular gap between the or each cylinder and its corresponding sleeve of 200 μm or less.

In a subsidiary aspect of the second broad independent aspect of this invention, the device comprises an annular gap between the or each cylinder and its corresponding sleeve of approximately 50 μm .

10

The selection of this magnitude of gap between the cylinder and the sleeve represents a complete departure from the prior art thinking that the gap size should be approximately 500 μm . However, once this inventive step has been taken of selecting such a lower value for the gap size, one of the advantages that become apparent is that it requires a smaller sample volume because of the cell's smaller pathlength. A further advantage of this configuration is a greater average orientation of the sample for example it was found that the average orientation of a DNA sample was ten times greater with the 50 μm pathlength.

15

According to a third broad independent aspect, the applicant presents an automated multi-sample spectrophotometry-based sampling system incorporating a device according to any of the preceding aspects and detailed with reference to the first broad aspect.

20

Brief Description of the Drawings

Figure 1 represents a cross-sectional view of a first embodiment of the invention.

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Figure 2 shows a cross-sectional view of a second embodiment of the invention.

Detailed Description of the Drawings

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Figure 1 presents a cell 1 incorporating a body 2 which has an essentially circular cross section and an essentially cylindrical body shape destined to house a drive means 3, a tube 4 and a rod 5. In its upper section the cell body incorporates a series of peripherally arrayed holes being sufficiently threaded around their surfaces to engage lid screws 6.

35

The cell can be closed and/or sealed at its upper end by a cell lid 7 which also comprises a series of peripherally arrayed holes to allow the passage of the screws 6 that ensure that the lid is releasably attached to the body of the cell.

- 5 The material used for both the cell lid and the cell body is preferably stainless steel. The invention envisages that other materials may be used, for example selected from a range of plastics.

10 The cell lid also incorporates a centre hole appropriately shaped to receive the rod 5 accompanied by its rod clamp screw 9 or another appropriate holder. The rod clamp screw should be easily assembled and disassembled to and from the cell lid 7 in order to allow rapid access to the inner chamber 10 of the cell for maintenance or substitution purposes.

15 At the lower end of the cell, driving means 11 are provided to rotate the spindle 3 from top to bottom the spindle incorporates three diameters which diminish in size. The lower section of the spindle which has the smallest diameter engages with the driving means 11 and is retained between a throat 12 of the body.

20 The middle section of the spindle, abuts at its lower end against the throat 12 and its peripheral surface is enshrouded by the bottom spacer 13 around which the bottom bearing 14 is placed.

25 The upper section of the spindle interacts with the upper surface of the bottom bearing 14. In this upper section of the spindle a recess is provided whose side walls extend in the axial direction of the cell. This recess receives the lower portion of the tube 4. The tube is retained within the spindle's recess by a grub screw or alternative holder to enable the tube and the spindle to rotate together while the measurements are taken.

30 The measurements are taken through windows 20 appropriately placed in the cell's body.

The upper portion of the tube 4 is surrounded by a top spacer 16 and a top bearing 17 which are arranged to facilitate the rotation of the tube around the rod.

The material selected for both the rod and the tube is quartz. The invention also envisages the use of other UV, visible or infra-red transparent materials including CaF_2 or plastics. The use of CaF_2 is particularly advantageous for readings within the infra-red spectrum.

- 5 The selection for the materials of the other inner cell components was also an important consideration as these components are required to be immune to water and solvent fatigue. In conventional systems with similar requirements, the person skilled in the art would select among others nylon and glass bearings designed to run in a liquid environment. However, this type of bearing has a high noise level and variable rotation.
- 10 The person skilled in the art would also consider other polymer bearings, however these would in the specific environment of the present invention, break down in a short working time-span. In this context, stainless steel bearings which are notorious for not being one hundred per cent water and rust resistant and also having a high level of friction would be immediately disregarded. However it was found that the stainless steel bearings were best
- 15 suited for the invention's device as these are robust and not deformable.

Figure 2 presents a cell 21 which is mounted on a base plate 22. The cell is split into three sections: the cell lid 27, the cell body 22 and a motor housing 38. The cell of this embodiment can be easily adapted to allow the cell body 22 to separate from the motor

20 housing 38. As illustrated in the drawing the rotor shaft 23 incorporates at its lower section a flexible coupling means 39 which would easily permit the above-mentioned separation of the cell body from the motor housing. The shaft 23 engages a recess at the bottom of crystal 25 so that crystal 25 rotates together with shaft 23 when the drive means or the motor rotate the shaft. This embodiment shows a sleeve 24 arranged around the

25 periphery of crystal 25. In this configuration, when the crystal 25 is rotated, sleeve 24 is essentially immobilised.

The annular gap between crystal 25 and sleeve 24 is advantageously selected to be approximately 50 μm . This reduces the volume of the sample and improves the overall

30 analysis of the sample. Other volumes could be employed, however this value of annular gap is deemed to be particularly advantageous.

The preferred material employed for both the crystal and its sleeve is either quartz or calcium fluoride (CaF_2), though other transparent materials may be adopted depending on sample chemistry and radiation range used.

- 5 The lid 27 can be disassembled as was the case for lid 7 in Figure 1. This operation is carried out to permit access to the cell's inner chamber 30 for cleaning purposes and also for sample loading.

10 The insertion of the sample may be carried out manually with pipettes or any other method so appropriate.

The cell is also provided with a drain hole 40 which is used to evacuate the sample after its analysis and for cleaning purposes.

- 15 Since the samples in this embodiment come into contact with the inner walls of the cell body 22, the upper bearing 17 and lower bearing 14 are additionally protected by shaft seals 33 and 36.

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CLAIMS:

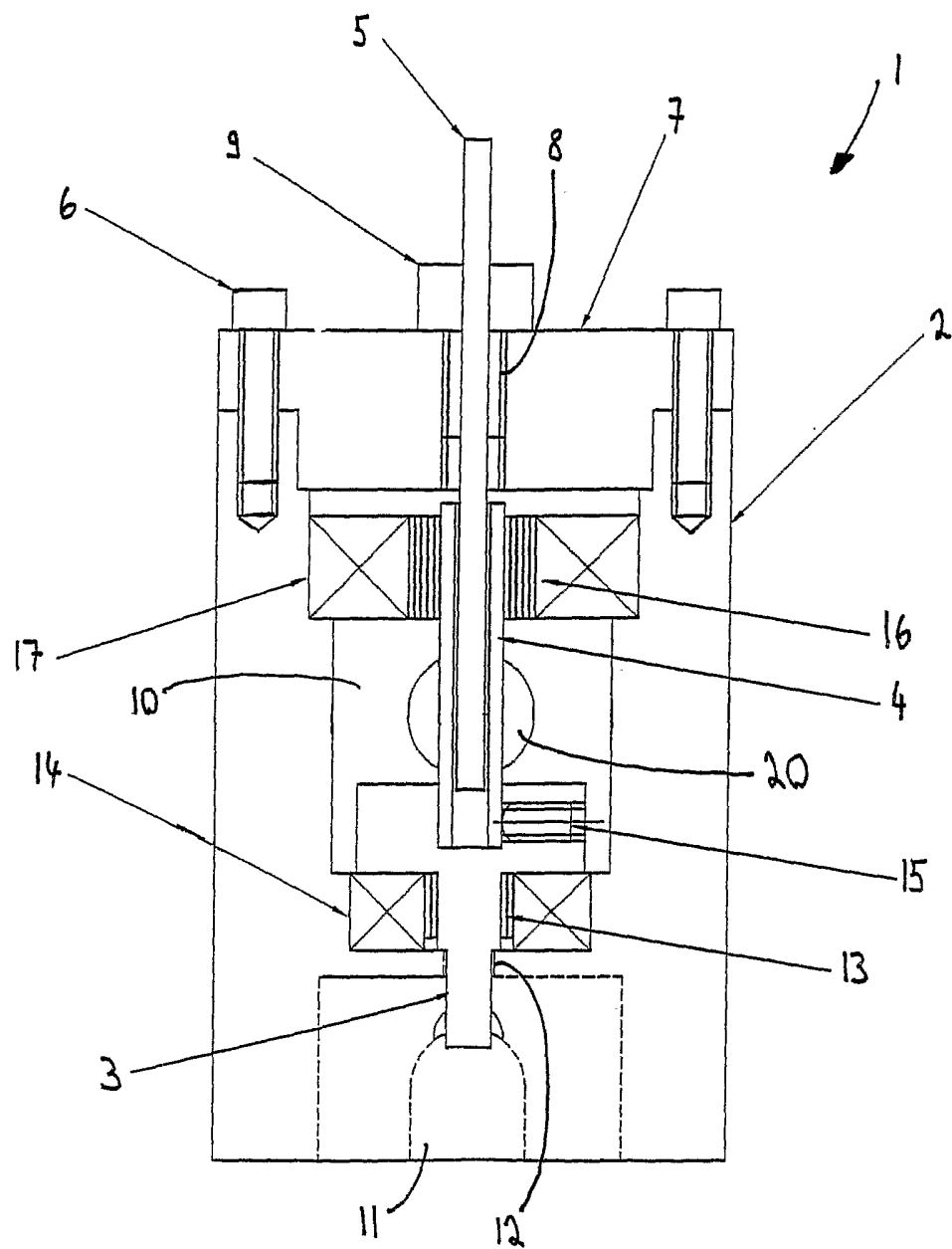
- 5 1. A device primarily for use in spectrophotometry, comprising one or more housings; one or more windows appropriately located in one or more walls of the or each housing; one or more tubes releasably or permanently mounted to a drive means; and one or more rods located partly or entirely within the or each corresponding tube when the device is in use, wherein said drive means are adapted to rotate the or each tube around its corresponding rod so that one or more measurements can be obtained for the or each sample inserted into the device.
- 10 2. A device according to Claim 1, wherein the or each tube is releasably mounted on the spindle.
- 15 3. A device according to either of Claims 1 and 2, wherein the or each rod is releasable without having to remove the housing top cover.
4. A device according to any preceding claim, wherein the or each rod is immobilised when the device is in use.
- 20 5. A device according to any preceding claim, wherein the drive means incorporates a spindle which is supported by at least one essentially horizontal surface and at least one essentially vertical surface of a corresponding bearing means.
- 25 6. A device according to Claim 5 incorporating respective upper and lower rotating bearings which remain undisturbed in the housing if and when the housing top cover is removed.
- 30 7. A device primarily for use in spectrophotometry, substantially as hereinbefore described with reference to and/or illustrated in any appropriate combination of the accompanying text and drawings.

8. A device primarily for use in spectrophotometry, comprising one or more housings; one or more windows appropriately located in one or more walls of the or each housing; one or more cylinder releasably or permanently mounted to a drive means; and one or more sleeves located partly or entirely around the or each cylinder when the device is in use, wherein at least the cylinder is a crystal of calcium fluoride (CaF_2), quartz, or other optically transparent material, as required, and said drive means is adapted to rotate the or each cylinder within the or each sleeve so that one or more measurements can be obtained for the or each sample inserted into the device.

9. A device according to Claim 8, comprising an annular gap between the or each cylinder and its corresponding sleeve of 200 μm or less.

10. A device according to Claim 8, comprising an annular gap between the or each cylinder and its corresponding sleeve of 50 μm .

11. An automated spectrophotometry-based sampling system incorporating a device according to any of the preceding claims.

FIGURE 1

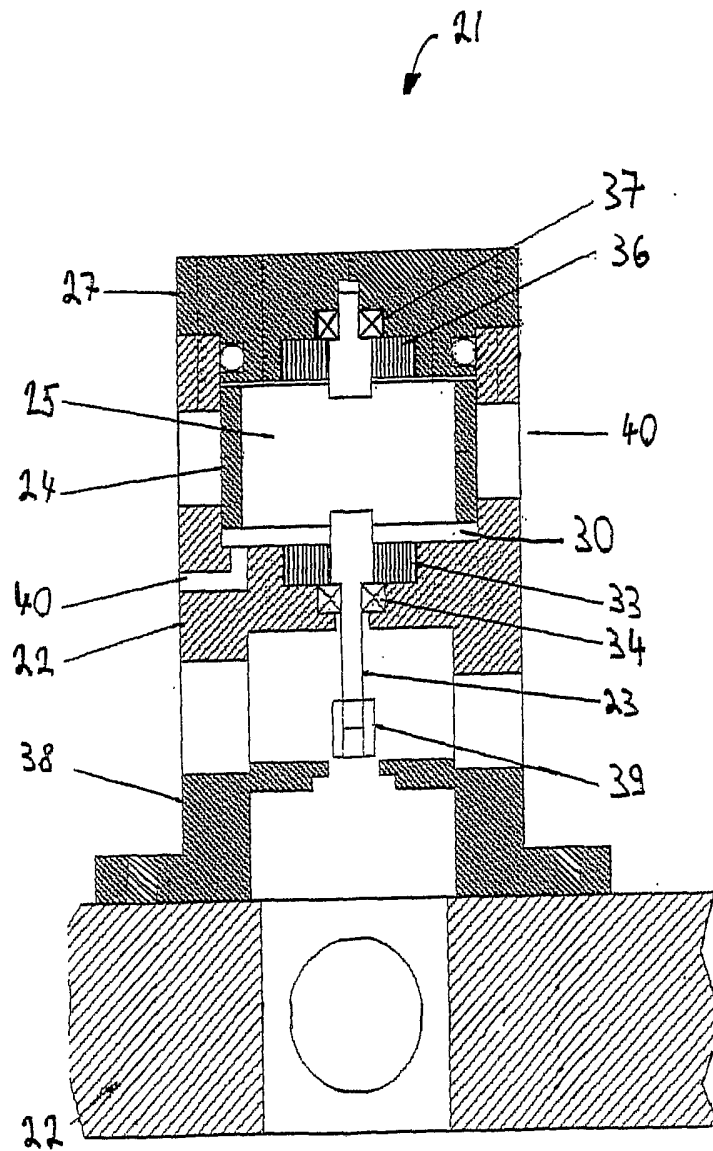


FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB 03/03227

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/19 G01N21/05

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)

IPC 7 G01N

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

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

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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X	RODGER A: "CIRCULAR DICHROISM AND LINEAR DICHROISM" ENCYCLOPEDIA OF ANALYTICAL CHEMISTRY, XX, XX, 2000, pages 10305-10333, XP001153376 paragraph '3.3.2!; figure 20 ----- -/--	1-11
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in 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
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- *O* document referring to an oral disclosure, use, exhibition or other means
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- *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

1 October 2003

Date of mailing of the international search report

14/10/2003

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 03/03227

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SEHLSTEDT U ET AL: "Interactions of the antiviral quinoxaline derivative 9-OH-B220 {2,3-dimethyl-6-(dimethylaminoethyl)-9-hydroxy-6H-indolo-2,3-biquinoxaline} with duplex and triplex forms of synthetic DNA and RNA" JOURNAL OF MOLECULAR BIOLOGY, LONDON, GB, vol. 278, no. 1, 24 April 1998 (1998-04-24), pages 31-56, XP004453976 ISSN: 0022-2836 page 52, column 2, line 12 - line 23 ---	1-11
P,X	WO 02 081744 A (DAFFORN TIMOTHY RICHARD ;UNIV CAMBRIDGE TECH (GB); RODGER ALISON) 17 October 2002 (2002-10-17) page 10, line 18 -page 12, line 14; figure 7 page 21, line 1 - line 21 -----	1-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 03/03227

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 02081744 A	17-10-2002	WO 02081744 A2	17-10-2002