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(54) Title: OCULAR IRRIGATING SOLUTION

(57) Abstract

There is disclosed an ocular irrigating solution for irrigating the eye during surgery comprising a source of bicarbonate ions an d a physiologically acceptable organic buffer which is an organic zwitterionic buffer having a buffering capacity within the range pH 6.8 to 8.0.

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OCULAR IRRIGATING SOLUTION

This invention relates to aqueous solutions for use in surgical procedures, and is particularly concerned with an ophthalmic irrigating solution useful for irrigating the human eye during surgery.

A description of the problems associated with surgical procedures, especially surgical procedures performed on the eye, and the historical development of tissue irrigating solutions may be found in EP-A-0076658.

The stated object of EP-A-0076658 is to provide a stable sterile ophthalmic irrigating solution which, in addition to correct electrolyte balance, provides factors necessary for continued metabolism in the endothelial cells, maintenance of the fluid transport pump system, and consequential maintenance of proper corneal thickness and clarity. This problem is stated to be achieved in EP-A-0076658 by providing a two-part solution system which includes a basic solution and an acidic solution which are individually stable and which, on mixing, form an ocular solution which contains the necessary factors to maintain endothelial cell integrity and corneal thickness during ocular surgery. The combined solution contains the necessary ions in a bicarbonate-phosphate buffer as well as oxidised glutathione and dextrose (d-glucose), the latter being present as an energy source.

There are problems associated with the solution system of EP-A-0076658. Firstly, such a system is relatively expensive because two separate solutions must be prepared and separately sterilised; this problem is not easy to overcome because certain of the ingredients of the system, particularly the oxidised glutathione and the glucose, are heat-labile and cannot therefore be sterilised by an autoclaving procedure as required by various regulatory authorities for

solutions exceeding about 500ml in volume which are to be used in surgical procedures. As a consequence, the two-part system of EP-A-0076658 is prepared, in practice, such that the non-labile components are present in the solution which contains the majority of the fluid which will form the final ocular solution, which is then bottled and autoclaved. The labile components are contained in the other solution of relatively small volume (below the threshold above which autoclaving is required) which may be sterilised by a filtration technique.

A second problem with the solution system of EP-A-0076658 is that its two-part nature can potentially lead to errors in forming the final ocular solution, a procedure which is normally conducted in a hospital.

HEPES has been proposed, in the 1980 article "Intraocular irrigating and replacement fluid", M.V. Graham et al, Trans. Ophthal. Soc. U.K. (1980) 100, p282-285, as a buffer for an intraocular irrigating solution. However, the 1983 article, "A Comparison of HEPES and Bicarbonate Buffered Intraocular Irrigating Solutions: Effects on Endothelial Function in Human and Rabbit Corneas", by Dayle H. Geroski et al, J. Toxicol - Cut & Ocular Toxicol 1(4), 299-309, (1982-83) concludes that HEPES is toxic to endothelial Na^+K^+ATPase and questions the prudence of using HEPES buffer in intraocular irrigating solutions.

It would be an advantage to provide a stable ophthalmic irrigating solution as a single solution capable of being sterilised by autoclaving.

It has now been found that a solution which is effective as an ophthalmic irrigating solution can be formed which does not require the glutathione ingredient previously believed to be essential, but does include a specific buffer to ensure that the

proper pH is maintained prior to and during use.

Thus, according to a first aspect of the present invention there is provided an ocular irrigating solution for irrigating the eye during surgery comprising, a source of bicarbonate ions and a physiologically acceptable organic buffer which is an organic zwitterionic buffer having a buffering capacity within the range pH 6.8 to 8.0.

The organic buffer preferably maintains the solution at a pH in the range 7.2 to 7.8 to match the physiological pH of 7.4.

Highly preferred as the organic buffer are the zwitterionic amino acids, such as N-2-[hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid], commonly referred to as HEPES, which has a pKa of 7.55 at 25°C. Other organic buffers in this family are N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid (BES), pKa=7.1; 3-[N-morpholino]propanesulfonic acid (MOPS), pKa=7.2 at 25°C; N-tris[hydroxymethyl]methyl-2-aminoethanesulfonic acid (TES), pKa=7.4 at 25°C; N-[2-hydroxyethyl]-piperazine-N'-[3-propanesulfonic acid] (EPPS), pKa=8.0 at 25°C; N-tris[hydroxymethyl]methyl-glycine (TRICINE), pKa=8.1 at 25°C.

The organic buffer should be present in the solution in an amount sufficient to buffer the solution over the duration of the surgical procedure. In practice, this means that the concentration of the buffer should be about 10 to 50 mmol/l.

The bicarbonate source is normally sodium bicarbonate. The bicarbonate source is preferably present in the solution to give a bicarbonate concentration of about 10 to 50 mmol/l, preferably from 15 to 25 mmol/ml to maintain the fluid pump system in the endothelium of the eye.

The ocular irrigating solutions of the present invention are preferably free from glutathione, which

has previously been considered essential for effective performance.

Hitherto it has been considered essential for ocular irrigating solutions to contain an energy source which is purportedly required as a substrate for the various metabolic pathways taking place in the cornea. It has now surprisingly been discovered that ocular irrigating solutions which are free from an energy source (such as glucose) are capable of supporting endothelial function and maintaining corneal thickness as well as solutions containing the energy source. Thus, irrigation solutions of the invention need not contain an energy source. This is of particular significance so far as glucose is concerned which tends to degrade at physiological pH over extended time periods. Therefore, preferred ocular irrigation solutions of the present invention do not contain glucose, or any other energy source which tends to degrade at physiological pH over extended time periods. If an energy source is to be present in an irrigation solution of the invention, a typical concentration is 2-10 mmol/l.

The solution of the invention preferably also contains other electrolytes necessary to maintain physiological function, such as Na^+, K^+, Ca^{2+}, and Cl^-, but not Mg^{2+}, which can lead to the formation of magnesium precipitates in some circumstances. These should be present at concentrations which will permit continued cellular integrity and metabolism. Typically, these electrolytes are present in the following concentrations:

Na^+	130 - 180 mmol/l
K^+	3 - 10 mmol/l
Ca^{2+}	up to 5 mmol/l
Cl^-	130 - 210 mmol/l

Preferably the concentration of Ca^{2+} is at least 0.05 mmol/l, and preferably no more than 0.1 mmol/l.

Moreover, the osmolality should be between approximately 250 - 350 mosmol/kg, preferably 290 - 320 mosmol/kg, to maintain osmotic stability of the cells.

Also normally present in the solution will be a source of phosphate ions, although primarily not for buffering purposes, as in EP-A-00766598, but for normal physiological function. The approximate concentration of phosphate in the solution is normally about 1 mmol/l.

The solution of the invention may be prepared by mixing the components together in aqueous solution, in the desired proportions. It may then be bottled and autoclaved in the normal manner.

One advantage of the invention is that it may be autoclaved without any deleterious effect. For this reason, components which would degrade to a significant extent under the chosen autoclave conditions should be excluded or reduced in amount to a point at which degradation is minimal. Typical autoclave conditions are 121°C for 15 minutes or 134°C for 3 min.

The ocular solution of the invention should preferably be free from nutrients of the type normally present in tissue culture media, namely: amino acids, vitamins, hormones, proteins, growth factors, lipids, nucleosides, minerals.

The solution of the invention may be used in a method of surgery performed on the human eye to replace fluid loss during the operation and to maintain corneal function. Thus according to another aspect of the invention, there is provided an aqueous solution, comprising a source of bicarbonate ions and a physiologically acceptable organic buffer which is an organic zwitterionic buffer having a buffering capacity within the range pH 6.8 to 8.0, for use in a surgical

method, preferably a surgical method performed on the eye.

The invention will now be illustrated by reference to the following Example and drawings in which:

Figure 1A shows the change in corneal thickness during assessment perfusion following 90 minutes exposure to the "UB-M2" solution in accordance with the invention and "BSS Plus";

Figure 1B shows the change in corneal thickness during perfusion with a solution in accordance with the invention "UB-M2" solution and with "BSS Plus".

Example 1

A prior art irrigating solution and an irrigating solution in accordance with the invention were tested in a masked laboratory experiment to evaluate their effectiveness. BSS Plus (which is in accordance with EP-A-0076658) was obtained as a two part system and made up as directed. The composition of these solutions along with those of aqueous humour and BSS are shown in Table 1.

Table 1

	Aqueous humour	BSS	BSS Plus	Invention
Na^+ (mM)	162.9	144.0	160.0	137.2
K^+ (mM)	2.2-3.9	10.0	5.0	5.4
Ca^{2+} (mM)	1.8	4.3	1.0	0.075
Mg^{2+} (mM)	1.1	3.2	1.0	-
Cl^- (mM)	131.6	127.2	130.0	121.2
HCO_3^- (mM)	20.2	-	25.0	20.0
HPO_4^{2-} (mM)	0.6	-	3.0	0.8
SO_4^{2-} (mM)	-	-	-	-
Acetate (mM)	-	28.6	-	-
Citrate (mM)	-	5.8	-	-
Lactate (mM)	2.5-4.5	-	-	-
Glucose (mM)	2.7-3.7	-	5.0	-
Glutathione	1.9 μM	-	0.3 mM	-
HEPES (mM)	-	-	-	20.0
Osmolality (mosmol/kg)	304	302	305	320
pH (20°C)	7.4	7.3	7.4	7.4

Corneas obtained from New Zealand White rabbits (3-4 kg) after an intravenous overdose of pentobarbitone sodium were secured on support rings and perfused as described in J. Physiol 1972; 221: 29-41, "The metabolic basis to the fluid pump in the cornea", Dikstein S. and Maurice DM. The paired corneas from each rabbit were randomly allocated, one to BSS Plus and one to the invention. The allocation was unknown to the person performing the experiment. The epithelial surface was covered with silicone oil to prevent changes in corneal thickness owing to evaporation.

The endothelial surface was perfused at 2.5 ml/h, a pressure of 15 cm H_2O and 35°C. During the first 90 minutes of perfusion, corneas were exposed to the intraocular irrigation solution. This was followed by a further 6 hours of perfusion during which endothelial

function was assessed.

Corneal thickness was measured with an ultrasonic pachymeter (DGH Technologies, Inc), every 30 minutes. The silicone oil was removed briefly to allow the measurements to be made. Each measurement was the mean of readings taken at four different sites of the central cornea.

Changes in corneal thickness during perfusion for 90 minutes with the irrigation solutions are shown in Figure 1A.

Corneal hydration and, thus, thickness are controlled by the endothelium through a pump leak mechanism. Removal of bicarbonate ions from the perfusate suppresses endothelial pump function and causes corneal swelling, although inhibition of the pump is not complete unless CO_2 is also removed from the perfusate. Pump function can be restored and the swelling reversed by returning bicarbonate to the perfusate.

After the 90 minute perfusion with one of the irrigation solutions, endothelial function was, therefore, assessed during a further 6 hours of perfusion with Tissue Culture Medium 199 (TC199). The first 2 hours of perfusion were with TC199 with Earle's salts (Sigma, M3769). This solution contained sodium bicarbonate (26 mmol/l), and should have supported endothelial pump function. Two hours of perfusion with TC199 with Hanks' salts (Sigma, M3274) then followed. This solution did not contain bicarbonate ions and, thus, should have caused corneal swelling, although the solution was not CO_2 free. For the final 2 hours, perfusion with TC199 Earle's was restored and, providing that the endothelium was undamaged, corneas should have thinned. Neither of the TC199 solutions contained phenol red, and their measured osmolalities (Roebling osmometer) were 290 and 288 mosmol/kg,

respectively, for TC199 with Earle's salts and TC199 with Hanks' salts.

Rates of change in corneal thickness both during perfusion with the irrigation solutions and during the three parts of the assessment perfusion were determined by regression analysis. Comparisons were made between groups by t-tests at the 5% level of significance. The results obtained are shown in Table 2 and are also illustrated graphically in Figure 1A.

Table 2

Irrigation solution[a]	Initial 90 min exposure to irrigation solution	Rate of change in corneal thickness (μm/h)[b] TC199 Earle's 0-2 h	TC199 Hank's[c] 2-4 h	TC199 Earle's 4-6 h
BSS Plus	-5.1(4.3)	+0.02(4.2)	+16.1(1.9)	-13.1(5.3)
Invention	-8.4(3.5)	+1.4 (4.7)	+17.7(2.0)	-13.4(3.7)

[a]Corneas were perfused for 90 minutes with an irrigation solution before the assessment perfusion with TC199.

[b]regression coefficient (SD), n=4: + indicates swelling, - indicates thinning.

[c]TC199 Hanks' does not contain HCO_3^-.

There were no differences at the 5% level of significance in rates of change in thickness between corneas exposed to BSS Plus and those exposed to the irrigating solution in accordance with the invention at any stage of the perfusion.

Example 2

An ocular irrigating solution in accordance with the invention was made up as in Example 1.

Corneas were dissected, mounted on support rings and perfused as in Example 1 except that the corneas were perfused continuously for a period of 7.5 hours with either BSS Plus or the invention. Paired corneas, from a single rabbit, were perfused, one with BSS Plus and the other with the invention. The allocation of

corneas to each solution was randomized and masked from the person performing the perfusion. Regression analysis showed no overall change (at the 5% level of significance) in thickness during the course of the perfusion nor was corneal thickness influenced by the type of irrigation solution (see Figure 1B).

In conclusion, Examples 1 and 2 demonstrate that the invention supports endothelial function at least as well as BSS Plus, despite the absence of components, such as glucose and glutathione, that are considered essential constituents of BSS Plus.

CLAIMS

1. An ocular irrigating solution for irrigating the eye during surgery comprising, a source of bicarbonate ions and a physiologically acceptable organic buffer which is an organic zwitterionic buffer having a buffering capacity within the range pH 6.8 to 8.0.

2. An ocular irrigating solution according to claim 1, wherein the organic buffer maintains the solution at a pH in the range 7.2 to 7.8.

3. An ocular irrigating solution according to claim 1 or 2, wherein the organic buffer is a zwitterionic amino acid.

4. An ocular irrigating solution according to claim 3, wherein the organic buffer is N-2-[hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid].

5. An ocular irrigating solution according to any preceding claim, wherein the concentration of the buffer is from 10 to 50 mmol/l.

6. An ocular irrigating solution according to any preceding claim, wherein the bicarbonate source is sodium bicarbonate.

7. An ocular irrigating solution according to claim 6, wherein the bicarbonate source is preferably present in the solution to give a bicarbonate concentration of about 10 to 50 mmol/l.

8. An ocular irrigating solution according to any preceding claim which does not contain glucose, or any other energy source which tends to degrade at physiological pH over extended time periods.

9. An ocular irrigating solution according to any preceding claim having been sterilised by an autoclaving procedure.

10. An ocular irrigating solution according to claim 1, for use in a surgical method performed on the eye.

11. A method of surgery performed on the human eye in which an ocular irrigating solution according to any one of claims 1 to 9 is employed to replace fluid loss during the operation and to maintain corneal function.

12. An ocular irrigating solution substantially as hereinbefore described, with reference to the accompanying examples.

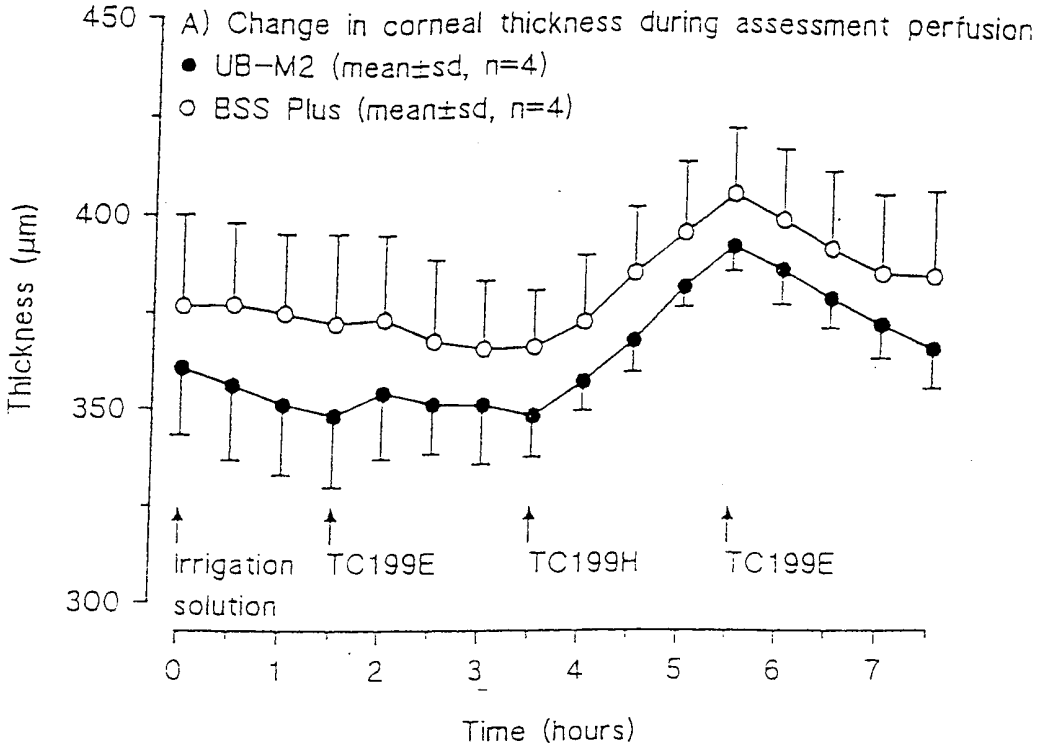


Figure 1A

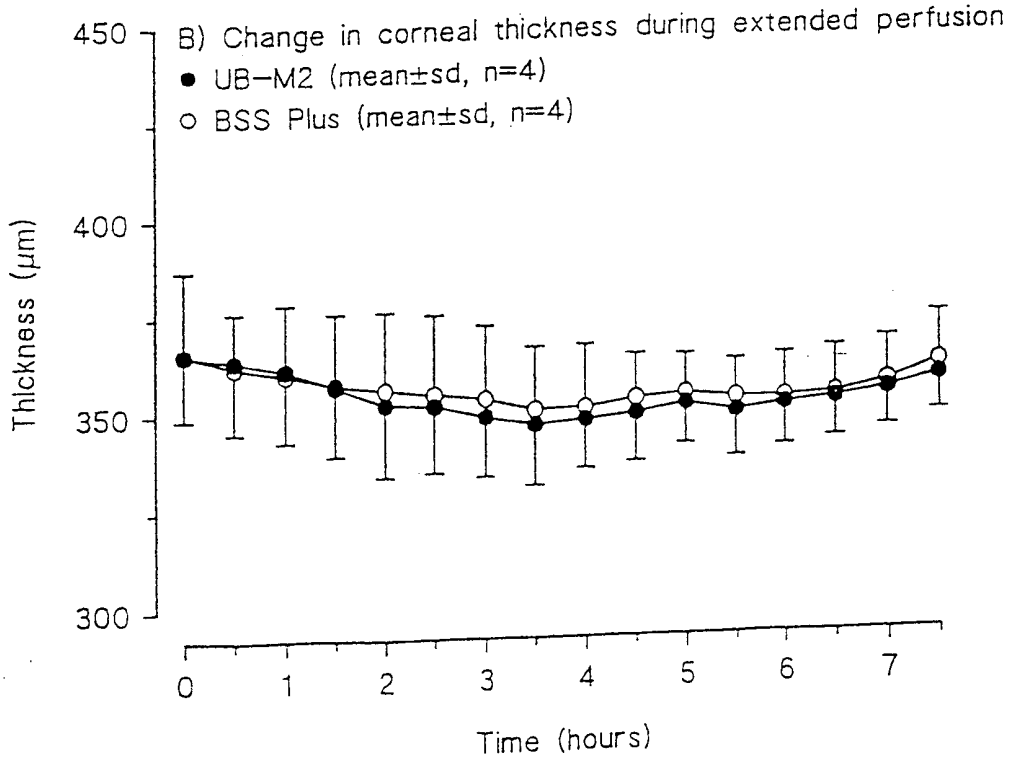


Figure 1B

INTERNATIONAL SEARCH REPORT

In ıtional Application No
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A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/08

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 6 A61K

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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 725 586 A (LINDSTROM ET AL.) 16 February 1988 (1988-02-16) column 1, line 10 - line 30 column 3, line 18 - line 36 column 4, line 8 - line 40 ---	1-12
X	EP 0 435 797 A (ANBEN) 3 July 1991 (1991-07-03) the whole document ---	1-3,5-12
X	FR 2 602 677 A (BLOMET) 19 February 1988 (1988-02-19) page 8; examples 2,3 ---	1-3
A	EP 0 778 021 A (TAISHO PHARMACEUTICAL CO. LTD) 11 June 1997 (1997-06-11) the whole document --- -/--	1-3,5-12

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

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"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	Date of mailing of the international search report
26 July 1999	30/07/1999

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INTERNATIONAL SEARCH REPORT

In ıtional Application No
PCT/GB 99/01066

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 064 320 A (WELSH NATIONAL SCHOOL OF MEDICINE) 17 June 1981 (1981-06-17) the whole document	1-12
A	DE 196 26 479 A (SCHRAGE) 8 January 1998 (1998-01-08) the whole document	1-12

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INTERNATIONAL SEARCH REPORT

.ernational application No.
PCT/GB 99/01066

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 11
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 11 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In ational Application No
PCT/GB 99/01066

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