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(56) Fremdragne publikationer:
ÜNLÜ K ET AL.: "Gentian violet solution for staining the anterior capsule", J CATARACT REFRACT SURG, Bd. 26, 1. August 2000 (2000-08-01), Seiten 1228-11232, XP002605866, & ALCON LABORATORIES: "BSS Plus", , Gefunden im Internet: URL:http://ecatalog.alcon.com/pi/BSSplus25 0_us_en.pdf> [gefunden am 2010-10-08]
EP-A1- 1 132 065
WO-A1-2004/035091
WO-A1-2010/060018
WO-A2-03/057259
US-A1- 2003 097 117
ÜNLÜ K ET AL.: "Gentian violet solution for staining the anterior capsule", J CATARACT REFRACT SURG, Bd. 26, 1. August 2000 (2000-08-01), Seiten 1228-11232, XP002605866, & ALCON LABORATORIES: "BSS Plus", , Gefunden im Internet: URL:http://ecatalog.alcon.com/pi/BSSplus25 0_us_en.pdf> [gefunden am 2010-10-08]
DE PAULA FIOD COSTA, E. ET AL.: 'Vital dyes and light sources for chromvitrectomy: comparative assessment of osmolarity, PH and spectrofotometry', [Online] 08 August 2008, Te Association for Research in Vision and Ophthalmology Gefunden im Internet: <URL:www.iovs.org/content/early/2008/08/08/ iovs.08-2285.pdf> [gefunden am 2013-04-02]
LECHNER, T AND MÜLLER B.K: 'Determination of the density of various dye solutions at 25Â°C and 35Â°C' 05 April 2013, Seiten 1 - 3
LESNICK-OBERSTEIN, S.Y ET AL.: 'IMPROVING THE STAINING CHARACTERISTICS OF TRYPAN BLUE IN

Fortsættes ...

VITREo-retinal surgery' INVESTIGATIVE OPHTHALMOLOGY AND VISUAL CCIENCE, [Online] Bd. 45, Nr. E-ABSTRACT 1984, 01 Januar 2004, ARVO
Peter Stalmans ET AL: "Toxic effect of indocyanine green on retinal pigment epithelium related to osmotic effects of the solvent", American Journal of Ophthalmology, vol. 134, no. 2, 1 August 2002 (2002-08-01), pages 282-285, XP055187000, ISSN: 0002-9394, DOI: 10.1016/S0002-9394(02)01468-X
ÜNLÜ K ET AL.: "Gentian violet solution for staining the anterior capsule", J CATARACT REFRACT SURG, vol. 26, 1 August 2000 (2000-08-01), pages 1228 - 11232, XP002605866
ALCON LABORATORIES: "BSS Plus", Retrieved from the Internet <URL:http://ecatalog.alcon.com/pi/BSSplus250_us_en.pdf> [retrieved on 20101008]
DE PAULA FIOD COSTA, E. ET AL.: "Vital dyes and light sources for chromvitrectomy: comparative assessment of osmolarity, PH and spectrofotometry", - 8 August 2008 (2008-08-08), Retrieved from the Internet <URL:www.iovs.org/content/early/2008/08/08/iovs.08-2285.pdf> [retrieved on 20130402]
LECHNER, T AND MÜLLER B.K: "Determination of the density of various dye solutions at 25Â°C and 35Â°C", 5 April 2013 (2013-04-05), pages 1 - 3
LESNICK-OBERSTEIN, S.Y ET AL.: "IMPROVING THE STAINING CHARACTERISTICS OF TRYPAN BLUE IN VITREo-retinal surgery", INVESTIGATIVE OPHTHALMOLOGY AND VISUAL CCIENCE, vol. 45, no. E-ABSTRACT 1984, - 1 January 2004 (2004-01-01)
PETER STALMANS ET AL: "Toxic effect of indocyanine green on retinal pigment epithelium related to osmotic effects of the solvent", AMERICAN JOURNAL OF OPHTHALMOLOGY, vol. 134, no. 2, 1 August 2002 (2002-08-01), pages 282 - 285, XP055187000, ISSN: 0002-9394, DOI: 10.1016/S0002-9394(02)01468-X
DR. M.K. SCHMID: "A new Method to Improve the Application of Dyes on the Retinal Surface Without Fluid-Air Exchange", VIDEO PRESENTATION EVRS CONGRESS IN PRAGUE, 9 September 2008 (2008-09-09)
Transcript of the presentation by Martin K Schmid
LECHNER, T AND MÜLLER B.K: 'Determination of the density of various dye solutions at 25°C and 35°C' 05 April 2013, Seiten 1 - 3

Dye solution

Field of the invention

The invention relates to a water-based biocompatible preparation for the in vivo selective staining of the internal limiting membrane (ILM) and/or of epiretinal membranes (ERM) in the human or animal eye according to claim 7.

The invention further relates to a water-based biocompatible preparation for use in surgical treatment on the eye, comprising the selective staining of the ILM and/or ERM in the human or animal eye and removal of the stained membrane according to claim 1

Background of the invention

Disorders of the eye, such as grey star (cataract), glaucoma (green star), age-related macular degeneration and diabetes-related retinopathy and also retinal changes or retinal detachment are on the increase, partly due to increased life expectancy. A vitrectomy is often indicated for the treatment of this and other diseases of the eye. In this case, it must be ensured that the retina is damaged as little as possible. In vitrectomy, a precautionary measure consists of removal of the internal limiting membrane (ILM) and, if appropriate, epiretinal membranes from the retina in order to relieve the macula of the suspected intravitreal tensile forces. For this purpose, the membranes are peeled off from the retina using tweezers. It is necessary that the surgeon can accurately distinguish as far as possible between the retina and membrane to be peeled off. For this purpose, the membranes to be peeled off should be made visible by as specific a staining as possible. Dyes suitable for staining have to meet many criteria. They must be biocompatible and non-toxic and must not damage the cells, they should be water-soluble, they should stain as specifically as possible and be easily rinsed off again. Dyes and methods of staining of the membranes mentioned have already been described but they are not yet completely satisfactory.

For instance, US 7,014,991 describes a method for staining ocular structures in the human eye, wherein the staining is carried out by injecting the dye indigotindisulphate into the relevant tissue. However, indigotindisulphate is cytotoxic.

Other dyes, such as Brilliant Blue G, Brilliant Blue R, Patent Blue V or methylene blue have also been proposed for use in the eye.

WO 03/057259 describes solutions which contain indocyanine green and are used for vascular imaging.

US 2003/0097117 describes devices for introducing dye solutions into the eye.

EP 1132065 describes stained viscoelastic compositions which may be used in eye operations as auxiliaries, e.g. to protect intraocular tissue, as placeholder or to facilitate intraocular manoeuvres, for example in a controlled capsulorhexis.

Ünlü et al. (2000), J. Cataract Refract. Surg. 26(8), 1228-1232 describes the use of the dye gentian violet to stain the anterior lens capsule in a cataract operation.

WO 2004/035091 describes dyes, in particular triphenylmethane dyes, for staining tissues, particularly membranes in the eye, for example the ILM.

WO 2010/060018 describes dyes for phototherapy.

Costa et al. (2009), Invest. Ophthalmol. Vis. Sci. 50(1), 385-391 describes solutions of water, BSS or 5% glucose, in which dyes have been dissolved, wherein the solutions are investigated in respect of in vitro aspects such as pH, osmolarity or photostability.

WO 2006/062233 describes Brilliant Blue G dyes as auxiliaries for staining the ILM and the lens capsular bag in eye operations.

Lesnik-Oberstein et al. (2004), ARVO, Invest. Ophthalmol. Vis. Sci., 45, E-Abstract 1984 describes a solution of trypan blue with 2.5% or 5% added glucose which may be used for staining the ERM in surgical procedures. Lesnik-Oberstein et al. (2007), Br. J. Ophthalmol. 91, 955-957 also describes a trypan blue solution with added glucose to stain the ERM, whereby carrying out air-fluid exchange (AFX) before the staining can be omitted.

WO 99/58159 describes various dyes, e.g. gentian violet or trypan blue, as auxiliaries in eye operations.

Rodrigues et al. (2005), Ophthalmologica 219, 251-262 describes indocyanine green solutions for use as stains in the treatment of macular holes and proposes adding a viscoelastic material or perfluorinated carboxylic acids to these solutions in order to prevent escape of the dye so that it remains in the solution.

Stalmans et al. (2002), American J. Ophthalmol., 134(2), 282-285 describes solutions which contain the dye trypan blue and 5% glucose and which can be used to stain the ILM.

During vitrectomy or a surgical procedure, the eye cavity is rinsed with a rinsing solution. One problem with the previously known dye solutions consists in the fact that the dye solution is distributed, diluted and rinsed out by the rinse solution. This has several disadvantages. Firstly, the view of the surgeon is marred if the rinse solution is coloured. Secondly, more dye solution is required than would be required only to stain the membrane.

In order to overcome this disadvantage, it has already been proposed to add a thickener to the dye solution, hyaluronic acid for example, which increases the viscosity of the dye solution. The increase in viscosity should cause the dye, due to reduced mobility, that is due to steric hindrance, not to transfer so readily in the rinse solution and to be more likely to reach the vicinity of the membrane to be stained. However, the high viscosity of the dye solution means that the dye can now only transfer poorly from the solution to the membrane such that the requirement for dye solution is once again higher than the amount which would be required only to stain the membrane.

The object of the invention, therefore, was to provide a preparation which can specifically stain membranes, in particular can selectively stain the membranes to be removed such as the internal limiting membrane (ILM) and/or epiretinal membranes (ERM) in the human or animal eye, which can be readily administered, migrates immediately after administration to the membrane and is distributed there, without excessively colouring the rinse solution. Furthermore, a preparation should be provided which neither leads to local irritation nor damage to the retina, and is non-cytotoxic, but is well tolerated.

This object is achieved by a preparation or a preparation for use in surgical treatment of the eye, as defined in the claims, especially in claim 1.

The dependent claims contain advantageous developments.

Detailed description of the invention

It has been found, surprisingly, that a preparation containing at least one dye selected from triphenylmethane dyes permits an effective and selective staining of the ILM and/or the ERM when the density of the preparation is adjusted in the range of 1.01 g/cm^3 to 1.5 g/cm^3,

preferably 1.01 g/cm^3 to 1.3 g/cm^3, wherein for adjusting the density no monosaccharide or reducing disaccharide is used, wherein the agent is a non-reducing disaccharide, a polysaccharide or a neutral polymer or a combination thereof, and wherein the osmolarity of the prepared solutions is in the range of 280-330 mosmol/L.

It has been found that a dye solution, having a higher density than water, sinks when it is injected into the region of the eye cavity in the context of a surgical treatment on the eye, whereby a rapid mixing with the rinse solution is avoided and it is distributed on the membrane after sinking and stains these. This prevents the dye from being rinsed away too quickly, and also the dye from marring the field of view.

The preparation according to the invention is based on water as solvent, wherein further solvents may optionally be present in relatively small amounts provided that they are homogeneously miscible with water and are biologically compatible. Suitable here are monohydric and polyhydric alcohols as are also used in the medical field. If another solvent is used, this is particularly preferably a glycol or glycerol. Mixtures of the solvents mentioned can also be considered. If a solvent is mixed with water, this should be used in a proportion of not more than 20% by weight, more preferably not more than 10% by weight. The preparation is preferably an isotonic solution.

Apart from water as solvent and the dye, which is explained in more detail below, the preparation according to the invention contains a density adjusting agent as essential constituent. The density adjusting agent must be biologically compatible, must be non-toxic and must be homogeneously miscible with water, optionally after addition of a small amount of a solubilizing agent such as alcohol, such that a clear transparent solution is formed. In addition, it must be compatible with the dye, i.e. it must not impair the solubility of the dye to a significant extent. When adjusting the preparation, the osmolarity is also to be considered in order not to cause damage to the tissue by osmosis. The osmolarity is in a range of 280-330 mosmol/l, preferably 300 mosmol/l.

Suitable liquids compatible with water are therefore those whose density is above the density of water.

An agent with which the density may be adjusted is a polysaccharide. Polysaccharides are suitable for increasing the density and are readily available. In addition, they are toxicologically safe and biologically compatible. Polysaccharides are here understood to mean

molecules which are composed of more than two, preferably more than 5, particularly preferably more than 10, saccharide units. Although generally monosaccharides and disaccharides can increase the density, only non-reducing disaccharides are used for increasing the density in accordance with the invention. The use of monosaccharides and reducing disaccharides can lead to undesired effects; for instance these can be cytotoxic, for example, in the amount required for increasing the density. Suitable non-reducing disaccharides according to the invention are sucrose or trehalose. Soluble starch derivatives such as hydroxyethyl starch and dextran may be mentioned as suitable polysaccharides. Suitable polysaccharides are those compounds which are neutral, have no reducing effects and do not degrade in aqueous solution.

Further agents for adjusting the density are neutral polymers such as polyether, polyvinyl alcohol, polyester, polyacrylic acid copolymer, polyvinylpyrrolidone.

Combinations of the agents mentioned are also very suitable to adjust the density of the preparation according to the invention.

The density of the preparation can be determined here by any conventional method which is generally known to those skilled in the art.

It has been found that increasing the density to 1.01 g/cm^3 already has the desired effect, namely that the dye solution rapidly sinks downwards following administration into the eye cavity and can then be distributed there on the membrane. This achieves a selective staining of the membrane without impairing the view of the operator. A density difference of less than 0.01 g/cm^3, based on water, is no longer sufficient to allow the dye preparation specifically to sink. The sinking then takes place as slowly as in the preparations of the prior art and leads to the problems listed above. If the density of the preparation is greater than 1.5 g/cm^3, it can lead to damage of the very sensitive retina due to the density.

Another important constituent of the preparation according to the invention is the dye which is a triphenylmethane dye. The dye used can be those compounds which can specifically and selectively stain the ILM and/or ERM such that the membrane is differentiated optically from the retina. Furthermore, the dye must be soluble in water or the mixture of water and a further solvent. It must neither be toxic, especially cytotoxic or damaging to cells, nor cause damage to the retina or develop toxic effects due to light reactions as in the case of ICG or

trypan blue. In addition, it should have a good staining ability in order to be able to keep the amount of dye low.

Proven to be advantageous are dyes from the group of the triphenylmethane dyes such as Brilliant Blue G, Brilliant Blue R, Brilliant Blue FCF, Patent Blue V, Bromophenol Blue, Lissamine Green SF, Lissamine Green G, Fast Green, Methyl Green, Brilliant Acid Green, Coomassie Violet R 200, Rosaniline.

Preference is given to using Brilliant Blue G, Brilliant Blue R, Brilliant Blue FCF, Patent Blue V, Methyl Green, Coomassie Violet R 200, Bromophenol Blue and/or Chicago Blue. Of the triphenylmethane dyes, particular preference is given to Brilliant Blue G, Coomassie Violet R 200 and Chicago Blue. Of the Brilliant Blue dyes, preference is given to Brilliant Blue G owing to its particularly good staining capacity. It can be used at a concentration of less than 0.3 g/l. This low concentration already leads to a sufficiently selective staining of ILM and/or ERM.

The preparation according to the invention preferably additionally contains a dye selected from the group consisting of azo dyes, cyanine dyes and/or natural dyes or mixtures thereof.

In order to further improve the advantageous properties of the preparation according to the invention, a viscosity adjusting agent can also be added to the preparation. It has been shown that the addition of an agent that increases the viscosity of the preparation according to the invention can result in an improvement of the cohesiveness such that the advantages obtained with the preparation according to the invention are again enhanced. The administered preparation sinks more rapidly due to the higher density and is distributed in the rinse solution to an even lesser degree since it is held together due to the increased viscosity until it impinges on the membrane. Since an advantageous effect is already achieved by adjusting the density, the viscosity does have to be increased so much that it leads to problems as exist in the prior art. Even a small increase in viscosity causes the drops emerging from the application device to form a more stable unit and they are therefore less easily able to be diluted, which prevents the dye incorporated in the preparation from being flushed out. Thus the dye is released only at the administration site by capillary effects at the membrane which is thereby stained. In this way, the dye can be brought specifically to the membrane.

As viscosity-regulating biocompatible agents, that is agents which adjust the viscosity, one or more from the following group may be used: polyether, polyvinyl alcohol, polyester, polyacrylic acid copolymer, polyvinylpyrrolidone and other polymers, polyhydric alcohols such

as glycerol, propylene glycol, butylene glycol, water-soluble cellulose derivatives such as methylcellulose, xanthan gum, starch, hyaluronic acid and respective derivatives thereof, chondroitin sulphate and sodium sulphate. The viscosity regulators used can also be those which not only increase the viscosity but also increase the density at the same time. In this case it is important to ensure that both parameters, i.e. the viscosity and the density, are in the desired range. In other words, an agent influencing the density and regulating the viscosity must not be used in an amount such that the finished preparation then has a density of more than 1.5 g/cm^3. The amounts suitable can be readily identified by those skilled in the art, if appropriate by routine experiments, and the relevant values are established in the preparation.

Particularly suitable as viscosity regulators are those agents which have a certain affinity for the dye used according to the invention and are characterized by a high spreadability. It has been found, surprisingly, that butylene glycol is an agent with which the viscosity may be regulated and which leads to a good spreadability. Addition of butylene glycol can therefore ensure that the administered preparation sinks downwards and as soon as it has reached the membrane it spreads out there and rapidly stains the membrane. Without being bound to a theory, this is explained in that on the one hand butylene glycol has an affinity to membranes and on the other hand, owing to lipophilic groups, the dye is well absorbed. When the butylene glycol- and dye-containing preparation reaches the membrane, the butylene glycol ensures that the dye can distribute rapidly on the membrane.

The viscosity of the preparation according to the invention is preferably adjusted such that the shear viscosity at 25°C and a shear rate 10 s^{-1} is in a range of 1 to 500 mPas. The shear viscosity at 25°C and a shear rate of 10 s^{-1} is adjusted preferably to a range from 50 to 275 mPas. The adjustment of the viscosity can be achieved using the viscosity-regulating agents already mentioned. If the viscosity is in a range of 1 to 500 mPas under the measurement conditions stated, the effects achieved with the preparation according to the invention are significantly enhanced. The preparation containing the selectively staining dye sinks down rapidly, without the dye being washed out to a notable extent with the rinse solution. The dye is therefore only released at the administration site by capillary effects at the membrane which is thereby stained. If the viscosity is lower than 1 mPas under the measurement conditions specified, the effect of the rapid settling of the preparation according to the invention cannot be additionally enhanced. There exists the possibility that at least a portion

of the dye is discharged with the rinse solution prior to the staining of the membrane and therefore is no longer available for staining the membrane. If on the other hand the dynamic viscosity at 25°C and a shear rate of 10 s^{-1} is above 500 mPas, the viscosity of the preparation is so high that the dye cannot be released optimally from the droplets which form. The ability of the dye preparation to spread, which causes a rapid, homogeneous staining of the membrane, is thereby significantly reduced. The membrane is not optimally wetted with the dye preparation and is therefore not stained so distinctly. A particularly good staining result is achieved when the dynamic viscosity at 25°C and a shear rate of 10 s^{-1} is in a range of 50 to 275 mPas.

It has been found that administration of dye solutions to the eye may cause problems. If the dye solution is administered with the syringes conventionally used, the pressure which is attained during the injection is too high, such that the dye can get behind the retina.

Syringes are preferably used in which cannula diameter, the ratio of barrel diameter to cannula diameter and the aspect ratio are adjusted such that damage is avoided. In accordance with the invention, syringes are preferably used in which the cannula diameter is very small in order to keep damage to the eye to a minimum. Furthermore, the barrel diameter is adapted to the cannula diameter such that the occurrence of a Venturi effect is largely avoided. In other words, the diameter of the barrel must also be as small as possible in the syringes provided for the administration so that the ratio of barrel diameter to cannula diameter is in the range from 10 to 2:1 to 0.2, preferably 20:1 to 4:1, particularly preferably 16:1 to 8:1. In addition, the syringe barrel should have an aspect ratio, i.e. ratio of barrel length to barrel diameter, in a range from 15 to 5:1.

Furthermore, a kit is described comprising a syringe with barrel and cannula containing a dye solution for the selective staining of the internal limiting membrane and/or epiretinal membranes in the human or animal eye, wherein the ratio of barrel diameter to cannula diameter is in the range from 10-2 to 1-0.2, preferably 20:1 to 4:1, particularly preferably 16:1 to 8:1. The ratio of barrel length to barrel diameter is preferably in a range from 15 to 5:1. As essential component, therefore, the kit has a syringe whose barrel diameter is adapted to the diameter of the cannula. It has been found that, at a smaller ratio of the diameter, no pressure can build up in the interior upstream of the cannula so that a uniform administration of the preparation according to the invention, that is an administration at uniform pressure

and constant rate, is ensured. The kit preferably contains a dye preparation according to the invention as described above.

For the kit or its syringe, a 19 to 27 gauge, particularly preferably 23 or 25 gauge, cannula is used. 19 to 27 gauge cannulae are suitable for injections into the eye. Their outlet opening is so small that they leave no appreciable damage at the injection site but are still large enough to administer the preparation according to the invention in the eye at a sufficient rate. If the diameter of the barrel of the syringe is adjusted accordingly, a build-up of pressure in the interior of the syringe or the cannula, which delivers the preparation into the eye at excessively high pressure during the injection such that the preparation is distributed further beyond the administration site, e.g. behind the retina, is avoided. 20, 23, 25 or 27 gauge cannulae, particularly 23 or 25 gauge cannulae, have proven to be particularly good with respect to the desired application. In a preferred embodiment, such a cannula is used with a syringe having a barrel diameter of 3 to 10 mm. Particular preference is given to 23 or 25 gauge cannulae if the dynamic viscosity of the preparation at 25°C and a shear rate of 10^{-1} is in a range of 1 to 500 mPas. In that specific case, the interaction between cannula and preparation is so good that a sufficiently large amount of the preparation according to the invention can be deposited uniformly at a sufficiently rapid rate at the administration site without there being any explosive discharge of the preparation from the cannula due to pressure build-up. In this way, injection of the preparation behind the desired application site is avoided, whereby an optimal staining of the membrane can be achieved.

The preparations according to the invention described above and the syringes provided for their administration enable specific membranes in the eye – ILM and/or ERM – to be stained. Depending on the dye used, it is possible either to stain only one type of membrane, i.e. only ILM or only ERM, or both types. In one embodiment, the preparation according to the invention can be used to effect a negative staining of the epiretinal membranes in order to allow these to be removed. In this embodiment, a solution of a dye is used, for example Brilliant Blue G, which selectively stains the ILM but not the ERM. In this manner, the non-stained membrane (ERM) can be differentiated from the stained membrane (ILM) and can therefore be readily removed.

The invention is further illustrated by the following examples which describe dye solutions with increased density and the preparation thereof, without being limited thereto.

Example 1

0.025 g of Brilliant Blue G, 5 g of sucrose, 0.19 g of disodium hydrogen phosphate, 0.03 g of sodium dihydrogen phosphate and 0.82 g of sodium chloride are weighed accurately and made up to 100 g with distilled water. The raw materials are treated in a glass bottle at a maximum of 60°C for 1 h, forming a homogeneous solution with a dye concentration of 0.25 g/l and a density of 1.023 g/cm^3.

Example 2

0.025 g of Brilliant Blue G, 5 g of trehalose, 0.19 g of disodium hydrogen phosphate, 0.03 g of sodium dihydrogen phosphate and 0.82 g of sodium chloride are weighed accurately and made up to 100 g with distilled water. The raw materials are treated in a glass bottle at a maximum of 60°C for 1 h, forming a homogeneous solution with a dye concentration of 0.25 g/l and a density of 1.023 g/cm^3.

Comparative Example 3

0.025 g of Brilliant Blue G, 0.19 g of disodium hydrogen phosphate, 0.03 g of sodium dihydrogen phosphate and 0.82 g of sodium chloride are weighed accurately and made up to 100 g with a mixture of distilled water and D_2O. The raw materials are treated in a glass bottle at a maximum of 60°C for 1 h, forming a homogeneous solution with a dye concentration of 0.25 g/l and a density of 1.018 g/cm^3.

Comparative Example 4

Dye+glycerol

0.025 g of Brilliant Blue G, 0.19 g of disodium hydrogen phosphate, 0.03 g of sodium dihydrogen phosphate and 0.82 g of sodium chloride are weighed accurately and made up to 100 g with a mixture of distilled water and 10% glycerol. The raw materials are treated in a glass bottle at a maximum of 60°C for 1 h, wherein a homogeneous solution with a dye concentration of 0.25 g/l and a density of 1.027 g/cm^3.

Example 5

Using the method as described in Examples 1 to 4, a dye solution was prepared with the following composition:

Substance	Target weight in g	Actual weight in g
Polyvinylpyrrolidone	6	6.0067

Substance	Target weight in g	Actual weight in g
Brilliant Blue G	0.0125	0.0125
$Na_2HPO_4*2H_2O$	0.095	0.0950
$NaH_2PO_4*2H_2O$	0.015	0.0159
NaCl	0.41	0.4100
Water	to 50 g	to 50 g

A homogeneous solution with a density of 1.028 g/cm^3 and a viscosity of 7.38 mPas was obtained.

Example 6

Using the method as described in Examples 1 to 4, a dye solution was prepared with the following composition:

Substance	Target weight in g	Actual weight in g
Methylcellulose E 10 M (2 wt.%)	25	24.9986
Brilliant Blue G	0.0125	0.0125
$Na_2HPO_4*2H_2O$	0.095	0.0956
$NaH_2PO_4*2H_2O$	0.015	0.0151
NaCl	0.41	0.4099
Water	to 50 g	to 50 g

A homogeneous solution with a density of 1.007 g/cm^3 and a viscosity of 142.79 mPas was obtained.

The dye solutions prepared in Examples 1 to 6 were used for staining the internal limiting membrane in the human or animal eye. It was found that all six solutions can be readily applied and immediately sank after administration and stained the ILM. The dye was more intense, with an identical amount of dye, than a comparative Brilliant Blue G solution, as known from DE 10255601.

PATENTKRAV

1. Vandbaseret, biokompatibelt præparat til anvendelse ved kirurgisk behandling i øjet omfattende den selektive farvning af membrana limitans interna (ILM) og/eller af epiretinale membraner (ERM) i øjet på mennesker eller dyr og fjernelse af den farvede membran indeholdende i det mindste et farvestof udvalgt af gruppen bestående af: triphenylmethanfarvestoffer, idet præparatet har en densitet i området fra 1,01 g/cm^3 til 1,5 g/cm^3, idet der til indstilling af densiteten ikke anvendes monosaccharid eller reducerende disaccharid, kendetegnet ved, at præparatet indeholder et middel til indstilling af densiteten, hvor midlet er et ikke-reducerende disaccharid, et polysaccharid eller en neutral polymer eller en kombination deraf, og at osmolariteten af den fremstillede opløsning ligger i et område fra 280-330 mosmol/l.

2. Præparat til anvendelse ifølge krav 1 endvidere indeholdende et farvestof udvalgt af gruppen bestående af azofarvestoffer, cyaninfarvestoffer og/eller naturfarvestoffer eller blandinger heraf.

3. Præparat til anvendelse ifølge krav 1 idet triphenylmethanfarvestoffet er Coomassie Violet R200.

4. Præparat til anvendelse ifølge et af de foregående krav til anvendelse som farvestof til en negativ visning af epiretinale membraner.

5. Præparat til anvendelse ifølge et af de foregående krav, kendetegnet ved, at præparatet ved 25°C og en forskydningshastighed på 10 s^{-1} har en dynamisk viskositet i området fra 1 til 500 mPas, fortrinsvis i et område fra 50 til 275 mPas.

6. Præparat til anvendelse ifølge et af de foregående krav, kendetegnet ved, at osmolariteten i de fremstillede opløsninger ligger i området fra 280-330 mosmol/l.

7. Vandbaseret, biokompatibelt præparat til in vivo selektiv farvning af membrana limitans interna (ILM) og/eller af epiretinale membraner (ERM) i øjet på mennesker eller dyr indeholdende i det mindste et triphenylmethanfarvestof, idet præparatet indeholder et middel, hvormed der indstilles en densitet i området fra 1,01 g/cm^3 til 1,5 g/cm^3, og idet der til indstillingen af densiteten ikke anvendes monosaccharid eller reducerende disaccharid, hvor midlet er et ikke-reducerende disaccharid, et polysaccharid eller en neutral polymer eller en kombination deraf,

kendetegnet ved, at osmolariteten af den fremstillede opløsning ligger i et område fra 280-330 mosmol/l.