

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 May 2009 (14.05.2009)

PCT

(10) International Publication Number
WO 2009/059645 A1

(51) International Patent Classification:
G01N 27/28 (2006.01) **G01N 27/36** (2006.01)

(21) International Application Number:
PCT/EP2007/062130

(22) International Filing Date:
9 November 2007 (09.11.2007)

(25) Filing Language: English

(26) Publication Language: English

(71) Applicant (for all designated States except US): **Metrogas AG** [CH/CH]; Chalchofenstrasse 7, CH-8910 Affoltern a/A. (CH).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HEULE, Martin** [CH/CH]; Laerchenstrasse 23, CH-8903 Birmensdorf (CH). **BOECK, Christian** [CH/CH]; Rossweid, CH-8926 Kappel a.A. (CH).

(74) Agents: **HEPP, Dieter** et al.; Friedtalweg 5, CH-9500 Wil (CH).

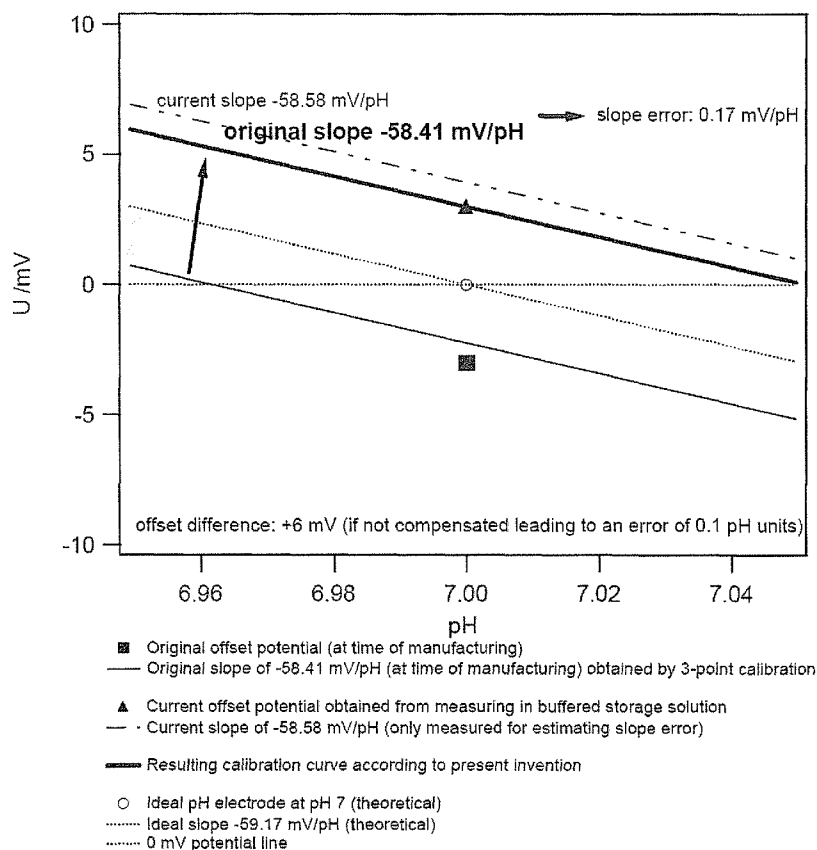
(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— with international search report

(54) Title: PH GLASS ELECTRODE FOR A DISPOSABLE CONTAINER

Fig. 2:



(57) Abstract: The invention provides a pH glass electrode in a storage compartment, for use in a disposable container or a component of a disposable container, wherein said storage compartment is filled with a sufficient amount of a buffered storage solution so as to cover at least the pH sensitive membrane(s) of the glass electrode during storage of the pH glass electrode in the storage compartment, and wherein said storage solution is selected such as to provide for a stable pH upon exposure of the storage solution to gamma rays. One-point calibration of the electrode is enabled due to the specified storage solution and a pre-determined slope of the potential of the electrode at the manufacturer's site.

WO 2009/059645 A1

pH Glass Electrode for a disposable container

The present invention relates to the technical field of pH glass electrodes especially for disposable containers such as bioprocess bags, and methods for testing and calibration of such pH glass electrodes.

The biopharmaceutical industry increasingly uses disposable liquid containers and tubing in their processes. Single-use systems promise a more cost-efficient biotechnological production. With fixed installations like steel tanks there are expensive cleaning and validation procedures necessary before using the system for production in order to meet regulatory requirements. Disposables are usually supplied in sterilized and clean state and with certificates meeting regulatory standards. Therefore the need of cleaning procedures necessary for fixed steel or plastic tanks like clean-in-place, steam-in-place and their validation is obsolete. Furthermore, the risk of cross-contamination is strongly minimised by using disposable equipment. There are a variety of plastic bags, filters, tubing etc. for virtually all stages of biotechnological production available, upstream processing, from media and buffer preparation to storage, cell fermentation, downstream processing and waste collection. A brief review of the current disposable bioprocess technology is given in BioProcess International, May 2007, p 44-51.

There is a growing need to monitor chemical parameters such as pH, conductivity, dissolved oxygen and many others also when using disposable equipment. This is done by using electrochemical, optical or other sensors. An overview of the state of the art in bioprocess monitoring can be found in BioProcess International, January 2007, p 32-41, and in US 2007/0159920. However, it is widely known and a matter of current discussion that there is a

multitude of additional requirements in the single use domain compared to using the chemical sensors which are well known from process analytical technology. The sensors need to be easy to handle, calibration-free and cost-efficient to allow for disposability. Especially, they need to be able to withstand the sterilisation treatment without affecting the analytical performance.

Irradiation by gamma rays is the most abundantly used procedure for sterilisation in the field of disposable bioprocess equipment. The standard procedure in the case of bioprocess bags is to fabricate the bags in a clean room environment, to package the bags ready for use and then to subject the whole package to gamma sterilisation to obtain sterility inside. The sterilised bags are then shipped to the bioprocessing site.

Mounting a sensor into the bag system after this sterilisation procedure is cumbersome. It requires sensor sterilisation, a sterile environment and an expert person to perform the mounting of the sensor. The sensor is preferably mounted earlier during the manufacturing process of the bag and sterilised together with the packaged bag. The sensor is not accessible anymore after sterilisation without the risk to break sterility. Therefore, the sensor cannot be taken out of the installation for calibration. This is a severe problem for sensors requiring calibration before use, especially for pH glass electrodes that are calibrated using dedicated buffer solutions. These issues apply to any prepackaged assembly that contain a potentiometric pH electrode.

For meeting the calibration needs of a pH electrode as stated above, there have been suggestions of automated calibration systems such as detailed in US 2002/0050460 A1. However, these are

complex installations with valves and tubing that require maintenance and special care in the case of sterile operation. For connecting to bioprocess disposables, these installations are too complex.

In cases where sensors are not compatible enough to be integrated with disposable containers, samples for off-site analysis are taken from the container to be tested. Care has to be taken that the sample is not changed by the sampling procedure and remains representative for the contents of the container. This type of validation can become expensive and takes a lot of experience. Therefore, it is highly desirable to integrate the sensor into the disposable container system.

On the basis of the considerations above the industry nowadays focuses on optical solutions. The plastic materials used for manufacturing the bioprocess disposables often is optically transparent. This allows for reading out the optical properties of a chemically sensitive material on the inside of a disposable system without endangering sterility. The chemically sensitive material has typically a very low mass and is often provided as a small adhesive patch that can be easily fixed on the inside. Sensing materials that withstand the gamma sterilisation process have been found and are in use today. Their optical properties are often stable enough so that calibration data obtained during the manufacturing of the sensing material is still valid for measurement months and years later. Therefore, such a system is perceived as "calibration-free" by the final user in biotechnology. A good example is given in US 6,602,716 B1 and references therein. It discloses a process for optical determination of pH, CO₂ and ionic compounds based on the decay times of luminescent complexes. The disadvantages of optical sensing are the limited measuring range from pH 4 to pH 8 in the case of the pH sensors

in US 6,602,716 B1, and other interferences e.g. of ionic strength on the signal.

In principle, pH glass electrodes are still the most robust and selective pH sensors available. For many applications such as media and buffer preparation in biotechnology, it would definitively be an advantage to use glass electrodes for pH measurement. pH glass electrodes are very well known and well accepted in standard biotechnology equipment such as steel tank fermentation etc. pH glass electrodes are abundantly used the heavily regulated field of biopharmaceutical production and fulfil current regulatory, quality and safety requirements such as GMP, cGMP and other regulatory systems. Sterilisation in those classic systems is usually done by autoclaving the electrodes mounted to the tank or bioreactor.

In 2006, a fermentation system with disposable bags called "Appliflex" was marketed by Applikon Biotechnology BV (Schiedam, The Netherlands), including the option to mount a single use sensor module consisting of a pH glass electrode, Clark-type dO sensor and a temperature sensor. The sensor module was sterilized by gamma irradiation, but had to be mounted under sterile conditions into a threaded port on the bag. The calibration had to be performed using external calibration solutions. Furthermore, the module could only be mounted on the top of the system with sensors pointing downwards due to the liquid-filled sensors.

It is thus an object of the present invention to overcome the above-mentioned shortcomings, especially to allow for pH calibration and measurement in disposable containers by means that are easier, contamination-free, access-free and which allow for broad-range pH detection.

This object is solved by a pH glass electrode in a storage compartment according to the independent claim 1, and related independent claims, i.e. the use of a buffered solution as a storage solution for a pH glass electrode, a method of testing a pH glass electrode, a method of calibrating a pH glass electrode, and a method of putting a pH glass electrode into operation.

According to the present invention, a pH glass electrode (for use in a disposable container or a component of a disposable container) is stored in a storage compartment. This storage compartment is filled with a sufficient amount of a buffered storage solution so as to cover at least the pH sensitive membrane(s) of the glass electrode during storage of the pH glass electrode in the storage compartment. Moreover, said storage solution is selected such as to provide for a stable pH upon exposure of the storage solution to gamma rays.

As used herein, "storage" shall mean to keeping of at least the pH sensitive glass of the electrode in a liquid milieu while no actual use of the electrode for pH measurement is being intended at that time.

A "disposable container" preferably is a disposable bioprocessing bag (cf. overview in BioProcess International, May 2007, p 44-51), but the invention is not limited to such bags. The pH glass electrode according to the invention could also be mounted in another piece of disposable equipment such as a transfer tubing setup or other disposable component in contact with a bioprocess fluid whose pH is of interest.

As used herein, a "storage compartment" is a housing that seals the pH sensitive glass membrane of the electrode leakproof from

the environment. Typically, such a storage compartment has an inner volume of no more than 50 cm³, preferably no more than 10 cm³, most preferably no more than 2 cm³. A very simple storage compartment is e.g. a flexible plastic cap that fits the pH glass electrode. Thus, the cap can be filled with the storage solution and is then pressed onto the pH electrode. After checking and calibrating, the cap is removed for measurement into the disposable container.

The pH glass electrode can be integrated in a disposable container such as a bioprocess bag system during the bag production process. Subsequently, the pH glass electrode undergoes gamma sterilisation with the fully packaged bag. The final user only has to attach the sensor cable to the pH meter to take the pH sensor into operation. Since the electrode is stored in a solution suited for a one-point calibration (as will be outlined below in more detail), there is no need to bring the electrode in contact with additional calibration solutions. The electrode will remain in its original storage compartment throughout the testing and/or calibration procedures, thereby maintaining sterility.

Typically, the liquid compartment in which the electrode and its storage solution are kept is very small and can be opened after calibration towards the inside of a bioprocess bag in order to get the pH electrode into contact with the whole bag volume. It should be unproblematic that the small amount of storage solution in the order of a few millilitres quantity is being dissolved in the media on the order of 100 to 1000 litres. This is especially the case with e.g. phosphate buffers which are contained in many buffer and media compositions, anyhow. Alternatively, the remainders of the storage solution may of course be flushed out with the first quantity of process solution from the

interior of the container. Another alternative would be to provide a storage compartment with fill and drain tubing. This tubing could be used to drain and dry the compartment before bringing the container's contents into contact with the sensor. The exact choice of procedure will evidently depend on the specific application, on the phase of the biotechnological production (upstream/downstream, etc.) and finally on the user and his specific regulatory requirements.

Thus, in brief, the object of the present invention is solved by storing the sensor in a small amount of buffered solution that substantially maintains its pH throughout the sterilisation process by gamma irradiation. Although the suggested process is not entirely calibration-free, it can be simplified by automatic means to a point where the user only has to connect the electrode to the pH measuring equipment and to enter (or automatically read out) two values only, i.e. the slope from an electrode certificate and the pH of the storage solution. For a valid calibration, an electrode slope value and the offset potential value are required. For the definitions and the basic science and technology of pH measurement, pH glass electrodes, origin of the electrode potential and general calibration procedures it is referred to the corresponding chapters in *Galster, pH Measurement*, VCH, Weinheim (DE), 1991 (included herein by reference). DIN 19268:2006 provides further details for pH electrode calibration (included herein by reference).

It was surprisingly found that the slope of a pH glass electrode remains sufficiently stable within a small error margin throughout the sterilisation process at 25 - 50 kGy and during storage time in the order of several months to a few years. However, the offset potentials may exhibit a significant change of several mV with time. Thus, at the time of use, knowing the well-defined pH

of the storage solution allows to determine the current value of the offset potential of the electrode.

It is already customary that pH electrodes are tested by the manufacturer and that a certificate informing about the results, i.e. offset potential (compared to the "ideal" pH glass electrode according to the Nernst equation) and slope, is issued to the customer. The slope taken from the certificate and the offset potential determined by the customer before use then form a set of calibration data for measurement.

The systematic error due to a changed slope will increase the more the pH to be measured differs from pH 7. However, according to experiments conducted in the course of the present invention, this error will typically not exceed 0.02 pH units even at pH 1 or pH 12.

According to a preferred embodiment, the combined pH glass electrode according to the invention has electrolyte gels in both the reference electrode and the measuring electrode; these gels do not flow. This allows for mounting and operating the electrode in any orientation, including the electrode pointing upwards. Such electrodes can be integrated at any location of the container. It is preferred to mount the electrodes at the bottom of the containers such that the electrode can be used to measure already during the first filling process as soon as both diaphragm and pH sensitive glass are immersed. This may be important in media and buffer preparation steps.

A pH glass electrode according to the invention comprises buffered storage solution, preferably containing:

- i) a first compound or combination of compounds that buffer(s) the storage solution at a substantially constant pH, despite irradiation with gamma rays; and
- ii) a second compound or combinations of compound that function(s) to adjust the ionic strength of the storage solution.

The compound(s) of lit. i) constitute a buffer system selected from the group consisting of phosphate buffers, carbonate buffers, borate buffers. Despite these three types of buffers being named specifically, the person of routine skill in the art will easily find further suitable buffer systems, be it organic or inorganic buffer systems, by simple routine experiments, e.g. by subjecting a buffer candidate to the desired gamma treatment and monitoring the change of pH. It is currently believed that inorganic buffers are generally more stable than organic buffers; phosphate buffers are currently preferred. Phosphates do not exhibit any measurable shift in pH up to a gamma irradiation dose of 49 kGy. For comparison: A very commonly used dose for bioprocess equipment is 25 kGy.

Preferably, the buffer system is adjusted to a pH of ≤ 6.5 , preferably 6.5; or ≥ 7.5 , preferably 7.5. This is advantageous because a pH in the above ranges results in an electrode potential different from 0 mV when the electrode is fully functional; however, a broken or otherwise short-circuited pH electrode could exhibit a potential of 0 mV, as well as an "ideal" electrode at pH 7. In that case, a false positive would occur, i.e. the user would take a container with a defective electrode into operation. To prevent this, the following procedure is applied. If the pH of the storage solution within the above pH ranges results in a stable sensor signal within a certain margin of preferably ± 15 mV around the expected Nernstian potential during the

electrode check, it can be reasonably assumed that the electrode is fully functional. For all measurements and calibration procedures, the temperature of the solutions shall be taken into account. Towards this end, slopes and measurements are routinely (and throughout all embodiments described herein below) temperature-compensated according to the Nernst equation.

The compound(s) of lit. ii) (above) comprise an alkali metal salt, preferably potassium chloride, wherein the concentration of the alkali metal salt in the storage solution substantially equals the concentration of the alkali metal salt in the reference electrolyte of the pH glass electrode. Typically, the storage solution is either saturated with the alkali metal salt, preferably potassium chloride; or contains the alkali metal salt, preferably potassium chloride, at a concentration of about 3 mol/l. Thereby, potassium chloride in the reference electrolyte is prevented from leaching out of the electrode by diffusion and the inner potential at the silver/silver chloride electrode is kept stable. It has to be noted that the addition of large amounts of salt to the storage solution also changes the hydrogen activity. The buffer solution pH is therefore adjusted to match the hydrogen activity to the target pH of the buffer solution.

As is evident from the above, a further aspect of the present invention relates to a disposable container or a component of a disposable container, equipped with a pH glass electrode as described above.

Yet another aspect of the present invention relates to the use of a buffered solution which is stable in pH upon exposure to gamma rays, as storage solution for a pH glass electrode. As

outlined above in any detail, the buffered storage solution preferably contains:

- i) a first compound or combination of compounds that buffer(s) the storage solution at a substantially constant pH, despite irradiation with gamma rays; and
- ii) a second compound or combinations of compound that function(s) to adjust the ionic strength of the storage solution.

Using such a solution for storage of a pH glass electrode in the context of disposable containers for the first time opens up the possibility of one-point calibration under sterile conditions, as outlined above.

In another aspect, the invention concerns a method of testing a pH glass electrode as described above, comprising the step of determining the potential of the pH glass electrode in the storage solution prior to opening the storage compartment. In brief, (shortly) before taking the disposable container with the pH electrode into operation, the electrode according to the present invention may be checked for good measuring condition by measuring the potential in its storage solution. This procedure will prevent the accidental use of a bag with a defective sensor.

Yet a further aspect of the invention concerns a method of calibrating a pH glass electrode as described above, comprising the steps of:

- i) Measuring the potential of the pH glass electrode in the buffered storage solution of defined pH, at a time t_1 , prior to opening the storage compartment;
- ii) Determining an expected reference potential of the pH glass electrode in the buffered storage solution of defined pH, said expected potential being either

- a) calculated theoretically, based on the Nernst equation; and/or
 - b) deduced from a suitable pre-determined data set correlating at least one measured potential at time $t_0 < t_1$ to a defined pH of a calibration standard;
- iii) Calculating a current offset potential from the results of steps i) and ii), compensating for the shift of the potential from t_0 to t_1 ; and
- iv) Linear offsetting (a) slope(s) of (a) pre-determined calibration plot(s) by the offset potential determined in step iii), wherein the calibration plot or a suitable pre-determined data set for generation of said calibration plot correlates the potential of the pH glass electrode with the pH of standard solutions at the time t_0 .

Typically, t_0 is a point in time at the manufacturer's site, before sterilization by gamma irradiation and/or installation into a disposable container; t_1 is typically a point in time at the end-user's site, after sterilization by gamma irradiation and installation into a disposable container. If the pH of the storage solution differs from 7.0, the offset potential U_{off} can be calculated by $U_{\text{off}} = U_m - U'_N * (7.0 - \text{pH}_{\text{sts}})$; wherein U_m is the measured potential according to step i), U'_N is the slope of the predetermined calibration plot at t_0 and pH_{sts} is the hydrogen ion activity of the storage solution (notation according to Galster, pH Measurement, VCH, Weinheim (DE), 1991 (p. 157; included herein by reference). Choosing pH 7 as the zero potential point is a common convention for the construction of pH Electrodes, e.g. according to DIN 19263:2006.

According to common practice, the pre-determined calibration plot or suitable pre-determined data set allowing for generation

of such a plot is derived from a two or three-point calibration, e.g. at pHs 4.0, 7.0 and 9.0.

As is readily apparent, the above aspects of the invention can be combined in a method of putting a pH electrode into operation, i.e. by a method comprising the steps of:

- i) Testing the pH glass electrode according to a method of testing as outlined above; and,
- ii) Calibrating the pH glass electrode according to a method of calibrating as outlined above,

wherein steps i) and ii) are performed prior to opening the storage compartment, i.e. still under sterile conditions and with the pH glass electrode being stored in the storage solution in the storage compartment.

The invention is henceforth described in more detail by means of a specific embodiment, in order to aid the skilled artisan in complete understanding of the invention; it is to be noted that the breadth of the invention is not to be limited to the presented embodiment.

- Fig. 1
- a) pH glass electrode in a storage compartment, schematically;
 - b) pH glass electrode in a storage compartment, installed in a disposable container, the storage compartment still being sealed leakproof from the interior of the container, schematically;
 - c) pH glass electrode in a storage compartment, installed in a disposable container, the storage compartment being opened towards the interior of the container, schematically.

Fig. 2 Graphical representation of a calibration process according to the present invention.

Fig. 1 a) represents a conventional pH glass electrode 1, here a combined pH glass electrode with an inner electrode 2 and an outer electrode 3; both electrodes are to be connected to a pH meter. The pH glass electrode according to the invention is stored in a storing solution 4 as outlined above, in a storage compartment 5 as outlined above.

The pH glass electrode of Fig. 1 a) is installed in a disposable container 6, as shown in Fig. 1 b), the storage compartment 5 still being sealed leakproof from the interior of the container 6. In this configuration, sterilization of the whole container assembly is performed, as well as (optional testing and) one-point calibration of the pH glass electrode 1 in the storage solution 4.

Thereafter, the storage compartment 5 can be opened towards the interior of the container 6, as is shown in Fig. 1 c), thereby enabling pH measurements in the container 6 with the glass electrode 1. Opening of the storage compartment 5 has to be sufficiently wide in order to provide for substantially free fluid transport from the inside of the bioreactor 6 towards the electrode 1. In the schematic drawings, enabled fluid communication between storage compartment 5 and the interior of the bioreactor 6 is illustrated schematically by the long-dashed line at the bottom of the storage compartment 5.

pH glass electrodes

Standard combined glass electrodes with a ceramic pin diaphragm were used for the experiments.

Buffer

The following buffer system in the storage compartment of the pH glass electrode has been used in the subsequent examples:

4.565 g KH_2PO_4 and 2.979 g Na_2HPO_4 were added to 500 ml of Millipore[®] grade water, resulting in a pH of 6.51. To this mixture, 111.8 g KCl were added. The pH was shifted to 5.81 due to the higher ionic strength. The pH was then readjusted to 6.50 by adding 9.57 ml of 1 M NaOH solution by means of an automated titration.

Table 1

Before Gamma treatment:

Sensor ⁽⁰⁾	Offset potential ⁽¹⁾ [mV]	Slope 4/7 ⁽²⁾	Slope 7/9 ⁽³⁾	Reference Temp. ⁽⁴⁾
10938502	-1.2	0.994	0.978	22.78
10938503	-2.3	0.992	0.983	22.78
10938504	-3	0.994	0.977	22.78
10938505	-1.6	0.993	0.980	22.78
10938506	-1.8	0.993	0.983	22.78
10835832	-5	0.996	0.974	22.46
10835833	-4.6	0.996	0.975	22.46
10835834	-3	0.997	0.973	22.46
10835835	-5.1	0.997	0.975	22.46
10835836	-4	0.995	0.978	22.46

After Gamma treatment⁽⁵⁾:

Sensor ⁽⁰⁾	Offset potential ⁽¹⁾ [mV]	Slope 4/7 ⁽²⁾	Slope 7/9 ⁽³⁾	Reference Temp. ⁽⁴⁾
10938502	7.4	0.997	0.977	26.37
10938503	9.5	0.994	0.972	26.37
10938504	7.4	0.997	0.981	26.37
10938505	5.7	0.996	0.975	26.37
10938506	7.7	0.997	0.981	26.37
10835832	9.3	0.998	0.973	26.41
10835833	3.4	0.996	0.976	26.41
10835834	0	0.998	0.973	26.41
10835835	1.4	0.999	0.976	26.41
10835836	8.6	0.998	0.973	26.41

Sensor ⁽⁰⁾	Δ Offset potential ⁽¹⁾ [mV]	Δ Slope 4/7 ⁽²⁾	Δ Slope 7/9 ⁽³⁾
10938502	8.6	0.004	-0.002
10938503	11.8	0.001	-0.010
10938504	10.4	0.003	0.004
10938505	7.3	0.003	-0.005
10938506	9.5	0.004	-0.002
10835832	14.3	0.002	-0.001
10835833	8	0.000	0.000

10835834	3	0.001	0.000
10835835	6.5	0.002	0.001
10835836	12.6	0.003	-0.005

- (0) Ten pH glass electrodes (identified by the 8 digit numbers) have been investigated under the regime of the present invention.
- (1) The offset potential has been calculated based on the difference of the measured potential at pH 7.0 and the theoretical potential at pH 7.0 ($\overset{!}{=} 0$). The resulting difference in the offset potential after Gamma treatment has been determined.
- (2,3) Three-point calibration has been performed with standard solutions of pH 4.0, 7.0 and 9.0. The resulting potentials at the different pHs were plotted against the respective pH (cf. Fig. 1, thin straight line), and the slopes were determined for the pH ranges 4-7 and 7-9, respectively. The values are given as a fraction of the ideal Nernstian slope. The resulting differences in the respective slopes after Gamma treatment have been determined, clearly indicating high stability with respect to gamma sterilisation treatment.
- (4) The term "Reference Temperature" denotes the temperature during the measurements which were used for potential correction according to Nernst.
- (5) Gamma treatment was performed by a specialist service provider (Studer Hard AG, Daeniken, Switzerland) with a total dose of 46-49 kGy.

Fig. 2 is a graphical representation of a calibration process according to the present invention. The inclined dotted line represents the pH dependent potential of an ideal pH glass electrode, calculated with the Nernst equation. Before shipping of the electrode, a calibration data set is generated, indicated

"original slope"; this calibration can be easily performed with standard solutions of well defined pH, e.g. a three-point calibration at pHs 4.0, 7.0 and 9.0 at the manufacturer's site. The same calibration was carried out after Gamma sterilization. As can be seen from Fig. 2 ("current slope"), the slope of the actual potential (U/mV, plotted against pH) only deviates by 0.17 mV/pH after Gamma treatment (which is a neglectable deviation), but the offset potential may change by several mV, e.g. here by + 6 mV. This difference in offset potential must be corrected, what can be easily achieved according to the invention by measuring the potential in the buffered storage solution and subsequent linear offsetting the complete prior calibration data set by this actual determined offset value.

Claims

1. A pH glass electrode in a storage compartment, for use in a disposable container or a component of a disposable container, wherein said storage compartment is filled with a sufficient amount of a buffered storage solution so as to cover at least the pH sensitive membrane of the glass electrode during storage of the pH glass electrode in the storage compartment, and wherein said storage solution is selected such as to provide for a stable pH upon exposure of the storage solution to gamma rays.
2. A pH glass electrode according to claim 1, wherein at least the storage compartment is sterile.
3. A pH glass electrode according to one of claims 1 to 2, wherein the electrolytes in both the reference electrode and the measuring electrode are gels.
4. A pH glass electrode according to one of claims 1 to 3, wherein the buffered storage solution comprises:
 - i) a first compound or combination of compounds that buffer(s) the storage solution at a substantially constant pH, despite irradiation with gamma rays; and
 - ii) a second compound or combinations of compound that function(s) to adjust the ionic strength of the storage solution.
5. A pH glass electrode according to claim 4, wherein the compound(s) of lit. i) constitute a buffer system selected from the group consisting of phosphate buffers, carbonate buffers, borate buffers.

6. A pH glass electrode according to claim 5, wherein the buffer system is adjusted to a pH of ≤ 6.5 , preferably 6.5; or ≥ 7.5 , preferably 7.5.
7. A pH glass electrode according to one of claims 4 to 6, wherein the compound(s) of lit. ii) comprise an alkali metal salt, preferably potassium chloride, wherein the concentration of the alkali metal salt in the storage solution substantially equals the concentration of the alkali metal salt in the reference electrolyte of the pH glass electrode.
8. A pH glass electrode according to claim 7, wherein the storage solution is either saturated with the alkali metal salt, preferably potassium chloride; or wherein the storage solution contains the alkali metal salt, preferably potassium chloride, at a concentration of about 3 mol/l.
9. A disposable container or a component of a disposable container, equipped with a pH glass electrode according to one of claims 1 to 8.
10. Use of a buffered solution which is stable in pH upon exposure to gamma rays, as storage solution for a pH glass electrode.
11. A method of testing a pH glass electrode according to one of claims 1 to 8, comprising the step of determining the potential of the pH glass electrode in the storage solution prior to opening the storage compartment.

12. A method of calibrating a pH glass electrode according to one of claims 1 to 8, comprising the steps of:
- i) Measuring the potential of the pH glass electrode in the buffered storage solution of defined pH, preferably pH 7, at a time t_1 , prior to opening the storage compartment;
 - ii) Determining an expected reference potential of the pH glass electrode in the buffered storage solution of defined pH, said expected potential being either
 - a) calculated theoretically, based on the Nernst equation; and/or
 - b) deduced from a suitable pre-determined data set correlating at least one measured potential at time $t_0 < t_1$ to a defined pH of a calibration standard;
 - iii) Calculating a current offset potential at the pH of the buffered storage solution, preferably pH 7, from the results of steps i) and ii), compensating for the shift of the potential from t_0 to t_1 ; and
 - iv) Linear offsetting (a) slope(s) of (a) pre-determined calibration plot(s) by an offset potential determined in step iii), wherein the calibration plot or a suitable pre-determined data set for generation of said calibration plot correlates the potential of the pH glass electrode with the pH of standard solutions at the time t_0 .
13. Method according to claim 12, wherein the pre-determined calibration plot or suitable pre-determined data set allowing for generation of such a plot is derived from a two-point or a three-point calibration.

14. A method of putting a pH electrode according to one of claims 1 to 8 in a disposable container or a component of a disposable container into operation, comprising the steps of:

- i) Testing the pH glass electrode according to a method of claim 11,
- ii) Calibrating the pH glass electrode according to a method of one of claims 12 to 13;

wherein steps i) and ii) are performed prior to opening the storage compartment.

Fig. 1:

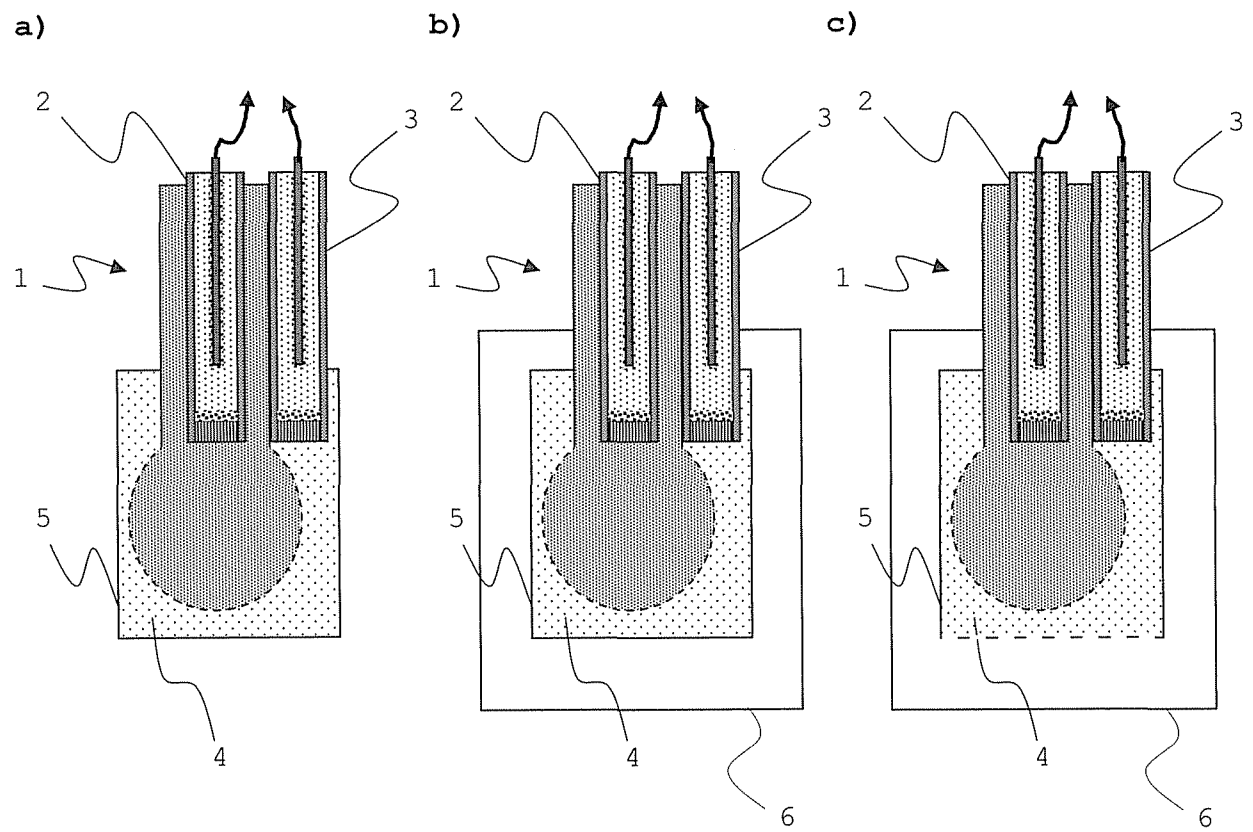
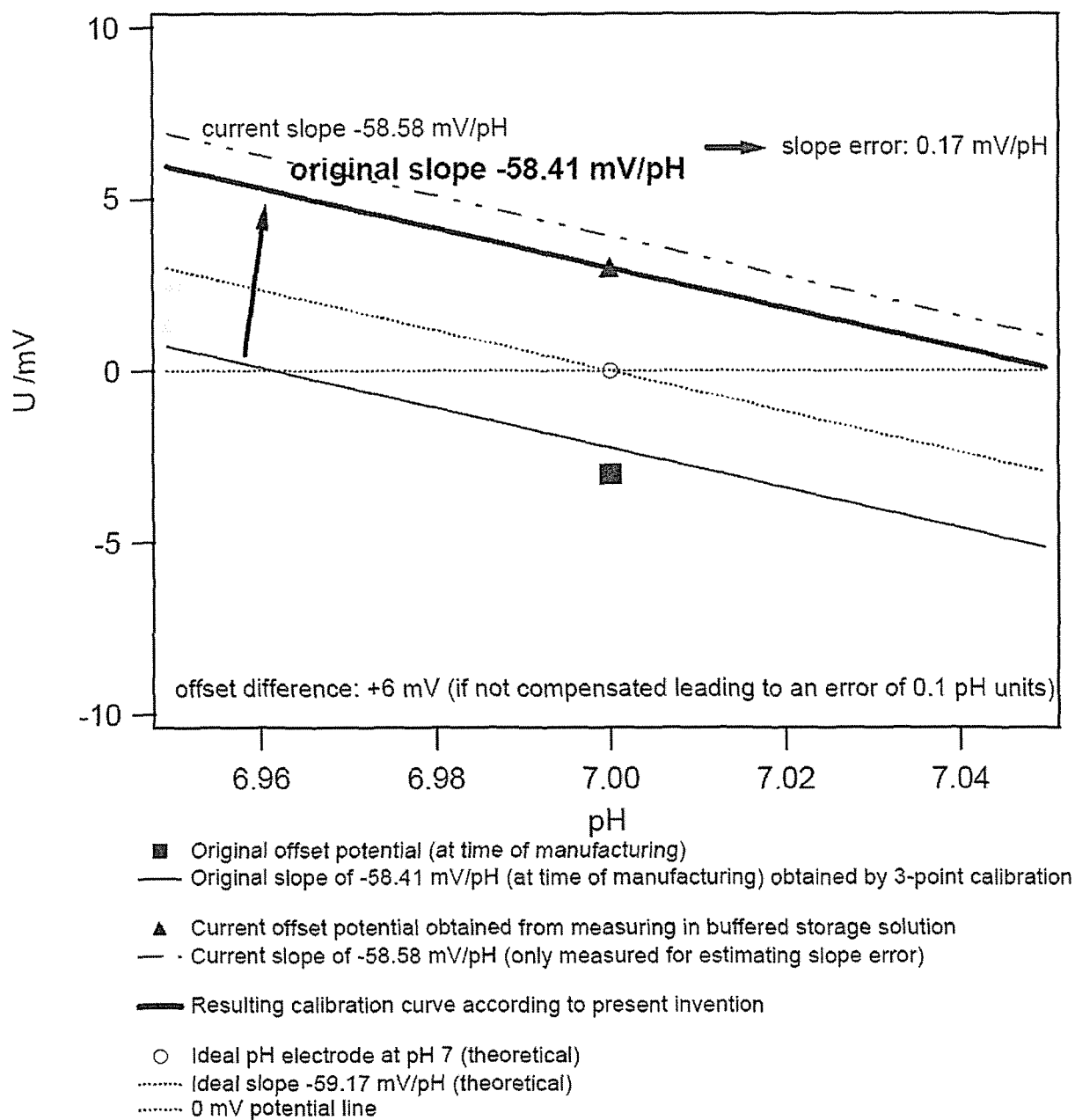


Fig. 2:



INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/062130

A. CLASSIFICATION OF SUBJECT MATTER

INV. G01N27/28 G01N27/36

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)

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/059286 A (TERUMO CARDIOVASCULAR SYS [US]; HACKER THOMAS [US]; TAKAGI MARK [US];) 15 July 2004 (2004-07-15) paragraphs [0028], [0048], [0053], [0059]	1-3, 10
Y	EP 0 753 737 A (HORIBA LTD [JP]) 15 January 1997 (1997-01-15) column 1, lines 18-21 column 4, lines 14-35 column 5, lines 7-10	1-14
Y	US 2004/121469 A1 (ROMNEY MATTHEW [US] ET AL) 24 June 2004 (2004-06-24)	1-9, 11-14
X	paragraphs [0009], [0012], [0017], [0021]	10
	----- -/--	

☒ Further documents are listed in the continuation of Box C.☒ See patent family 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
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *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

9 July 2008

Date of mailing of the international search report

25/07/2008

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Stussi, Elisa

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2007/062130

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	THERMO ELECTRON CORPORATION: "Users Guide ROSS pH & ROSS Sure-Flow pH Electrode"[Online] 2005, pages 1-11, XP002487330 Retrieved from the Internet: URL:www.wiselab.appstate.edu/technical_information/ross_electrode.pdf> [retrieved on 2008-07-07] pages 7,9	1-14
Y	"ORION MATERIAL SAFETY DATA SHEET" THERMO ELECTRON COMPANY, [Online] 3 May 1999 (1999-05-03), pages 1-2, XP002487331 Retrieved from the Internet: URL:www.msds hazcom.com/WEB_DOCS/MISC/PDF/WCD00179.PDF> [retrieved on 2008-07-07] page 1	1-14
Y	WO 2005/104706 A (BAXTER INT [US]; BAXTER HEALTHCARE SA [CH]; HYCLONE LAB INC [US]; KUNA) 10 November 2005 (2005-11-10) paragraphs [0005], [0007], [0010], [0014], [0019], [0068], [0073], [0078], [0138], [0167]	9,14
A	US 2007/159920 A1 (BAUMFALK REINHARD [DE] ET AL) 12 July 2007 (2007-07-12) cited in the application paragraphs [0065], [0072], [0082]	1-14
A	US 2003/150726 A1 (WEST STEVEN J [US] ET AL) 14 August 2003 (2003-08-14) paragraph [0053]; figure 5	1-14
A	EP 1 615 023 A (METROGLAS AG [CH]) 11 January 2006 (2006-01-11) the whole document	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2007/062130

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 2004059286	A	15-07-2004	AU 2003302258 A1	22-07-2004
EP 0753737	A	15-01-1997	DE 69626153 D1	20-03-2003
			DE 69626153 T2	15-01-2004
			JP 3391945 B2	31-03-2003
			JP 9021777 A	21-01-1997
			US 5939610 A	17-08-1999
US 2004121469	A1	24-06-2004	AU 2003301191 A1	22-07-2004
			WO 2004058373 A2	15-07-2004
WO 2005104706	A	10-11-2005	AU 2005237512 A1	10-11-2005
			AU 2005240969 A1	17-11-2005
			BR PI0510291 A	30-10-2007
			BR PI0510376 A	06-11-2007
			CA 2559496 A1	17-11-2005
			CA 2559537 A1	10-11-2005
			CN 1997730 A	11-07-2007
			CN 1946835 A	11-04-2007
			EP 1756259 A2	28-02-2007
			EP 1763575 A2	21-03-2007
			JP 2007534335 T	29-11-2007
			KR 20070015178 A	01-02-2007
			KR 20070006864 A	11-01-2007
			WO 2005108546 A2	17-11-2005
US 2007159920	A1	12-07-2007	DE 102006001623 A1	12-07-2007
US 2003150726	A1	14-08-2003	NONE	
EP 1615023	A	11-01-2006	US 2006006075 A1	12-01-2006