

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
2 July 2009 (02.07.2009)

PCT

(10) International Publication Number
WO 2009/082187 A2

- (51) International Patent Classification:
G01N 27/30 (2006.01) *G01N 27/26* (2006.01)
- (21) International Application Number:
PCT/MY2008/000191
- (22) International Filing Date:
19 December 2008 (19.12.2008)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
PI 20072298 21 December 2007 (21.12.2007) MY
- (71) Applicant (for all designated States except US): **MIMOS BERHAD** [MY/MY]; Technology Park of Malaysia, Bukit Jalil, 57000 Kuala Lumpur (MY).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **AHMAD, Mohd, Rais** [MY/MY]; C/O Mimos Berhad, Technology Park Malaysia, Kuala Lumpur 57000 (MY). **ABD.AZIZ, Aiman, Sajidah** [MY/MY]; c/o MIMOS BERHAD, Technology Park Malaysia, 57000 Kuala Lumpur (MY).
- (74) Agent: **SIAW, Timothy**; Shearn Delamore & Co., 7th Floor, Wisma Hamzah-Kwong Hing, No. 1, Leboh Ampang, 50100 Kuala Lumpur (MY).

- (81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, 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, ST, 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, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- of inventorship (Rule 4.17(iv))

[Continued on next page]

(54) Title: REFERENCE ELECTRODE WITH IMMOBILIZED INTERNAL ELECTROLYTE

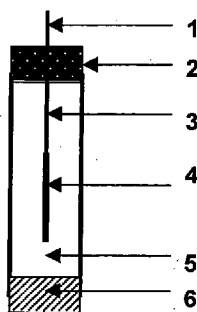


Figure 1

(57) Abstract: The present invention relates to the method of manufacturing of maintenance-free silver-silver chloride reference electrode wherein the immobilized gel internal electrolyte (5) is made of fluorinated quaternary ammonium chloride salts immobilized to the acrylic hydrogel polymer backbone. Migration and loss of chloride ion is prevented and thus results in negligible variation of chloride ion concentration, and prolonged life of the reference electrode.



WO 2009/082187 A2



Published:

- *without international search report and to be republished
upon receipt of that report*

REFERENCE ELECTRODE WITH IMMOBILIZED INTERNAL ELECTROLYTE

Field of the Invention

5 The present invention relates to the method of manufacturing of maintenance-free miniaturized reference electrode for field deployment.

Background of the Invention and Prior Art

Reference electrodes are widely used in many different types of
10 electrochemical experiments including measurements with pH electrodes, ion-selective electrodes and cathodic protection instrumentation to access and control the corrosion rate of metallic structures. The role of the reference electrode is to provide a stable potential against which other potentials are measured or controlled. Reference electrodes based on silver-silver chloride
15 cell are among the most widely used. The conventional double junction reference electrodes contain salt bridge and junctions separating the internal reference electrolyte and the external analyte solutions. This type of reference cell uses liquid electrolyte that contains chloride ion, and the ionic species gradually migrate to the analyte solution. Commercially available
20 conventional double junction silver-silver chloride reference electrodes are large, contains liquid electrolyte that must be regularly maintained and not suitable for prolonged field deployment. Regular maintenance that includes thorough cleaning and replacement of electrolyte solution is necessary to ensure accurate measurement.

25

Unlike conventional reference electrode, the present invention provides a junction-free reference electrode which is economically viable for mass production, maintenance-free, suitable for dry application such as in-situ soil analysis and environmental monitoring. The present invention
30 proposes a maintenance-free silver-silver chloride reference electrode with constant concentration of the chloride ion internal reference which is achieved by immobilizing ionic monomer salts to moisture-retaining hydrogel

media wherein the cationic charge is immobilized through covalent chemical bonding to hydrophilic polymer matrix, mixed with gel media. The proposed liquid-free reference electrode is expected to have a prolonged lifetime and constant reference voltage because the reference chloride ion, in the gel media, undergoes negligible concentration variation over a long period of operation. The proposed liquid-free reference electrode can be manufactured at low cost and can be deployed for field applications.

United States granted patent No. 5,833,825 discloses a conventional silver-silver chloride reference electrode using conventional metal chloride (NaCl) reference electrolyte. This invention is equipped with protective polyvinyl alcohol coating at the tip of the cell. This polymer coating is hydrolysable which means that it can be easily removed before use by soaking in water. It helps prevent leakage and loss of electrolyte solution after being manufactured but before use.

United States patent application No. 10/396,401 discloses a reference electrode based on substituted acrylate monomer. The substituents in the acrylate monomer contain alkyl, ether, hydroxyl, amine and silylether groups. The electrolyte is based on conventional potassium chloride salt solution.

United States granted patent No. 4,908,117 discloses a silver-silver chloride reference electrode wherein the migration of chloride electrolyte is prevented by coating the electrode with chloride-containing polymer partially quaternized by triethylamine. This layer is further coated by additional co-polymer coating.

Summary of the Invention

The present invention discloses a maintenance-free silver-silver chloride reference electrode which comprises platinum wire (1), epoxy (2), silver wire (3), silver-silver chloride electrode (4), immobilized gel internal electrolyte (5) and a micro-porous polymer (6). The platinum wire (1) is

designed for conducting current from the reference electrode to the electronic circuits. The end of the platinum wire (1) is soldered with gold male connector for connection to electronic board. The opposite end of the platinum wire (1) is soldered with silver wire (3). The epoxy (2) such as silicone capsule is designed for securing the silver wire (3) and the silver-silver chloride electrode (4) suspended in the immobilized gel internal electrolyte (5). The epoxy (2) is also designed for preventing loss of moisture of the immobilized gel internal electrolyte (5) and for encapsulating the upper part of the reference electrode. The silver wire (3) is coated with silver chloride coating and is designed for undergoing oxidation reaction to produce silver ion. The silver-silver chloride electrode (4) is designed for undergoing reduction reaction by silver ion to produce silver. The immobilized gel internal electrolyte (5) is designed for providing constant concentration of chloride ion to capture free silver ion for producing silver chloride precipitate. The immobilized gel internal electrolyte (5) comprises hydrogel monomers, fluorinated quaternary ammonium chloride salts, cross-linking agents and solvents. The cross-linking agents are N,N'-methylene-bisacrylamide, N,N,N',N'-tetraethylenediamine and ammonium persulfate. The solvents are deionized water, glycerol and sorbitol. The immobilized gel internal electrolyte (5) is derived from fluorinated quaternary ammonium chloride salt I, II and III. The immobilized gel internal electrolyte I comprises dimethylacrylamide, fluorinated quaternary ammonium chloride salt I, cross-linker, deionized water and glycerol. The immobilized gel internal electrolyte II comprises acrylamide, fluorinated quaternary ammonium chloride salt II, cross-linker, deionized water and sorbitol. The immobilized gel internal electrolyte III comprises hydroxyethylmethacrylate, fluorinated quaternary ammonium chloride salt III, cross-linker, deionized water and glycerol. The micro-porous polymer (6) such as ceramic filter is designed for securing the immobilized gel internal electrolyte (5) at the tip of the reference electrode from direct contact with analyte solutions and for encapsulating the lower end of the reference electrode.

Brief Description of the Drawings

Figure 1 illustrates the maintenance-free silver-silver chloride reference electrode.

5 Detailed Description of the Invention

The present invention relates to the method of manufacturing of maintenance-free silver-silver chloride reference electrode, wherein the internal chloride ion concentration is maintained at constant level through immobilization of fluorinated quaternary ammonium chloride salts. Migration
10 or loss of chloride ion is significantly reduced with the immobilization of fluorinated quaternary ammonium chloride salts and the reference electrode gives stable measurement in potentiometric setup. The maintenance-free silver-silver chloride reference electrode is suitable for field deployment in the following applications:

- 15 i. In-situ soil macronutrient analysis.
- ii. Plant nutrient uptake.
- iii. Environmental analysis.

The maintenance-free silver-silver chloride reference electrode
20 consists of platinum wire (1), epoxy (2), silver wire (3), silver-silver chloride electrode (4), immobilized gel internal electrolyte (5) and micro-porous polymer (6). The end of the platinum wire (1) is soldered with gold male connector for connection to electronic board. The opposite end of the platinum wire (1) is soldered with silver wire (3). The upper part of the
25 reference electrode is encapsulated with epoxy (2) and other protective materials, such as silicone capsule to secure the silver wire (3) and the silver-silver chloride electrode (4) which are suspended in the immobilized gel internal electrolyte (5). The silver chloride coating is deposited on the silver wire (3) by means or electroplating process. The immobilized gel internal
30 electrolyte (5) comprises hydrogel monomers, fluorinated quaternary ammonium chloride salts, cross-linking agents and solvents. The present invention proposes an immobilized gel internal electrolyte (5) with constant

concentration of the chloride ion internal reference which is achieved by immobilizing ionic monomer salts to moisture-retaining hydrogel media wherein the cationic charge is immobilized through covalent chemical bonding to hydrophilic polymer matrix, mixed with gel media. The

5 immobilized gel internal electrolyte (5) is derived from fluorinated quaternary ammonium chloride salt I, II and III. These fluorinated quaternary ammonium chloride salts I, II and III can be immobilized to the hydrogel acrylic backbone and used as key ingredient in the electrolyte compositions. These fluorinated quaternary ammonium chloride salts I, II and III are derived from fluorinated

10 acrylamide IV, fluorinated acrylamide V and fluorinated amino acrylate VI respectively. The immobilized gel internal electrolyte I comprises dimethylacrylamide, fluorinated quaternary ammonium chloride salt I, cross-linker, deionized water and glycerol. The immobilized gel internal electrolyte II comprises acrylamide, fluorinated quaternary ammonium chloride salt II,

15 cross-linker, deionized water and sorbitol. The immobilized gel internal electrolyte III comprises hydroxyethylmethacrylate, fluorinated quaternary ammonium chloride salt III, cross-linker, deionized water and glycerol. The lower end of the reference electrode can be protected by micro-porous polymer (6) such as ceramic filter to secure the immobilized gel internal

20 electrolyte (5) and minimize the direct contact of the cell surface of reference electrode with analyte solutions.

The platinum wire (1) is designed to conduct current from the reference cell to the electronics circuits.

25

The epoxy (2) is designed to secure the silver wire (3) and the silver-silver chloride electrode (4) suspended in the immobilized gel internal electrolyte (5) and to prevent loss of moisture of the immobilized gel internal electrolyte (5). The epoxy is also designed to encapsulate the upper part of

30 the reference electrode.

The silver wire (3) is designed to undergo oxidation reaction to produce silver ion.

5 The silver-silver chloride electrode (4) is designed to undergo reduction reaction of the silver ion to produce metallic silver.

10 The immobilized gel internal electrolyte (5) is designed to provide constant concentration of chloride ion to capture free silver ion for the deposit of silver chloride.

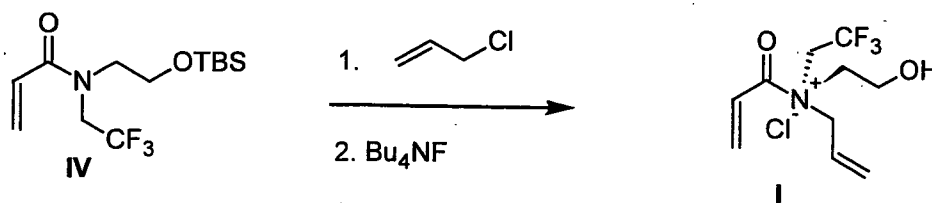
15 The micro-porous polymer (6) is designed to secure the immobilized gel internal electrolyte (5) at the tip of the reference electrode from direct contact with analyte solutions. The micro-porous polymer is also designed to encapsulate the lower end of the reference electrode.

Preparation of silver-silver chloride electrode:

20 The 1.5 cm silver wire (3) is soldered to the 2 cm platinum wire (1), washed with isopropyl alcohol and dried in the oven for 2 hours. Silver chloride coating is deposited on the silver wire (3) by electrochemical means wherein the silver wire (3) is immersed in 1M of potassium chloride solution with regulated 3.0V supply over 2 minutes to produce a silver-silver chloride electrode (4).

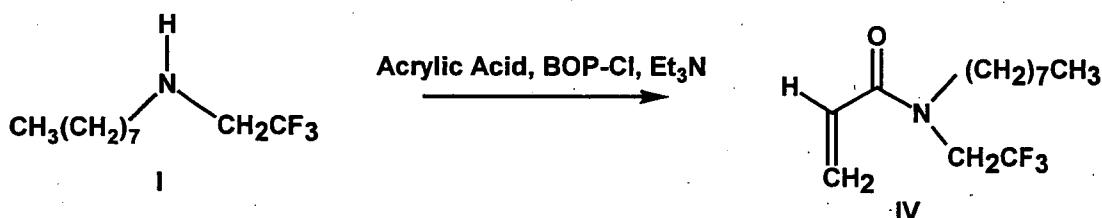
Synthesis of fluorinated quaternary ammonium chloride salt I:

25 The fluorinated quaternary ammonium chloride salt I is synthesized from fluorinated acrylamide IV, allyl chloride and tetrabutylammonium fluoride (Bu_4NF). The silyl protection group is removed following a standard procedure using tetrabutylammonium fluoride.

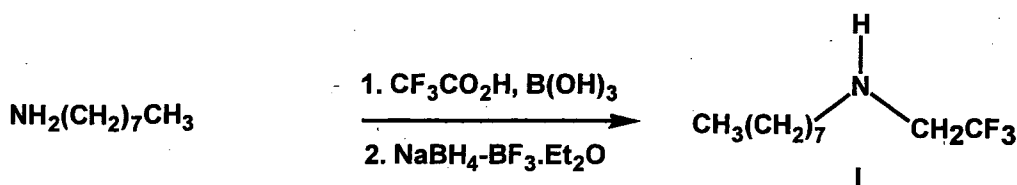


An oven-dried 250-mL three-neck, round-bottomed flask is equipped with rubber septum, a reflux condenser, mechanical stirrer and a nitrogen inlet. The flask is flushed with steady flow of nitrogen and charged with 0.075 mol of fluorinated acrylamide IV in 50 mL of freshly distilled tetrahydrofuran (THF). The flask is chilled in an ice-water bath and 0.08 mol of allyl chloride in 30 mL of freshly distilled THF is added dropwise over 30 minutes. The mixture is stirred for additional 1 hour at ice-water bath temperature and then the bath is removed to allow the mixture to warm to ambient temperature. The reaction is heated at gentle reflux for 20 hours. Deionized water (50 mL) is added, the layers are separated and the organic layer is washed twice with 25 mL portion of brine. The organic layer is dried with sodium sulfate and the solvent is removed by distillation. The residue is recrystallized in ethanol to give 0.06 mol of fluorinated quaternary ammonium chloride salt I.

The fluorinated acrylamide IV as used in the preparation of fluorinated quaternary ammonium chloride salt I is synthesized from fluorinated amine I, acrylic acid, coupling reagent BOP-Cl and triethylamine.

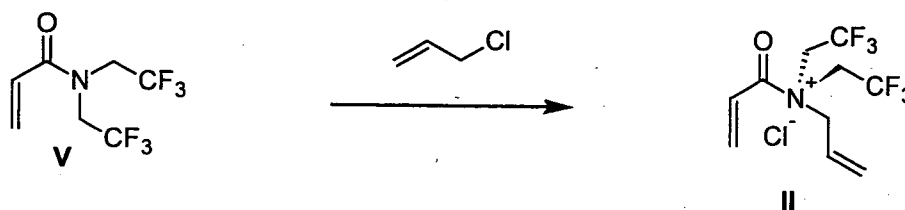


The fluorinated amine I as used in the preparation of fluorinated acrylamide IV is synthesized from trifluoroacetic acid, boric acid, $\text{NaBH}_4\text{-BF}_3\text{.Et}_2\text{O}$ and primary amine $\text{NH}_2(\text{CH}_2)_n\text{CH}_3$ wherein $n = 7, 11$.



Synthesis of fluorinated quaternary ammonium chloride salt II:

5 The fluorinated quaternary ammonium chloride salt II is synthesized from fluorinated acrylamide V and allyl chloride.

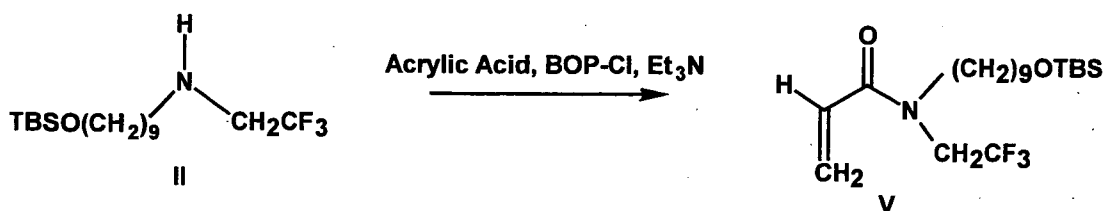


10 An oven-dried 250-mL three-neck, round-bottomed flask is equipped with rubber septum, a reflux condenser, mechanical stirrer and a nitrogen inlet. The flask is flushed with steady flow of nitrogen and charged with 0.075 mol of fluorinated acrylamide V in 50 mL of freshly distilled tetrahydrofuran (THF). The flask is chilled in an ice-water bath and 0.08 mol of allyl chloride in 30 mL of freshly distilled THF is added dropwise over 30 minutes. The mixture is stirred for additional 1 hour at ice-water bath temperature and then the bath is removed to allow the mixture to warm to ambient temperature. The reaction is heated at gentle reflux for 20 hours. Deionized water (50 mL) is added, the layers are separated and the organic layer is washed twice with 25 mL portion of brine. The organic layer is dried with sodium sulfate and the solvent is removed by distillation. The residue is recrystallized in ethanol to give 0.06 mol of fluorinated quaternary ammonium chloride salt II.

15

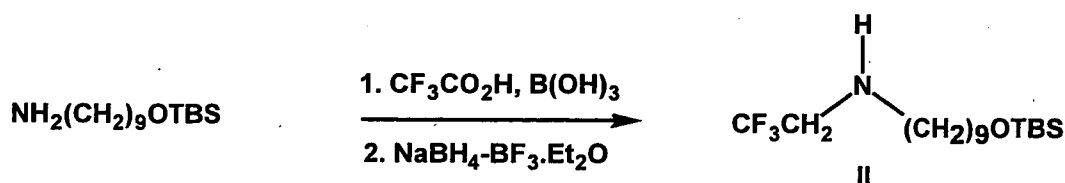
20

25 The fluorinated acrylamide V as used in the preparation of fluorinated quaternary ammonium chloride salt II is synthesized from fluorinated hydroxylamine II, acrylic acid, coupling reagent BOP-Cl and triethylamine.



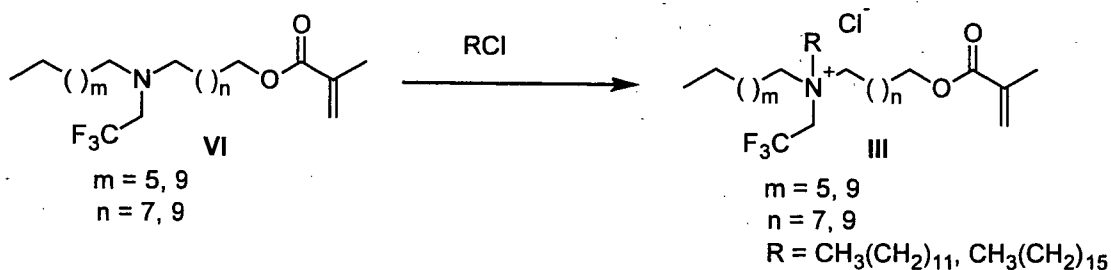
The fluorinated hydroxylamine II as used in the preparation of fluorinated acrylamide V is synthesized from trifluoroacetic acid, boric acid, NaBH₄-

5 BF₃.Et₂O and hydroxylamine NH₂(CH₂)_nOH wherein n = 9, 11.



10 Synthesis of fluorinated quaternary ammonium chloride salt III:

The fluorinated quaternary ammonium chloride salt III is synthesized from fluorinated amino acrylate VI and alkyl chloride (RCl).



15

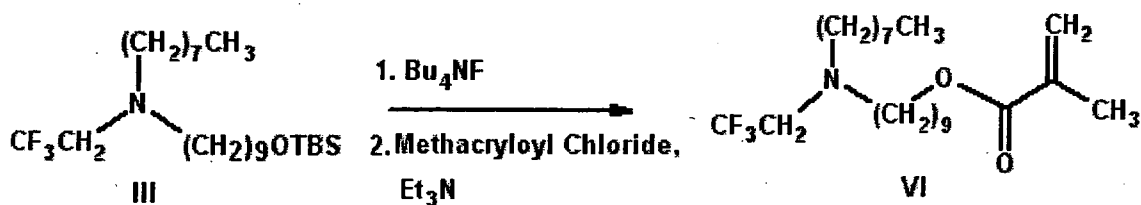
An oven-dried 250-mL three-neck, round-bottomed flask is equipped with rubber septum, a reflux condenser, mechanical stirrer and a nitrogen inlet. The flask is flushed with steady flow of nitrogen and charged with 0.075 mol of fluorinated amino acrylate VI in 50 mL of freshly distilled

20 tetrahydrofuran (THF). The flask is chilled in an ice-water bath and 0.08 mol of alkyl chloride (RCl) in 30 mL of freshly distilled THF is added dropwise over 30 minutes. The mixture is stirred for additional 1 hour at ice-water bath temperature and then the bath is removed to allow the mixture to warm to

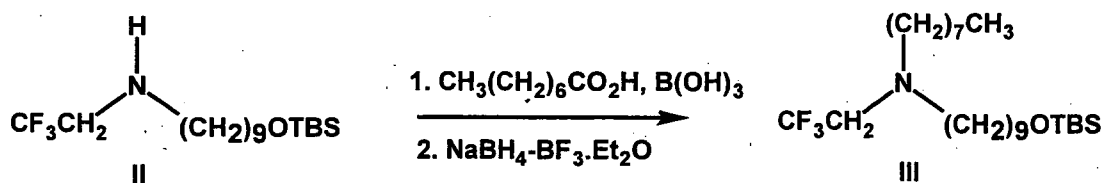
ambient temperature. The reaction is heated at gentle reflux for 20 hours.

Deionized water (50 mL) is added, the layers are separated and the organic layer is washed twice with 25 mL portion of brine. The organic layer is dried with sodium sulfate and the solvent is removed by distillation. The residue is recrystallized in ethanol to give 0.06 mol of fluorinated quaternary ammonium chloride salt III.

The fluorinated amino acrylate VI as used in the preparation of fluorinated quaternary ammonium chloride salt III is synthesized from fluorinated hydroxylamine III, tetrabutylammonium fluoride (Bu_4NF), methacryloyl chloride and triethylamine.



The fluorinated hydroxylamine III as used in the preparation of fluorinated amino acrylate VI is synthesized from fluorinated hydroxylamine II, boric acid, $\text{NaBH}_4\text{-BF}_3\text{.Et}_2\text{O}$ and carboxylic acid $\text{CH}_3(\text{CH}_2)_n\text{COOH}$ wherein $n = 6, 10$.



Synthesis of fluorinated amine I, hydroxylamines II and III:

An oven-dried 250-mL three-neck, round-bottomed flask is equipped with rubber septum, a Dean-Stark trap topped with a reflux condenser fitted with a nitrogen inlet, and a Teflon-coated magnetic stirring bar. The flask is flushed with steady flow of nitrogen and charged with appropriate carboxylic

acids such as trifluoroacetic acid (30 mmol), boric acid (0.30 mmol), and 100 mL of toluene. The colorless reaction mixture is stirred for 30 minutes and solution of appropriate amines such as excess of n-octylamine (32 mmol) is added dropwise to the reaction mixture. The mixture is heated at gentle reflux
5 for 20 hours. The mixture is allowed to cool to ambient temperature and then the mixture is poured with stirring into a 200 mL of hexane. Precipitation of a white solid is observed. Stirring is continued for an additional 30 minutes and then the precipitates are filtered off under vacuum suction.

10 The diamide intermediate is reduced to the corresponding amines using $\text{NaBH}_4\text{-BF}_3\cdot\text{Et}_2\text{O}$ mixture as described in the following:

A solution of boron trifluoride etherate ($\text{BF}_3\cdot\text{Et}_2\text{O}$) (0.007 mmol) in freshly distilled anhydrous tetrahydrofuran (THF) (20mL) is added cautiously
15 to a solution of sodium borohydride (NaBH_4) (0.02 mmol) and amide intermediate (0.015 mmol) in 30mL of anhydrous THF. The flask is flushed with steady flow of nitrogen flow and the addition is done at ambient temperature. The reaction mixture is heated to gentle reflux. Thin Layer Chromatography (TLC) is performed to monitor the progress of the reaction.
20 Upon completion of the reaction, the flask is chilled in an ice-water bath and the reaction mixture is quenched with dropwise of water. After stirring for an additional 30 minutes, water is added and the organic layer is separated, washed three times with brine and dried with magnesium sulfate (MgSO_4). The amine-borane complex is dissolved in 50 mL of water and 10 mL of 10%
25 of sodium hydroxide solution is added followed by 10 mL of 30% hydrogen peroxide. The reaction mixture is extracted with ethyl acetate and the solvent is removed by distillation to yield fluorinated amines.

Synthesis of fluorinated acrylamides IV and V:

30 An oven-dried 250-mL three-neck, round-bottomed flask is equipped with rubber septum, a Dean-Stark trap topped with a reflux condenser fitted with a nitrogen inlet, and a Teflon-coated magnetic stirring bar. The flask is

charged with 30 mmol of acrylic acid, 30 mmol of appropriate fluorinated amines such as amine I and 40 mmol of triethyl amine in 50 mL of freshly distilled dichloromethane. Coupling reagent BOP-Cl (30 mmol) in 20 mL of anhydrous dichloromethane is added. The colorless reaction mixture is heated at reflux for 20 hours. The mixture is washed twice with 20 mL portions of sodium bicarbonate and twice with 20 mL portions of distilled water. The solvent is removed by distillation through Vigreux column. The residue is purified by running through silica gel column to give 25 mmol of white solid of fluorinated acrylamides.

10

Synthesis of fluorinated amino acrylate VI:

An oven-dried 250-mL three-neck, round-bottomed flask is equipped with rubber septum, a Dean-Stark trap, a reflux condenser, addition funnel and a Teflon-coated magnetic stirring bar. The flask is charged with appropriate hydroxyl amines such as hydroxylamine III (20 mmol) and 25 mL of freshly distilled THF. Freshly distilled triethylamine (23 mmol) is added to the mixture. Methacryloyl chloride (20 mmol) is added dropwise while the flask is chilled in an ice-water bath. The mixture is stirred for 1 hour and the reaction is quenched with 50 mL of sodium bicarbonate solution. The organic layer is washed twice with 50 mL portions of deionized water and dried with anhydrous magnesium sulfate (MgSO_4). The solvent is removed by distillation through Vigreux column and the residue is purified through silica gel column to yield 18 mmol of fluorinated acrylates.

15

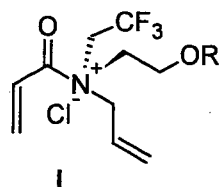
20

Preparation of immobilized gel internal electrolyte I:

The immobilized gel internal electrolyte I is prepared with the use of fluorinated quaternary ammonium chloride salt I. The fluorinated quaternary ammonium chloride salt I is freshly prepared and purified before being immobilized to hydrogel media. The fluorinated quaternary ammonium chloride salt I has the formula:

25

30



R = H, CH₃, CH₂CH₃, CF₃

4 mL of deionized water and 6 mL of glycerol is added into a 50-mL beaker. The mixture is stirred gently with a Teflon-coated magnet bar for 10 minutes and 5 mL of dimethylacrylamide is added to the stirred mixture. The freshly prepared fluorinated quaternary ammonium chloride salt I (24% of total weight) is added gradually to the mixture under gentle stirring over 10 minutes. Stirring is continued until solid material is completely dissolved. 0.2g of N,N'-methylene-bisacrylamide, 0.05 mL of N,N,N',N'-tetraethylenediamine and 0.01g of ammonium persulfate are added to the mixture while gentle stirring is continued. Stirring is continued for additional 2 hours before the gel mixture is transferred into the reference electrode chamber.

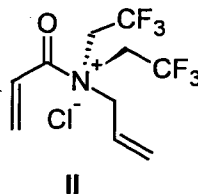
Table 1. Composition of immobilized gel internal electrolyte I based on fluorinated quaternary ammonium chloride salt I.

Component	% Weight
Dimethylacrylamide	25%
Fluorinated quaternary ammonium chloride salt I	24%
Cross-linker	3%
Deionized water	20%
Glycerol	28%

Preparation of immobilized gel internal electrolyte II:

The immobilized gel internal electrolyte II is prepared with the use of fluorinated quaternary ammonium chloride salt II. The fluorinated quaternary ammonium chloride salt II is freshly prepared and purified before being

immobilized to hydrogel media. The fluorinated quaternary ammonium chloride salt II has the formula:



4 mL of deionized water and 9 mL of sorbitol is added into a 50-mL beaker. The mixture is stirred gently with a Teflon-coated magnet bar for 10 minutes and 5 mL of acrylamide is added to the stirred mixture. The freshly prepared fluorinated quaternary ammonium chloride salt II (15% of total weight) is added gradually to the mixture under gentle stirring over 10 minutes. Stirring is continued until solid material is completely dissolved. 0.4g of N,N'-methylene-bisacrylamide, 0.1 mL of N,N,N',N'-tetraethylenediamine and 0.01g of ammonium persulfate are added to the mixture while gentle stirring is continued. Stirring is continued for additional 2 hours before the gel mixture is transferred into the reference electrode chamber.

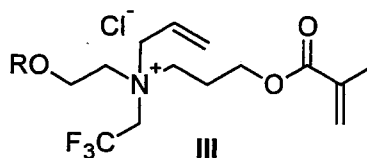
Table 2. Composition of immobilized gel internal electrolyte II based on fluorinated quaternary ammonium chloride salt II.

Component	% Weight
Acrylamide	25%
Fluorinated quaternary ammonium chloride salt II	15%
Cross-linker	4%
Deionized water	20%
Sorbitol	36%

Preparation of immobilized gel internal electrolyte III:

The immobilized gel internal electrolyte III is prepared with the use of fluorinated quaternary ammonium chloride salt III. The fluorinated quaternary

ammonium chloride salt III is freshly prepared and purified before being immobilized to hydrogel media. The fluorinated quaternary ammonium chloride salt III has the formula:



R = H, CH₃, CH₂CH₃, CF₃

5

4 mL of deionized water and 7.5 mL of glycerol is added into a 50-mL beaker. The mixture is stirred gently with a Teflon-coated magnet bar for 10 minutes and 6.5 mL of 2-hydroxyethyl methacrylate is added to the stirred mixture. The freshly prepared fluorinated quaternary ammonium chloride salt III (20% of total weight) is added gradually to the mixture under gentle stirring over 10 minutes. Stirring is continued until solid material is completely dissolved. 0.2g of N,N'-methylene-bisacrylamide, 0.05 mL of N,N,N',N'-tetraethylenediamine and 0.01g of ammonium persulfate are added to the mixture while gentle stirring is continued. Stirring is continued for additional 2 hours before the gel mixture is transferred into the reference electrode chamber.

15

Table 3. Composition of immobilized gel reference electrolyte III based on fluorinated quaternary ammonium chloride salt III.

Component	% Weight
Hydroxyethyl methacrylate	27%
Fluorinated quaternary ammonium chloride salt III	20%
Cross-linker	3%
Deionized water	20%
Glycerol	30%

20

Assembly of maintenance-free silver-silver chloride reference electrode:

The end of the platinum wire (1) of the reference electrode is soldered to gold male connector. The silver-silver chloride electrode (4) is fitted into a 5-uL pipette tip and secured at the silver-platinum junction with acrylic and epoxy encapsulation materials (2). The lower end of the reference electrode is temporarily closed with paraffin film wherein the immobilized gel internal electrolyte (5) is transferred into the reference electrode under vacuum through the opened upper part of the reference electrode. The immobilized gel internal electrolyte (5) is polymerized into gel form at room temperature within a few hours.

Characterization of reference electrode performance:

An electrode setup comprises commercially available double-junction Orion reference electrode and the maintenance-free silver-silver chloride reference electrode of the present invention wherein both the electrodes are connected to Orion ionmeter. The double-junction Orion reference electrode is used as a stable reference for the above said combined electrodes.

Potential difference of the electrode setup is observed through a period of 60 hours to record the response variation. If the present invention is stable, less than 2mV variation is observed in buffered solutions at pH 4, 7 and 10 and in potassium chloride and potassium nitrate solutions over 10^{-7} M and 1 M concentration range.

Claims

1. A silver-silver chloride reference electrode comprising:
 - a platinum wire (1);
 - 5 - an epoxy (2);
 - a silver wire (3);
 - a silver-silver chloride electrode (4);
 - an immobilized gel internal electrolyte (5); and
 - a micro-porous polymer (6).
- 10 2. A silver-silver chloride reference electrode according to claim 1 which is maintenance free.
3. A silver-silver chloride reference electrode according to claim 1 wherein
15 the platinum wire (1) is designed for conducting current from the reference electrode to the electronic circuits.
4. A silver-silver chloride reference electrode according to claim 1 wherein
20 the end of platinum wire (1) is soldered with gold male connector for connection to electronic board.
5. A silver-silver chloride reference electrode according to claim 1 wherein the opposite end of platinum wire (1) is soldered with silver wire (3).
- 25 6. A silver-silver chloride reference electrode according to claim 1 wherein the epoxy (2) is designed for
 - securing the silver wire (3) and the silver-silver chloride electrode (4)
 - suspended in the immobilized gel internal electrolyte (5).
 - preventing loss of moisture of the immobilized gel internal electrolyte
 - 30 (5).
 - encapsulating the upper part of the reference electrode.

7. A silver-silver chloride reference electrode according to claim 1 wherein the epoxy (2) is silicone capsule.
8. A silver-silver chloride reference electrode according to claim 1 wherein the silver wire (3) is designed for undergoing oxidation reaction to produce silver ion.
9. A silver-silver chloride reference electrode according to claim 1 wherein the silver wire (3) is suspended in the immobilized gel internal electrolyte (5).
10. A silver-silver chloride reference electrode according to claim 1 wherein the silver wire (3) is coated with silver chloride coating.
11. A silver-silver chloride reference electrode according to claim 1 wherein the silver-silver chloride electrode (4) is designed for undergoing reduction reaction of the silver ion to produce metallic silver.
12. A silver-silver chloride reference electrode according to claim 1 wherein the silver-silver chloride electrode (4) is suspended in the immobilized gel internal electrolyte (5).
13. A silver-silver chloride reference electrode according to claim 1 wherein the immobilized gel internal electrolyte (5) is designed for providing constant concentration of chloride ion to capture free silver ion for producing silver chloride precipitate.
14. A silver-silver chloride reference electrode according to claim 1 wherein the immobilized gel internal electrolyte (5) comprises hydrogel monomers, fluorinated quaternary ammonium chloride salts, cross-linking agents and solvents.

15. The immobilized gel internal electrolyte (5) according to claim 14 wherein the cross-linking agents are N,N'-methylene-bisacrylamide, N,N,N',N'-tetraethylenediamine and ammonium persulfate.
- 5 16. The immobilized gel internal electrolyte (5) according to claim 14 wherein the solvents are deionized water, glycerol and sorbitol.
17. A silver-silver chloride reference electrode according to claim 1 wherein the immobilized gel internal electrolyte (5) is immobilized gel internal
10 electrolyte I.
18. The immobilized gel internal electrolyte I according to claim 17 comprises dimethylacrylamide, fluorinated quaternary ammonium chloride salt I, cross-linker, deionized water and glycerol.
- 15 19. A silver-silver chloride reference electrode according to claim 1 wherein the immobilized gel internal electrolyte (5) is immobilized gel internal electrolyte II.
- 20 20. The immobilized gel internal electrolyte II according to claim 19 comprises acrylamide, fluorinated quaternary ammonium chloride salt II, cross-linker, deionized water and sorbitol.
21. A silver-silver chloride reference electrode according to claim 1 wherein
25 the immobilized gel internal electrolyte (5) is immobilized gel internal electrolyte III.
22. The immobilized gel internal electrolyte III according to claim 21
30 comprises hydroxyethylmethacrylate, fluorinated quaternary ammonium chloride salt III, cross-linker, deionized water and glycerol.

23. A silver-silver chloride reference electrode according to claim 1 wherein the micro-porous polymer (6) is designed for

- securing the immobilized gel internal electrolyte (5) at the tip of the reference electrode from direct contact with analyte solutions.
- 5 - encapsulating the lower end of the reference electrode.

24. A silver-silver chloride reference electrode according to claim 1 wherein the micro-porous polymer (6) is ceramic filter.

10

15

20

25

30

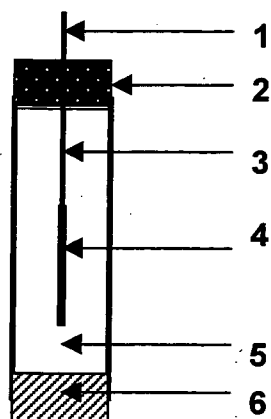


Figure 1