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[54] **POTENTIOSTATIC PREPARATION OF MOLECULAR ADSORBATES FOR SCANNING PROBE MICROSCOPY**

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[58] **Field of Search** 250/307, 304, 250/440.1, 306; 361/234; 204/153.1, 400, 403

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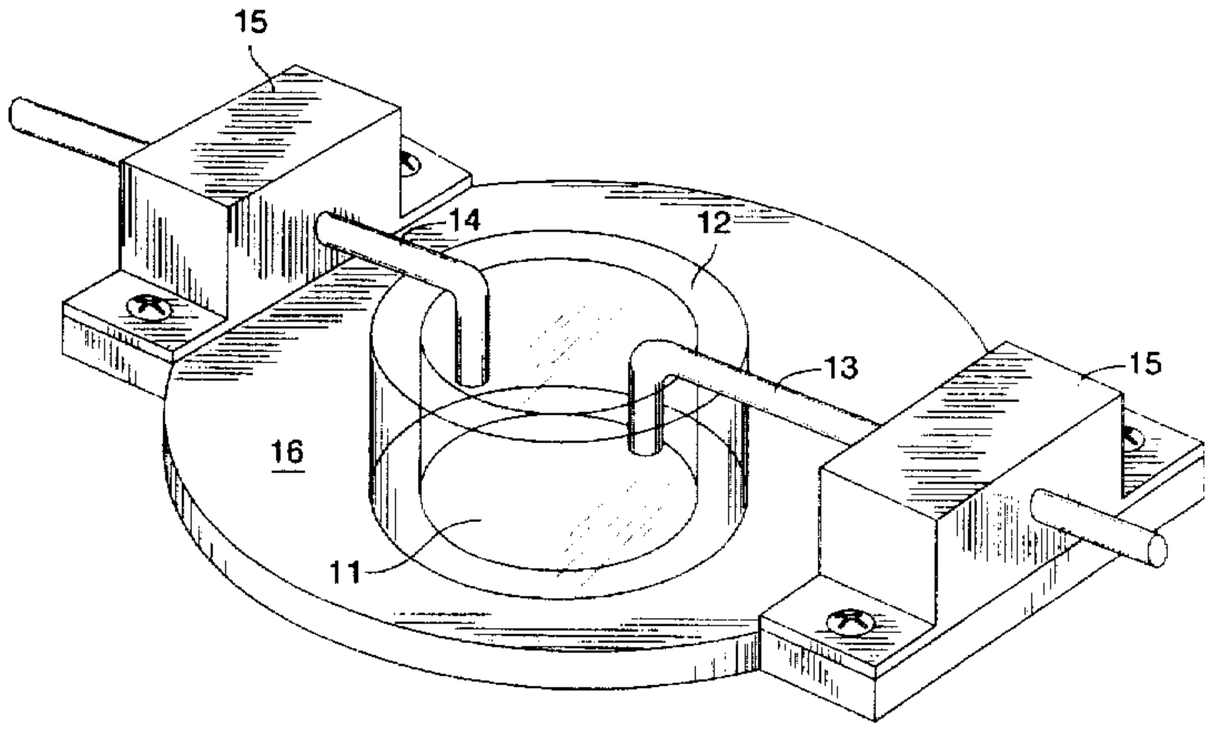
[57] **ABSTRACT**

[A method of**]** *An apparatus and method for* preparing molecular adsorbates for scanning probe microscopy by potentiostatic methods. Negatively charged molecules are deposited upon and held to a substrate with an electrochemical cell having a gold substrate, a platinum wire counter electrode and a silver wire reference electrode. The polymer to be observed is dissolved into a buffer solution which is non-reactive with the substrate which is gold (**111**).

40 Claims, 1 Drawing Sheet



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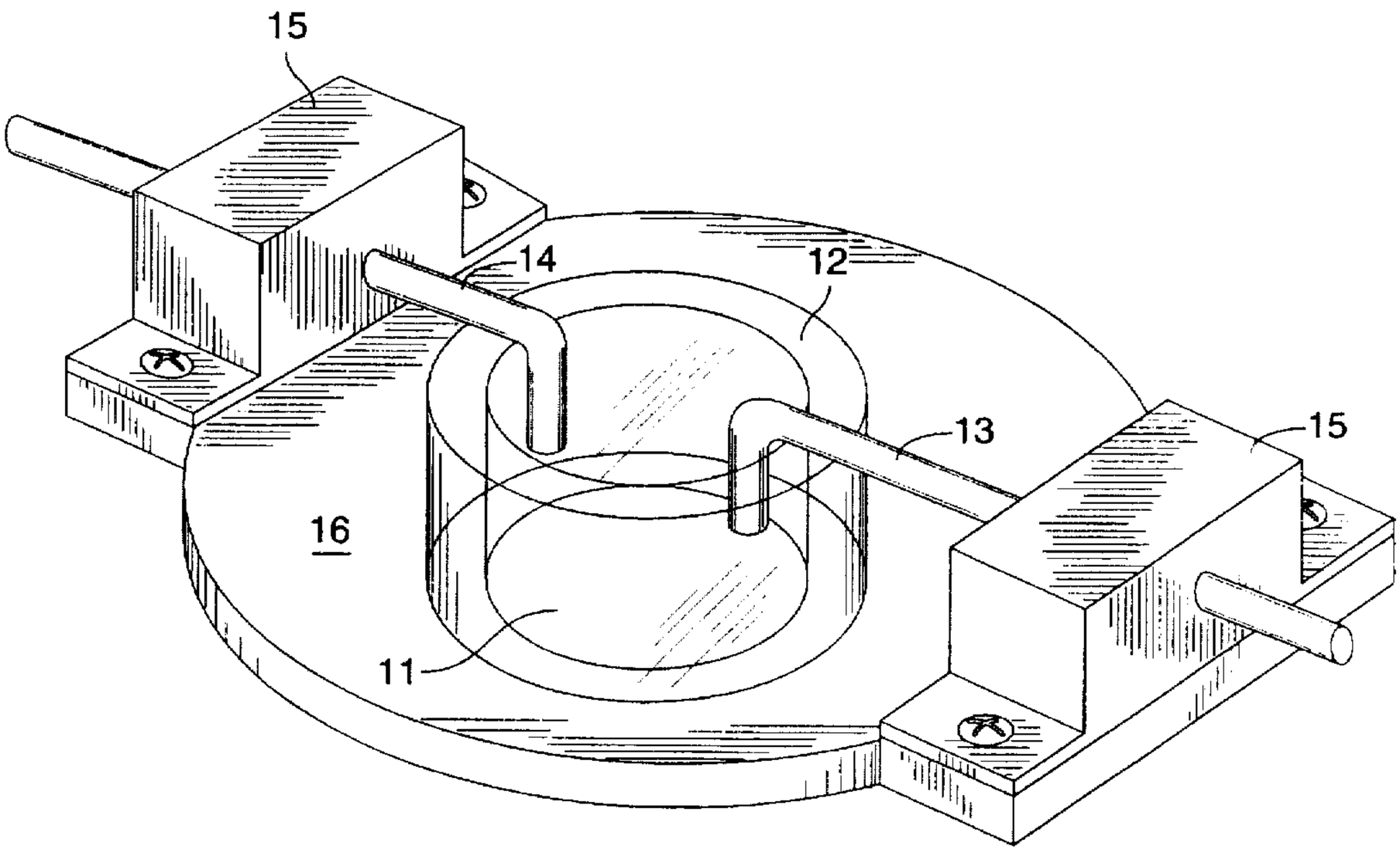

FIG. 1

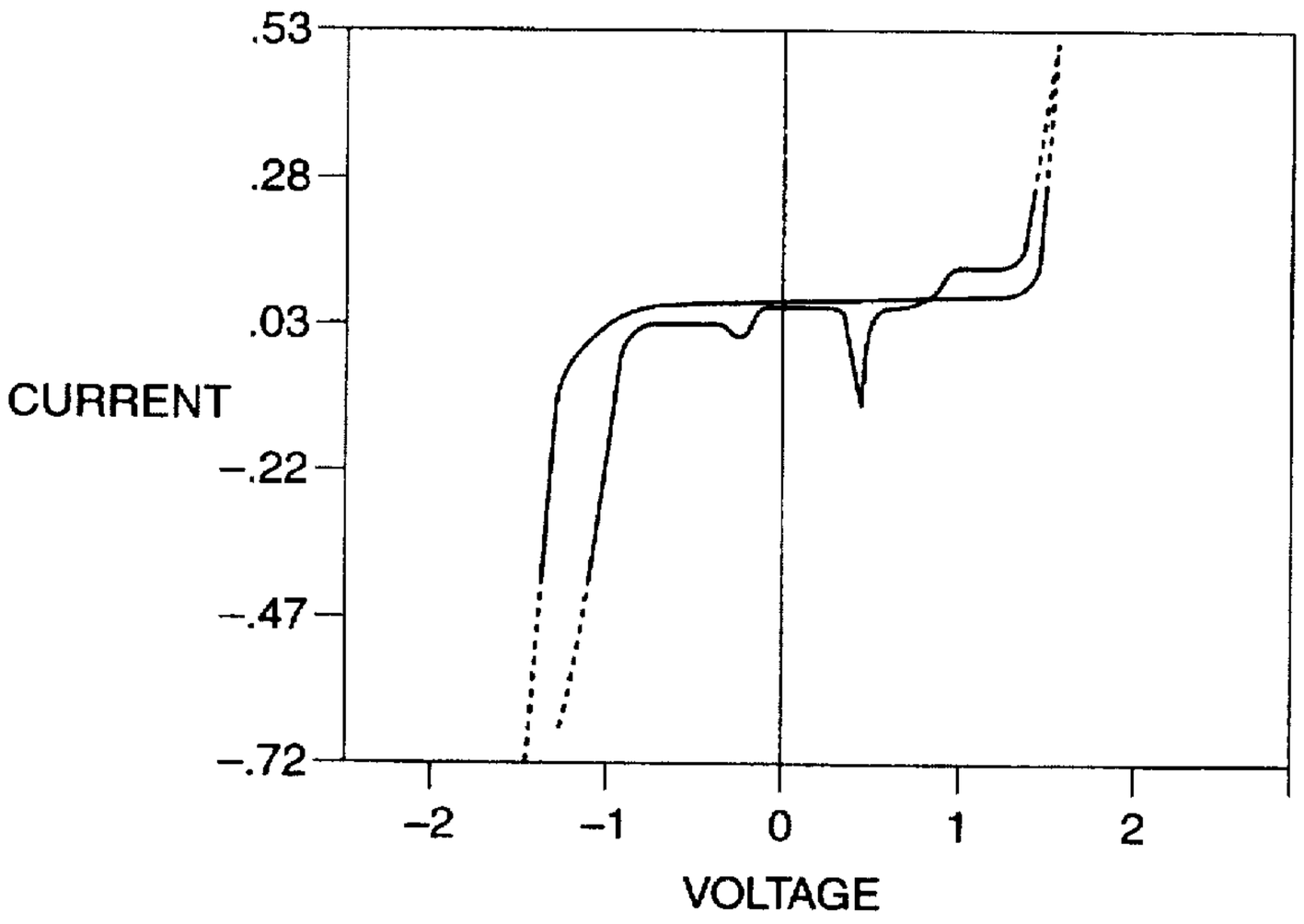

FIG. 2

POTENTIOSTATIC PREPARATION OF MOLECULAR ADSORBATES FOR SCANNING PROBE MICROSCOPY

Matter enclosed in heavy brackets [ ] appears in the original patent but forms no part of this reissue specification; matter printed in italics indicates the additions made by reissue.

INTRODUCTION

[This] *The* present invention relates generally to scanning probe microscopy and more particularly to *an apparatus and method for* the potentiostatic preparation of molecular adsorbates for study with scanning probe microscopes.

This invention was made with Government support under contract No. N00014-90-J-1655 awarded by the Department of the Navy and grant DIR 89-20053 awarded by the National Science Foundation. The Government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Various ways have heretofore been proposed for chemically reacting molecules with a metal substrate in an electrochemistry cell. In some cases, the prior methodology allows molecules to be bonded strongly enough so that they can be imaged in a scanning tunnelling microscope (STM) or in [the] *an* atomic force microscope (AFM). However, in the case of negatively charged molecules, such as DNA, it is extremely difficult to get them to adhere to an electrode because most metal surfaces are intrinsically negatively charged and, as such, repel the molecule. Thus a clear need exists for new and improved technology for the potentiostatic preparation of negatively charged [molecules] *molecular* adsorbates such as DNA to enable them to be properly bonded to *a* suitable substrate so *that* they can be imaged in *a* scanning tunnelling microscope [(STM)] or *an* atomic force [microscopes (AFM)] *microscope*. It is [toward] *to* this end that the present invention is directed.

BRIEF SUMMARY OF THE INVENTION

The present invention is predicated upon the discovery of a remarkably simple procedure for getting negatively charged molecules onto a substrate and holding them there. The new methodology is based in part on the concept that DNA (or any other negatively charged molecule) can be attracted to a surface which is positively charged by virtue of its interaction with an electrolyte ([See: Lindsay et al, 1988] *See: Lindsay, S. M., and Barris, B., "Imaging DNA Molecules on a Metal Surface Under Water by STM", Journal of Vacuum Science and Technology. Vol. A6, Pages 544–547 (1988)*). What is new and unexpected is that the same forces that attract the molecules to the surface are capable, in the practice of the present invention, of holding such molecules in place on that surface for study in an [SPM] *STM* or an AFM. More particularly, the present invention relates to the potentiostatic preparation of molecular adsorbates for scanning probe microscopy in an electrochemical cell having a gold substrate, a platinum wire counter electrode and a silver wire reference electrode placed upon the microscope. The polymer to be observed is dissolved into a buffer solution which is non-reactive with the gold in the substrate and thereafter quickly deposited upon the substrate. Once the cell is filled, the reference electrode and the counter [electrodes] *electrode* are connected and a stable layer of adsorbate is formed on the gold

electrode where it can be readily scanned with the microscope probe.

Accordingly, the principle object of the present invention is to provide new and improved methodology for preparing molecular adsorbates for scanning probe microscopy.

Another object of the present invention is to provide methodology especially adapted to potentiostatically prepared negatively charged molecular adsorbates for scanning probe microscopy.

These and still further objects as shall hereinafter appear are readily fulfilled by the present invention in a remarkably unexpected manner as will be readily discerned from the following detailed description of an exemplary embodiment thereof especially when read in conjunction with the accompanying drawing [in which like parts bear like numerals throughout the several views].

BRIEF DESCRIPTION OF THE DRAWINGS

In the drawings:

FIG. **1**[.] is a schematic drawing of a simple electrochemical cell *according to the present invention*; and

FIG. **2** is a cyclic voltammogram of a solution used for deposition of DNA taken in situ using a phosphate buffer solution adjusted to pH=6 with NaOH and containing [5 micrograms 5/ml] *5 micrograms per milliliter* of DNA.

DESCRIPTION OF PREFERRED EMBODIMENT

A small electrochemistry cell is mounted on an STM or AFM as described in my prior U.S. Pat. No. 4,868,396. A sketch of the current simplified cell is shown in FIG. **1**. The substrate is Au (**111**), the counter electrode a platinum wire and the reference electrode a silver wire. It has been discovered that silver wires produce results that are identical to Ag/AgCl/KCl reference electrodes in these particular solutions, but are much easier to use and do not cause chlorine contamination of the substrate.

One practical arrangement of an electrochemistry cell is shown in FIG. **1**. Referring to FIG. **1**, the electrochemistry cell is designated by the general reference **10** and comprises a gold-on-mica substrate **11**[,] *and* a glass cell **12**, having a polished bottom that forms a seal against the gold substrate. A platinum (Pt) wire counter electrode **13** and a silver (Ag) wire reference electrode **14** extend into cell **12**. Each of these wires are longer than needed and a fresh cut surface is introduced into the cell for each experiment by advancing the [electrode] *electrodes* **13**, **14** in [its] *their* respective electrode [holder] *holders* **15**, **15**. A stainless steel plate **16** is glued to the lower exterior surface of glass cell **12** to hold down the substrate **11** and make electrical contact with the gold.

Cleanliness is critical. As will appear, an excess length of wire is used for each wire electrode. Before starting the next run, the used portion of the wire is cut away and a portion of the fresh wire is advanced into the cell. A fresh gold substrate is also used for each run.

In one practice of the present invention, a substrate is loaded onto the SPM. Clean reference and counter electrodes are placed into a clean glass cell on the substrate as shown in FIG. **1**. The polymer is dissolved into a buffer solution that does not react with the gold over the appropriate range of substrate potentials. One suitable buffer solution for use with Au (**111**) between −1.2 and [=] + 1.3 V (vs. the Ag reference) is NaH_2PO_4[.] (10 mM adjusted to pH6 with NaOH). A cyclic voltammogram taken in situ is

shown in FIG. 2. For sparse coverage of the electrode, a solution that, at full adsorption, gives less [that] *than* a monolayer coverage of the macromolecule is used. For example, in a 50 microliter cell (0.5 cm^2 electrode area) less than 5 micrograms of DNA per mL of solution are required.

The solution is placed onto the substrate as quickly as possible (to minimize contamination) and, once the cell is full, the reference and counter electrodes are connected. Any positive potential (in case of DNA) between the potentials at which reactions occur (from –0.2 V vs. Ag to +0.6 V vs. Ag; the DNA bases oxidize at higher voltage) may be applied to the substrate. There are some small reversible phosphate [absorptions] *adsorptions* at lower potentials, but in the double-layer region macromolecules can be seen in stable arrangements all the way up to about 1 volt. The voltage employed in any given reading is correlated to the reference electrode. The voltage required for deposition is dependant upon the salt solution used. The values reported herein are for the phosphate buffer solution.

If the solutions are free of contamination and, in the case of the STM, the tip is well insulated, a very stable layer of [absorbate] *adsorbate* is formed on the gold electrode. It may be scanned in situ repeatedly with no sign of sample movement or degradation. Indeed this is the salient feature of this invention, namely that [the] an adsorbate, when under potentiostatic control, is remarkably stable. Furthermore, the adsorbate layer may be lifted [on and off] *off and placed back on* the electrode surface at will simply by cycling the substrate potential between a positive value and –0.2 V (vs. Ag). Of course, these conditions represent a much diminished disruption of the solvated structure of the polymers compared to methods where the adsorbate is chemically reacted onto the substrate.

The coverage of the substrate, even with the simple layout shown in FIG. 1, is remarkably homogeneous. The whole problem of molecular microscopy is now reduced to scanning an area large enough to contain a few molecules (as calculated from the expected coverage, given the cell geometry and sample concentration) and then applying a suitable potential. Furthermore, reactions and various dynamic processes may be studied simply by allowing them to proceed in the cell (using components and a potential that avoid irreversible reactions) and then applying [and] *an* attractive charge to the substrate.

Finally it should be noted that the problem of contamination is greatly reduced. Only those molecules that satisfy conditions for physical adsorption appear in the image. In contrast a vacuum or ambient image would show all contaminants.

This method can also be used to hold positively charged molecules providing the reaction current due to dissolved oxygen is eliminated. This may be done *by* using degassed solutions and by operating in an inert gas [enviroment] *environment*.

Experiments demonstrate that this method yields excellent high resolution images of macromolecular [absorbates] *adsorbates* in both the STM and the AFM. The electrodes and buffers herein disclosed are intended as representative preferred materials and not by way of limitation thereon.

From the foregoing, it is readily apparent that a useful embodiment of the present invention has been herein described and illustrated which fulfills all of the [aforestated] *aforementioned* objectives in a remarkably unexpected fashion. It is of course understood that such modifications, alterations and adaptations as may readily occur to the artisan confronted with this disclosure are intended within the spirit of this disclosure which is limited only by the scope of the claims appended hereto.

Accordingly, what is claimed is:

1. A method of preparing molecular adsorbates for scanning probe microscopy comprising: loading a substrate into a cell; placing the cell on a scanning probe microscope; placing a clean reference electrode in said cell; placing a clean counter electrode in said cell in spaced relationship to said reference electrode; dissolving a polymer containing negatively charged molecules into a buffer solution that is inert relative to said substrate; filling said cell with said polymer [contained] *containing* buffer solution; activating said reference electrode and said counter electrode and applying a potential to said substrate to deposit and secure said polymer onto said substrate for examination by said microscope.

2. A method according to claim **1** in which said substrate is gold (**111**).

3. A method according to claim **1** in which said reference electrode is silver wire.

4. A method according to claim **1** in which said counter electrode is platinum wire.

5. A method according to claim **1** in which said buffer solution is NaH_2PO_4 at a pH of 6.

6. A method according to claim **3** in which said electrodes are activated to a voltage of from about –1.2 up to about + 1.3.

7. A method according to claim **5** in which said electrodes are activated to a voltage of from about –1.2 up to about + 1.3.

8. A method according to claim **2** in which said reference electrode is silver wire.

9. A method according to claim **8** in which said counter electrode is platinum wire.

10. A method according to claim **9** in which said buffer solution is NaH_2PO_4 at a pH of 6.

11. A method according to claim **10** in which said electrodes are activated to a voltage of from about –1.2 up to about + 1.3.

12. A method of preparing molecular adsorbates for scanning probe microscopy comprising:

loading a substrate into a cell;

placing the cell on a scanning probe microscope;

placing a clean reference electrode in said cell;

placing a clean counter electrode in said cell in spaced relationship to said reference electrode;

dissolving a polymer containing charged molecules into a buffer solution that is inert relative to said substrate;

filling said cell with said polymer containing buffer solution;

activating said reference electrode and said counter electrode; and

applying a potential between said substrate and said counter electrode to deposit and secure said polymer onto said substrate for examination by said microscope.

13. A method according to claim 12 in which said substrate comprises gold.

14. A method according to claim 12 in which said reference electrode is silver wire.

15. A method according to claim 12 in which said counter electrode is platinum wire.

16. A method according to claim 12 in which said buffer solution is NaH_2PO_4 at a pH of 6.

17. A method according to claim 13 in which said substrate is activated to a voltage in a range of about –1.2

volts up to about +1.3 volts with respect to said reference electrode by application of a suitable voltage to said counter electrode.

18. A method according to claim 14 in which said substrate is activated to a voltage in a range of about −1.2 volts up to about +1.3 volts with respect to said reference electrode by application of a suitable voltage to said counter electrode.

19. A method according to claim 15 in which said substrate is activated to a voltage in a range of about −1.2 volts UP to about +1.3 volts with respect to said reference electrode by application of a suitable voltage to said counter electrode.

20. A method according to claim 16 in which said substrate is activated to a voltage in a range of about −1.2 volts up to about +1.3 volts with respect to said reference electrode by application of a suitable voltage to said counter electrode.

21. A method according to claim 13 in which said reference electrode is silver wire.

22. A method according to claim 15 in which said reference electrode is silver wire.

23. A method according to claim 13 in which said counter electrode is platinum wire.

24. A method according to claim 16 in which said counter electrode is platinum wire.

25. A method according to claim 23 in which said buffer solution is NaH_2PO_4 *at a pH of 6.*

26. A method according to claim 25 in which said substrate is activated to a voltage in a range of about −1.2 volts up to about +1.3 volts with respect to said reference electrode by application of a suitable voltage to said counter electrode.

27. An electrochemical fluid cell and substrate assembly for use in studying molecular adsorbates with a scanning probe microscope, said cell comprising:

an electrically conductive substrate;

an electrically insulating cell wall having an inner boundary defining a fluid container open at its top, said fluid container having a bottom boundary defined by said substrate;

said substrate having a portion extending under and beyond said inner boundary;

a reference electrode extending from beyond said fluid container into said fluid container through said top;

a counter electrode extending from beyond said fluid container into said fluid container through said top and maintained in spaced relationship to said reference electrode;

a voltage potential capable of being applied to said substrate by connecting said voltage potential to said electrically conductive substrate at said portion extending under and beyond said inner boundary.

28. An electrochemical fluid cell and substrate assembly according to claim 27 wherein said substrate comprises gold.

29. An electrochemical fluid cell and substrate assembly according to claim 27 wherein said reference electrode is silver wire.

30. An electrochemical fluid cell and substrate assembly according to claim 27 wherein said counter electrode is platinum wire.

31. An electrochemical fluid cell and substrate assembly according to claim 28 wherein said reference electrode is silver wire.

32. An electrochemical fluid cell and substrate assembly according to claim 28 wherein said counter electrode is platinum wire.

33. An electrochemical fluid cell and substrate assembly according to claim 28 wherein said reference electrode is silver wire and said counter electrode is platinum wire.

34. An electrochemical fluid cell and substrate assembly for use in studying molecular adsorbates with a scanning probe microscope, said cell comprising:

an electrically conductive substrate;

an electrically insulating cell wall having an inner boundary defining a fluid container open at its top, said fluid container having a bottom boundary defined by said substrate;

said substrate in electrical contact with a conductor having a portion extending under and beyond said inner boundary;

a reference electrode extending from beyond said fluid container into said fluid container through said top;

a counter electrode extending from beyond said fluid container into said fluid container through said top and maintained in spaced relationship to said reference electrode;

a voltage potential capable of being applied to said substrate by connecting said voltage potential to said electrically conductive substrate at said portion of said conductor extending under and beyond said inner boundary.

35. An electrochemical fluid cell and substrate assembly according to claim 34 wherein said substrate comprises gold.

36. An electrochemical fluid cell and substrate assembly according to claim 34 wherein said reference electrode is silver wire.

37. An electrochemical fluid cell and substrate assembly according to claim 34 wherein said counter electrode is platinum wire.

38. An electrochemical fluid cell and substrate assembly according to claim 35 wherein said reference electrode is silver wire.

39. An electrochemical fluid cell and substrate assembly according to claim 35 wherein said counter electrode is platinum wire.

40. An electrochemical fluid cell and substrate assembly according to claim 35 wherein said reference electrode is silver wire and said counter electrode is platinum wire.

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