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(54) **Title:** ELECTROSPRAY DEPOSITING SYSTEM FOR BIOLOGICAL MATERIALS

(57) **Abstract:** An electrospray (ES)-based deposition system enabling the coating an impervious substrate, such as a glass slide, with biological materials in a vacuum. Distilled water or a buffer is used as the solvent; no other solvents are used thereby eliminating hazardous waste from the process. Movement across differential pumping stages causes evaporation of the solvent occurs resulting in shrinkage of the remaining constituents with an increase of the charge density. The resulting ion beam enters a vacuum chamber and the beam impinges on the substrate, whereby a thin layer is deposited thereon. The spray can be focused to a specific area allowing patterning of the substrate if desired. The amount of coating can be controlled and a specified number of coats of the same or different molecules can be added to the surface.

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Electrospray Depositing System for Biological Materials

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to co-pending U.S. Patent Application **60/654,735** filed February 18, 2005, which is fully incorporated herein by reference.

GOVERNMENT SUPPORT

This invention was developed under support from the U.S. Army Research, Development and Engineering Command (RDECOM) under grant DAAD13-00-C-0037; the U.S. government has certain rights in the invention.

BACKGROUND OF THE INVENTION

The present invention relates generally to the field of preparing a substrate for use in immunoassays. Specifically, the invention provides a method of directly coating biological materials on a glass surface.

A "sandwich" immunoassay measures an analyte that is bound between two antibodies; namely the capture antibody and the detection antibody. Sandwich immunoassays are utilized as a tool to specifically identify and/or detect analytes such as bacteria, fungi, viruses, and protozoa in samples. Capture molecules, such as antibodies, bind to the target cells and capture them while other debris and non-target cells in the sample are washed away.

In assays that require capture molecule attachment to an impervious substrate such as a glass slide, the glass surface must be coated with silane. A cross-linker molecule is used to cross-link the silane to the capture molecule. The chemistry involved in the silanization and cross-linking process is tedious and must be performed in the absence of oxygen using toluene, which is flammable, as the solvent. The toluene/silane contaminated reagents must then be discarded as hazardous waste.

Electrospray Ionization (ESI) involves injecting and focusing a charged stream of particles held in solution into a vacuum environment. The electrospray (ES) process allows for the deposition of a film of the desired particles onto a substrate. In the ESI process, the solute molecules are directed toward the substrate based not only on their kinetic energy, but based on their movement through a pressure differential. This differential is established such that the stream of solute molecules will move toward the target chamber, which is held at a lower

pressure than at the point of injection. To achieve this pressure difference, differential pumping stages are used. One example of the equipment used to accomplish this consists of two rotary vane pumps, one on each side of an orifice. The orifice size and pumping speeds are be balanced to achieve a good transmission rate of solute molecules, while preserving the pressure differential necessary to guide the solute to the target.

SUMMARY OF INVENTION

The present invention includes an electrospray-based process using in-vacuum deposition of the constituents of a sandwich immunoassay. In this process, solutions containing the constituents as solute are injected into vacuum through an orifice in the vacuum chamber. As the injected beam passes through consecutive vacuum stages, the solvent is extracted and a molecular beam containing only the solute remains. This beam is directed towards the substrate and a film is deposited.

In a first embodiment the present invention provides a method of preparing a substrate for use in an assay. In the first step, a substrate solution comprising a capture molecule is prepared. The substrate solution is then converted to an electrospray as it passes through consecutive vacuum stages. The electrospray is then deposited on the substrate. In alternate embodiments the substrate solution further comprises a solvent, such as distilled water or a buffer.

The converting step may take many forms, but by way of example, further comprises the steps of providing a differential pumping mechanism having an entry portal, a plurality of differential pumping chambers and a vacuum chamber, wherein the substrate is placed. A capillary is placed in fluid communication with the entry portal. The substrate solution is then passed through the capillary toward the entry portal where it then continues the plurality of differential chambers into the vacuum chamber.

Many variations of this embodiment are envisioned. For example, the capillary may be spaced apart from the entry portal, defining an area there between. The solution is ionized as it exits the capillary in embodiments where the capillary is held at a higher voltage relative to entry portal. The area between the capillary and entry portal can also be flooded with N_2, creating a plenum to prevent the intrusion of contaminants.

In a second embodiment, the present invention includes a method of capturing an analyte present in a sample. A substrate having at least one analyte-specific capture molecule thereon is placed in a vacuum chamber. An analyte solution is then prepared comprising the sample. As with the previous embodiment, the analyte solution is converted to an electrospray and deposited on the substrate.

In a variation of the second embodiment, the analyte solution further comprises a solvent; such as distilled water or a buffer. Moreover, the analyte solution may further comprise a detection molecule capable of conjugating to the analyte.

The previous embodiments can be combined in succession to form yet another embodiment. In this third embodiment the invention provides a method of capturing an analyte, present in a sample, on a substrate. In this embodiment substrate solution comprising an analyte-specific capture molecule is prepared and converting into an electrospray. The substrate-electrospray is then deposited on the substrate. The analyte solution is then prepared. As before, the analyte solution is converted into an electrospray and deposited on the substrate in a vacuum.

BRIEF DESCRIPTION OF THE DRAWINGS

For a fuller understanding of the nature and objects of the invention, reference should be made to the following detailed description, taken in connection with the accompanying drawings, in which:

Fig. 1 is a schematic of one possible electrospray (ES) deposition system that can be used in performing the inventive method.

Fig. 2 is a block diagram of the preparation step.

Fig. 3 is a block diagram of the capturing step.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

In the following detailed description of the preferred embodiments, reference is made to the accompanying drawings, which form a part hereof, and within which are shown by way of illustration specific embodiments by which the invention may be practiced. It is to be understood that other embodiments may be utilized and structural changes may be made without departing from the scope of the invention.

The present invention includes an electrospray (ES)-based deposition system enabling the coating an impervious substrate, such as a glass slide, in a vacuum. The ES process directly introduces macromolecules from solution into high vacuum. This has led to recent applications in mass spectroscopy of heavy molecules and is now routinely used in commercially available mass spectroscopy setups. ES has also been used for thin-film deposition and the fabrication of microassays at ambient pressure for a variety of

macromolecular materials including DNA, proteins, polymers, and other materials. See Dam, *et al. Photoelectron spectroscopic Investigation of In-Vacuum-Prepared Luminescent Polymer Thin Films Directly from Solution,* Journal of Applied Physics 97, 024909 (2005), which is incorporated herein by reference, for a discussion of the deposition of macromolecular materials in vacuum.

ES, however, has not been used to prepare substrates under high-vacuum conditions for traditional sandwich assays. The ability to deposit constituents in a vacuum provides great benefit for ELISA assaying techniques, including, but are not limited to, no hazardous waste is generated, such as silane contaminated toluene, and reduced preparation time. Furthermore, the technique enables patterning and mass selection of the deposited material, *i.e.* complex molecular structures can be deposited without contamination or intersolubility issues (since the solvent is extracted before the molecules are deposited on the substrate).

In one embodiment, the solvent used is distilled water or a buffer. No hazardous waste remains after the process since no other solvent is used. The spray can be focused to a specific area of the substrate allowing patterning of the surface if so desired. The amount of coating is controlled and a specified number of coats of the same or different molecules can be added to the substrate.

In another embodiment of the instant invention, shown in FIG. 1, a constituent solution is ejected from capillary **20** in front of a first skimmer orifice **30a** at a predetermined distance, here about 10 mm. In alternate embodiments capillary **20** is held at a high voltage, *e.g.* s1–5 kVd, relative to first skimmer **30a**. The differential in voltage results in the ionization of the sprayed constituent solution. Area **25** between capillary **20** and first skimmer **30a** is flooded with N2 at slight overpressure (relative to atmosphere) to prevent entry of ambient contaminants into first differential pumping stage **40a**. Once the solution spray enters differential pumping stages **40**, rapid evaporation of the solvent occurs resulting in a shrinkage of the remaining constituents, with an increase of the charge density further helping the separation between solute and solvent molecules. Once the resulting ion beam enters main vacuum chamber **50**, most of the solvent molecules have been captured in the differential pumping stages and a relatively clean beam of solute molecules results. This beam impinges on the substrate, and a thin layer is deposited thereon.

The fabrication of immunoassays using ES based thin film deposition in vacuum is, at first glance, counterintuitive. Protein molecules are generally thought to depend on a hydration

shell to retain their shape. Conventional wisdom dictates that dehydration occurring during the deposition process in vacuum would result in denaturing of the molecules preventing the fabrication of a functional immunoassay. Surprisingly, the unexpected findings of the present invention demonstrate that denaturing does not occur and that fully functional immunoassays are produced with ES deposition in vacuum.

An illustrative embodiment is shown Figs 2 and 3. Fig. 2 illustrates the preparation step, wherein the capture molecules adhere to the surface of the substrate and maintain their biological activity. In frame **A**, Substrate solution **60** is prepared comprising capture molecule **65** and solvent **70**. In this illustrative embodiment capture molecule **65** is Streptavidin, (1 mg), suspended in 10 ml distilled water (solvent **70**). As substrate solution **60** passes differential pumping stages **40** (see Fig. 1), frame **B**, solvent **70** begins rapid evaporation resulting in a shrinkage of the remaining constituents, namely capture molecule **65**. This process leads to an increase of the charge density further helping the separation between solute and solvent molecules. Most solvent molecules have are captured in the differential pumping stages and a relatively clean beam **65a** of solute (capture) molecules **65** results. Solute beam **65a** impinges on substrate **100** and a thin layer is deposited thereon (frame **C**).

Fig. 3 illustrates the capturing step. Analyte solution **75**, frame $\mathbf{A^1}$, is prepared comprising detection molecule **80**, the analyte to be assayed **85** and solvent **90**. Here, detection molecule **80** is Biotin which is conjugated to analyte **85** *E. coli* O157:H7 antibody (1 mg). These constituents are suspended in solvent **90**, in this example 10 ml distilled water. In frame $\mathbf{B^1}$, as with the preparation step, solvent **90** begins to evaporate creating analyte beam **75a**. Analyte solution **70** passes through the ES device (Fig. 1) in the same manner as substrate solution **60**. When resulting analyte beam **75a** enters main vacuum **50** (Fig.1), analyte **85** binds to its specific target; capture molecule **65** (frame $\mathbf{C^1}$).

Both solutes are deposited using the ES method in vacuum. In the inventor's experiments a control slide was prepared in the traditional manner, discussed above, and consisted of a silanized slide cross-linked to streptavidin (100 μg/ml) and patterned using a silicon stamp with biotin labeled anti-*E. coli* O157:H7 antibody at 20 μg/ml.

Slides were prepared following the embodiment shown in Figs. 2 & 3 as discussed and used in an immunoassay. *E. coli* O157:H7 cells suspended in phosphate buffered saline (PBS) were added to the slide by flowing a sample of cells over the slide and incubating for 10-15 minutes at 24°C. The slides were washed with PBS. A solution containing Cyanine 5 (Cy5) labeled

anti *E. coli* O157:H7 antibody (10 μg/ml) was allowed to flow over the slide in channels and incubated for 5 - 10 minutes. The slide was washed with PBS, and then all PBS was pumped away. The slide was viewed by directing a 635 nm laser diode to the edge of the slide to excite the Cy5 molecules. A CCD camera was used to view the emission from the Cy5 molecules as described by Rowe et al. (1999) Rowe-Taitt, Golden et al. (2000), and Rowe-Taitt, Hazzard et al.(2000).

Photoemission spectroscopy measurements revealed that the layer thickness of the immunoassay sandwich was about 4-10 Å, corresponding to approximately mono-molecular layer thickness.

It will be seen that the objects set forth above, and those made apparent from the foregoing description, are efficiently attained and since certain changes may be made in the above construction without departing from the scope of the invention, it is intended that all matters contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall there between. Now that the invention has been described,

What is claimed is:

1. A method of preparing a substrate for use in an assay, comprising the steps of:

providing a substrate solution comprising a capture molecule;

converting the substrate solution into an electrospray; and

depositing the electrospray on the substrate in a vacuum.

2. The method of claim 1 wherein the substrate solution further comprises a solvent.

3. The method of claim 2 wherein the solvent is distilled water.

4. The method of claim 1 wherein the converting step further comprises the steps of:

providing a differential pumping mechanism having an entry portal, a plurality of differential pumping chambers and a vacuum chamber;

providing a capillary in fluid communication with the entry portal;

passing the substrate solution through the capillary toward the entry portal; and

passing the substrate solution through the plurality of differential chambers into the vacuum chamber.

5. The method of claim 4 wherein the capillary is spaced apart from the entry portal, defining an area therebetween.

6. The method of claim 4 wherein the capillary is held at a higher voltage relative to entry portal.

7. The method of claim 4 wherein the area between the capillary and entry portal is flooded with N_2.

8. The method of claim 1 wherein the substrate is a glass slide.

9. A method of capturing an analyte present in a sample, comprising the steps of:

providing a substrate having at least one analyte-specific capture molecule thereon;

providing an analyte solution comprising the sample;

converting the analyte solution into an electrospray; and

depositing the electrospray on the substrate in a vacuum.

10. The method of claim 9 wherein the analyte solution further comprises a solvent.

11. The method of claim 10 wherein the solvent is distilled water.

12. The method of claim 9wherein the analyte solution further comprises a detection molecule capable of conjugating to the analyte.

13. The method of claim 9 wherein the converting step further comprises the steps of:

providing a differential pumping mechanism having an entry portal, a plurality of differential pumping chambers and a vacuum chamber;

providing a capillary in fluid communication with the entry portal;

passing the substrate solution through the capillary toward the entry portal; and

passing the substrate solution through the plurality of differential chambers into the vacuum chamber.

14. The method of claim 13 wherein the capillary is spaced apart from the entry portal, defining an area therebetween.

15. The method of claim 13 wherein the capillary is held at a higher voltage relative to entry portal.

16. The method of claim 13 wherein the area between the capillary and entry portal is flooded with N_2.

17. The method of claim 9 wherein the substrate is a glass slide.

18. A method of capturing an analyte, present in a sample, on a substrate comprising the steps of:

providing a substrate solution comprising an analyte-specific capture molecule;

converting the substrate solution into an electrospray;

depositing the substrate-solution electrospray on the substrate in a vacuum;

providing an analyte solution comprising the sample;

converting the analyte solution into an electrospray; and

depositing the analyte-solution electrospray on the substrate in a vacuum.

19. The method of claim 18 wherein the substrate solution further comprises a solvent.

20. The method of claim 19 wherein the solvent is distilled water.

21. The method of claim 18 wherein the analyte solution further comprises a solvent.

22. The method of claim 21 wherein the solvent is distilled water.

23. The method of claim 18 wherein the converting steps further comprise the steps of:

providing a differential pumping mechanism having an entry portal, a plurality of differential pumping chambers and a vacuum chamber;

providing a capillary in fluid communication with the entry portal;

passing the substrate solution through the capillary toward the entry portal; and

passing the substrate solution through the plurality of differential chambers into the vacuum chamber.

24. The method of claim 23 wherein the capillary is spaced apart from the entry portal, defining an area there between.

25. The method of claim 23 wherein the capillary is held at a higher voltage relative to entry portal.

26. The method of claim 23 wherein the area between the capillary and entry portal is flooded with N_2.

27. The method of claim 18 wherein the substrate is a glass slide.

Fig. 1

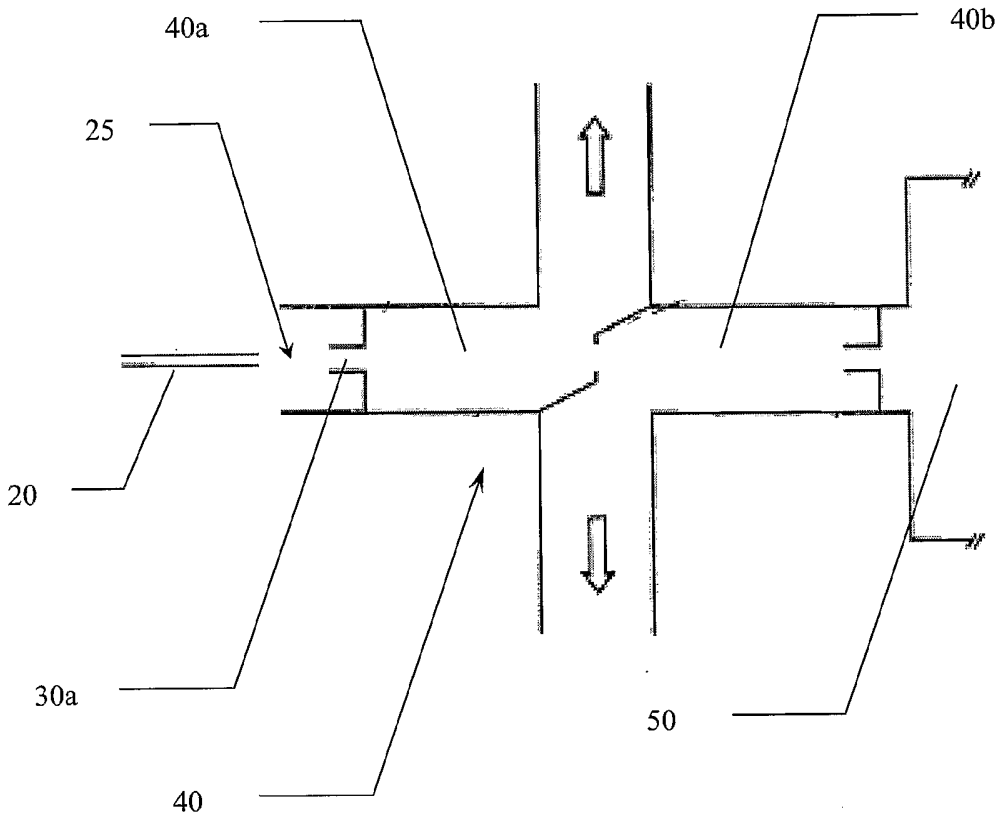
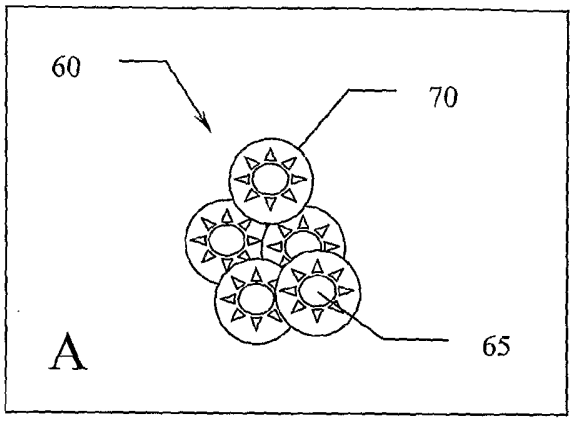
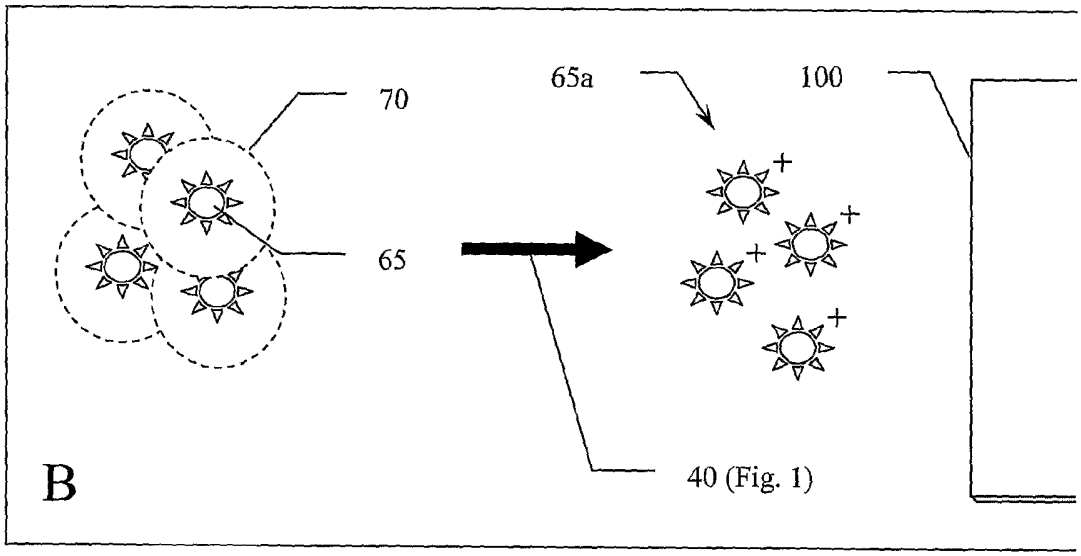


Fig. 2





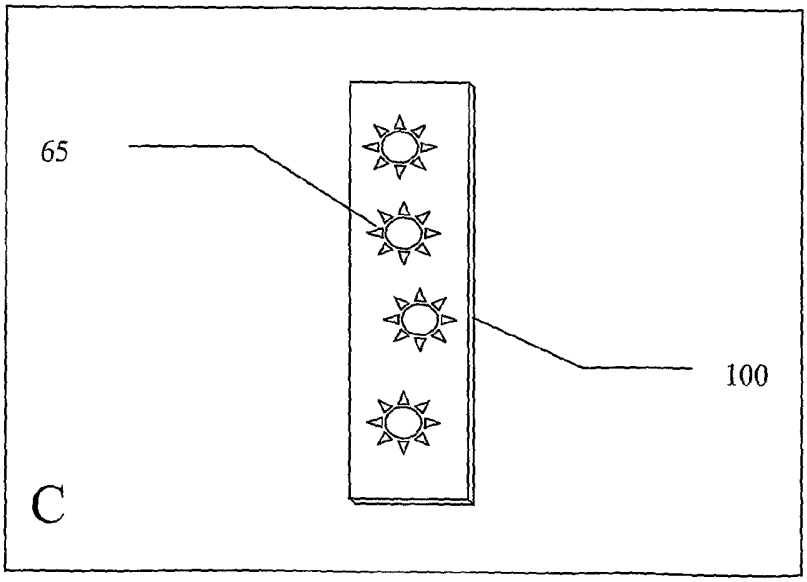


Fig. 3