

both reduced relative to the unetched ink. The low contact angle for water allows reagents to be adsorbed on the electrode by application of the reagents in a small volume of aqueous buffer and allowing the small volume to spread evenly over the electrode surface. Surprisingly, we have found that excellent assays may also be carried out on unetched carbon ink electrodes despite the presence of polymeric binders in the ink. In fact, in some applications requiring high sensitivity or low-non specific binding it is preferred to use unetched carbon ink electrodes so as to minimize the surface area of exposed carbon and therefore minimize background signals and loss of reagents from non-specific binding of reagents to the exposed carbon. Depending on the ink used and the process used to apply the ink, the electrode surface may not be easily wettable by aqueous solutions. We have found that we can compensate for the low wettability of the electrodes during the adsorption of reagents by adding low concentrations of non-ionic detergents to the reagent solutions so as to facilitate the spreading of the solutions over the electrode surface. Even spreading is especially important during the localized immobilization of a reagent from a small volume of solution. For example, we have found that the addition of 0.005-0.04% Triton X-100® allows for the spreading of protein solutions over unetched carbon ink surfaces without affecting the adsorption of the protein to the electrode and without disrupting the ability of a dielectric film applied on or adjacent to the electrode (preferably, a printed dielectric film with a thickness of 0.5-100 micrometers, or more preferably 2-30 micrometers, or most preferably 8-12 micrometers and having a sharply defined edge) to confine fluids to the electrode surface. Preferably, when non-ionic detergents such as Triton X-100® g are used to facilitate spreading of reagents (e.g., capture reagents) onto unetched screen-printed electrodes (i.e., so as to allow the immobilization of the reagents), the solutions containing the reagents are allowed to dry onto the electrode surface. It has been found that this drying step greatly improves the efficiency and reproducibility of the immobilization process.

[0115] The efficiency of the immobilization of reagents on carbon ink electrodes, especially unetched carbon ink electrodes, may exhibit some variability due to different levels of contamination of the electrodes surface. This effect is particularly pronounced when certain dielectric inks are used to form assay domains on the electrodes. We have found that we can improve the immobilization efficiencies and lower the variability by pre-washing the electrode surfaces, preferably with a surfactant solution.

[0116] The contamination of carbon ink electrodes by certain dielectric inks was observed by quantitatively assessing the surface wetting properties of the electrodes by measuring the contact diameter, where the larger the contact diameter, the better the wetting. A comparison of three alternative carbon surfaces with different dielectric layers is depicted in Table 1. As shown by the data in Table 1, washing the electrode surfaces can significantly increase the wetting properties (contact diameter) of carbon surfaces contacting the 451 dielectric (presumably by removing contamination of the electrode surface associated with the printing of the 451 dielectric, e.g., by migration of components of the dielectric ink on to the electrode surface).

TABLE 1

Comparison of Contact Diameters on Carbon Electrode Surfaces for Three Different Dielectric Materials (Mean 50 nL water drop diameter at 400 μ s open time)	
Surface	Contact Diameter, inches*
<u>No pre-treatment:</u>	
Carbon with 451 dielectric	0.0366
Carbon with Nazdar dielectric	0.0461
Carbon with PD039A dielectric	0.0457
<u>Pre-treated:</u>	
Carbon with 451 dielectric	0.0438
Carbon with Nazdar dielectric	0.0463
Carbon with PD039A dielectric	0.0448

[0117] in one embodiment, a method of decontaminating the carbon electrode surfaces may be employed wherein the electrode surfaces are soaked in an aqueous 0.5% Triton X-100 solution for several hours, subsequently rinsed with deionized water, then soaked in deionized water for approximately one hour and finally dried. The Triton solution preferably removes the contaminants from the surface and the deionized water removes the adsorbed surfactant. This method of decontamination is an effective cleaning procedure that enhances the differences between the retreating contact angles on the carbon and the dielectric inks.

[0118] FIG. 6 demonstrates the results of the decontamination procedure. Specifically, FIG. 6 depicts images of ECL from an ECL label over carbon ink electrodes, the exposed areas of the electrode being defined by a dielectric film. FIG. 6a is the ECL image without decontamination and FIG. 6b is the ECL image after decontamination with Triton X-100 in accordance with the present embodiment. These ECL images show that the treatment process greatly reduces the variation in ECL intensity over the surface of the electrode, the patchiness of ECL on the untreated electrode presumably being caused by patches of contamination on the surface.

[0119] Electrodes can be derivatized with chemical functional groups that can be used to attach other materials to them. Materials may be attached covalently to these functional groups, or they may be adsorbed non-covalently to derivatized or underivatized electrodes. Electrodes may be prepared with chemical functional groups attached covalently to their surface. These chemical functional groups include but are not limited to COOH, OH, NH₂, activated carboxyls (e.g., N-hydroxy succinimide (NHS)-esters), poly-(ethylene glycols), thiols, alkyl ((CH₂)_n) groups, and/or combinations thereof. Certain chemical functional groups (e.g., COOH, OH, NH₂, SH, activated carboxyls) may be used to couple reagents to electrodes. For further reference to useful immobilization and bioconjugation techniques see G. Hermanson, A. Mallia and P. Smith, *Immobilized Affinity Ligand Techniques* (Academic Press, San Diego, 1992) and G. Hermanson, *Bioconjugate Techniques* (Academic Press, San Diego, 1996).

[0120] In preferred embodiments, NHS-ester groups are used to attach other molecules or materials bearing a nucleophilic chemical functional group (e.g., an amine). In a