Saptasense Protocol

CHIT-crGO Modified SPEs

Background: In this protocol, we will be modifying carbon screen printed electrodes (SPEs) with chitosan (CHIT) and chemically reduced graphene oxide (crGO) and then attaching our aptamers via a glutaraldehyde linker.

Reduced graphene oxide is a nanomaterial being used for its high electrical conductivity, high surface area, and monolayer-forming properties. Reduced graphene oxide is a derivative of (oxidized) graphene oxide, which is a derivative of graphite. Graphite is a relatively abundant form of hexagonal carbon crystal sheets stacked on top of each other. It has several important applications, but its single-layer form, graphene, is used much more often. Unfortunately, synthesis and production of graphene from its multilayer form, graphite, is difficult on larger scales. Therefore, researchers have been focusing on derivatives of graphene that are much easier to form monolayers: graphene oxide (GO) and reduced graphene oxide (rGO). GO and rGO are easily dispersible in solvents making it easy to form monolayers. Importantly, GO has a marked reduction in conductive properties, making it less suitable for applications such as sensing. Reduction of GO to rGO restores most of the conductivity. Therefore, for our sarcosine aptasensor we will be using rGO for its enhanced conductivity, low-cost, ease of production, high surface area, and monolayer-forming properties.

Chitosan (CHIT) is a polysaccharide derived from treating the chitin shells of crustaceans with a strong base. It is often employed for its biocompatibility, film-forming properties, and ease of modification of functional groups. We are incorporating CHIT into our sensor because it will form a thin film with the rGO, and allow us to attach our aptamers covalently through a glutaraldehyde linker.

<u>Materials</u>
Chitosan
Glutaraldehyde
Reduced Graphene Oxide
Carbon SPE
ddH2O (MilliQ water)
PBS
Acetic Acid

<u>Instruments</u>
Sonicator
Potentiostat & SPE adaptor

Methods:

- Immerse the screen printed electrode in 0.5M H2SO4. Run CV using the potentiostat. Scan for 20 cycles at the speed of 100mV/s in the voltage range of -0.2V to 1.0V
- Rinse with PBS several times and leave to dry
- Make 5mL of 1% acetic acid

    - Add 50uL of acetic acid to 4,950uL ddH2O (that's 4 mL plus 950uL)
- Make 2 mL of a 2000ug/mL solution of chitosan in 1% acetic acid
    - Add 4mg (4000ug) to 2mL of 1% acetic acid

- Sonicate for 45 minutes to disperse
- Add in 4mg (4000ug) of lyophilized rGO to the CHIT-acetic acid solution
- Sonicate for 15 more minutes to disperse
- Pipette 5uL of CHIT-rGO solution onto the working electrode of the SPE. Incubate for 30 minutes.
- Rinse the electrode with PBS and leave it to dry
- Prepare a solution of 2.5% glutaraldehyde (5mL should suffice to immerse the SPEs)
    - WORK UNDER A FUME HOOD
    - Add 500uL 25% glutaraldehyde to 450uL ddH2O
- Immerse in 2.5% glutaraldehyde for 15 min
    - WORK UNDER A FUME HOOD
- Rinse the electrode with PBS and leave it to dry
- Prepare a solution of 0.1uM aptamer in ddH2O
    - Start by reconstituting aptamer #1 in ddH2O
        - Add 130.8uL for a 1mM solution
        - VORTEX THOROUGHLY for a while (>30 seconds) to fully resuspend the aptamers!! Centrifuge briefly to collect resuspension on bottom of the tube.
        - Take 10uL and add 90uL of ddH2O in a separate tube for a 100uM solution
        - VORTEX THOROUGHLY and centrifuge briefly
        - Take 10uL and add 990uL of ddH2O in a separate tube for a 1uM solution
        - VORTEX THOROUGHLY and centrifuge briefly
        - Take 10uL and add 90uL of ddH2O in a separate tube for a 0.1uM solution
        - VORTEX THOROUGHLY and centrifuge briefly
    - Start by reconstituting aptamer #2 in ddH2O
        - Add 91.3uL of H2O for a 1mM solution
        - VORTEX THOROUGHLY for a while (>30 seconds) to fully resuspend the aptamers!! Centrifuge briefly to collect resuspension on bottom of the tube.
        - Take 10uL and add 90uL of ddH2O in a separate tube for a 100uM solution
        - Mix
        - Take 10uL and add 990uL of ddH2O in a separate tube for a 1uM solution
        - Mix

        - Take 10uL and add 90uL of ddH2O in a separate tube for a 0.1uM solution

            - Mix
- Pipette 2.0uL of 0.1uM aptamer onto the working electrode and incubate at 25C for 120 minutes
- Rinse the electrode with PBS and leave it to dry
- Store in the refrigerator at 4C before use