Optimised Immobilisation of Cholesterol Oxidase (CO6)

Assay reagents (final concentrations)

Reagent Mix	Unit	Diluent	Stock solutions (Predilution)	Working Concentration
Hydroquinone	mM	20mM acetic acid	40	0.24
HRP	U/mL	0.1M phosphate buffer pH 7	7.5	3
Cholesterol Esterase	U/mL	0.1M phosphate buffer pH 7	18	1.2
Cholesterol Oxidase	U/mL	20mM TRIS-HCL pH 7 + 1% trehalose (Dry)	6 (Dry)	0.6
Cholesterol/cholesteryl acetate	mM	15mM sodium cholate + 1% triton x 100	3.2	0.16
Sodium cholate	mM	0.1M phosphate buffer pH 7	15	0.75
Triton X - 100	%	0.1M phosphate buffer pH 7	1	0.05

All solutions were tested using carbon screen printed electrodes.

Testing procedure:

1. **Drying conditions:** Drying buffer was 20mM TRIS-HCL pH 7.5 + 1% trehalose which was used as the lyophilisation buffer by BBI. Deposited 6U/mL deposition on electrode surface was dried at 40°C for 30 minutes in a box oven. Optical test was same deposition but dried in 96-well plate.
2. **Testing procedure**: Diluted and mixed together to quantities stated in table above. Dry enzyme deposits were resuspended manually with a pipette in mix which did not contain cholesterol oxidase but contained other enzymes. Reaction mix was then incubated for 10 minutes at room temperature.
3. **Electrochemical measurement:** Applied -0.25V for 60 seconds and measured current.
4. **Analysis:** Averaged current between 10 – 20 seconds for each replicate performed.

Results

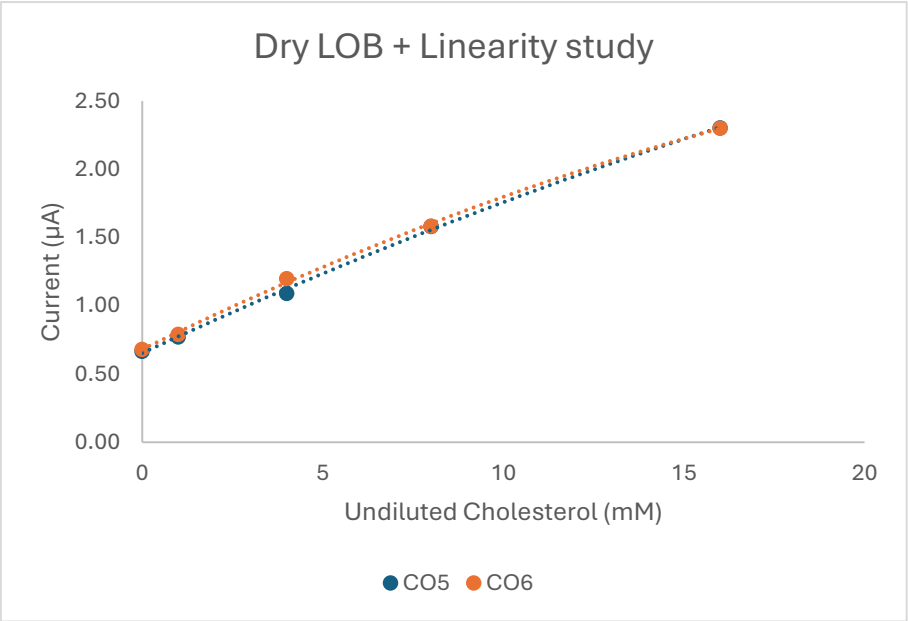

Figure shows the dose response for dried enzyme following resuspension