1st Call for H.F.R.I. Research Projects for the Support of Faculty Members and Researchers and the Procurement of high-cost Research Equipment

Title:	Development of sustainable chemoenzymatic processes for optically pure amines from alcohols or alkynes
Project Acronym:	CEPOPA
Project No:	664
P.I.:	Associate Prof. Ioannis Pavlidis
Deliverable	D3.1 Optimized protocols for efficient immobilization of ATAs on GO

The following protocol is the optimal procedure established during immobilization experiments. A typical workflow is described herein and it is a modified established protocol (Shariat et al. 2018)

- Weigh 10 mg of graphene oxide (GO) and dissolve it in 10 mL of potassium phosphate buffer solution (50 mM, pH 7.5) in a 50 mL centrifugal Falcon® tube.
- The solution is stirred, and the tube was placed in an ultrasonic bath for 3 hours to ensure complete exfoliation of the carrier.
- Prepare a 6% v/v glutaraldehyde (GA) solution in water phosphate.
- Mix glutaraldehyde solution with graphene oxide, using a peristaltic or a syringe pump for 4 hours at a flow rate of 2.5 mL/h.
- The solution is centrifuged for 30 minutes at 4°C, 15000 g.
- The supernatant was discarded, and the pellet was re-suspended in 10 mL of potassium phosphate buffer solution (50 mM, pH = 7.5).
- Repeat centrifugation for 15 minutes at 4°C, 15000 g, and the washing process is repeated.
- After washing, the supernatant was discarded, and the carrier was re-suspended in an enzyme solution (1 mL) prepared to maintain a 1:2 w/w ratio of enzyme to carrier. Also, Tween-20 was added during the incubation in 1% v/v.
- Incubate for 1-3 hours at room temperature.
- The solution is centrifuged, and 20 μL are used from the supernatant to measure concentration and activity.
- Finally, the material along with immobilized enzyme is centrifuge washed 3 times for 20 minutes at 4°C, 13000 rpm. In the first wash, buffer also contains 300 mM NaCl. The non-immobilized supernatant is filtered and stored in the refrigerator for reuse.
- The enzyme-GO complex is stored in 1 mL phosphate buffer solution with 0.1 mM PLP at 4°C for further experiments

*<u>*Mitigation plan-comparative covalent immobilization protocol in commercial carriers*</u>

- Initially, the quantity of beads to be weighed was calculated so that there would be 50 mg of enzyme in 1 g of beads.

- Washes were performed using a buffer solution with PLP (50 mM KPi, pH 7.5, 0.1 mM PLP). Four washes were conducted using a buffer volume such that the ratio of beads to solution was 1:2 w/v.
- A solution of 1% w/v glutaraldehyde was added. The volume added was adjusted to maintain a ratio of beads to solution at 1:4 w/v for 1% GA.
- Incubation was carried out for 1-2 hours under stirring at 500 rpm and 25°C. After one hour, the incubation was halted, and washes with PLP buffer were performed at least 4 times.
- Enzyme addition was done in a ratio ensuring 50 mg of enzyme corresponded to 1 g of beads. It was crucial for the enzyme to be in powder form to maintain the required ratio. Hence, the enzyme underwent lyophilization and was dissolved in the appropriate concentration
- The freeze-dried enzyme was dissolved in deionized water before addition (50 mg of enzyme per 1 g of beads).
- Incubation at room temperature continued until immobilization occurred at the desired rate (>95%).

Literature:

- Shariat, S.Z.A. et al. (2018) Immobilization of lactoperoxidase on graphene oxide nanosheets with improved activity and stability. *Biotechnol Lett* **40**, 1343–1353.