



## Fabrication of a Novel Colorimetric Paraoxon Ethyl Biosensor Using CUPRAC Reagent as a Chromogenic Reagent

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Copper(II)-bis-neocuproin complex ( $[\text{Cu}(\text{Nc})_2]^{2+}$ ), which was first developed by Apak et al. in 2004 and known as CUPRAC reagent, has been used as a useful chromogenic reagent in many colorimetric sensor studies.<sup>1-3</sup> However, according to our literature search, a paraoxon ethyl (POE) biosensor using the CUPRAC reagent based on acetylcholine esterase (AChE) inhibition has not yet been reported. First, an enzymatic reaction takes place between AChE and its substrate, acetylthiocholine (ATCh), followed by a colorimetric reaction between the enzymatically produced thiocholine (TCh) and the CUPRAC reagent. While  $[\text{Cu}(\text{Nc})_2]^{2+}$  reduces to a yellow-orange cuprous complex ( $[\text{Cu}(\text{Nc})_2]^+$ ) which gives maximum absorbance at 450 nm, TCh oxidizes to its disulfide form. However, the absorbance of  $[\text{Cu}(\text{Nc})_2]^+$  is proportionally decreased depending on the increase in the concentration of POE due to the inhibition of AChE by POE. Based on this strategy, the linear response range of a colorimetric biosensor was found to be between 0.15 and 1.25  $\mu\text{M}$  POE with a detection limit of 0.045  $\mu\text{M}$ . The fabricated biosensor enabled the selective determination of POE in the presence of some other pesticides and metal ions. Acceptable recovery results were obtained from water samples spiked with POE.

**Keywords:** Acetylcholine esterase; Paraoxon ethyl; Colorimetric biosensor; CUPRAC

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### References

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