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**Fully integrated ready-to-use paper-based electrochemical biosensor to detect nerve agents**

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Paper-based biosensor; Screen-printing; Reagentless; Enzyme inhibition; Nerve Agents; Paraoxon

**Abstract**

Paper-based microfluidic devices are gaining large popularity because of their uncontested advantages of simplicity, cost-effectiveness, limited necessity of laboratory infrastructure and skilled personnel. Moreover, these devices require only small volumes of reagents and samples, provide rapid analysis, and are portable and disposable. Their combination with electrochemical detection offers additional benefits of high sensitivity, selectivity, simplicity of instrumentation, portability, and low cost of the total system. Herein, we present the first example of an integrated paper-based screen-printed electrochemical biosensor device able to quantify nerve agents. The principle of this approach is based on dual electrochemical measurements, in parallel, of butyrylcholinesterase (BChE) enzyme activity towards butyrylthiocholine with and without exposure to contaminated samples. The sensitivity of this device is largely improved using a carbon black/Prussian Blue nanocomposite as a working electrode modifier. The proposed device allows an entirely reagent-free analysis. A strip of a nitrocellulose membrane, that contains the substrate, is integrated with a paper-based test area that holds a screen-printed electrode and BChE. Paraoxon, chosen as nerve agent simulant, is linearly detected down to 3  $\mu\text{g/L}$ . The use of extremely affordable manufacturing techniques provides a rapid, sensitive, reproducible, and inexpensive tool

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