Fast Quantification of Enzyme Activity by Electroanalysis

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Technology description

The internally calibrated electrochemical continuous enzyme assay (ICECEA, patent pending) was developed for the fast determination of enzyme activity unit (U). The assay depends on the integration of enzyme-free preassay calibration with the actual enzyme assay in one continuous experiment. Such integration resulted in a uniquely shaped amperometric trace that allowed for the selective picomolar determination of redox enzymes. The ICECEA worked because the preassay calibration did not interfere with the enzyme assay allowing both measurements to be performed in succession in the same solution and at the same electrode. The method displayed a good accuracy (relative error, <3%) and precision (relative standard deviation (RSD), <3%) when tested with different working electrodes (carbon nanotubes/chitosan, glassy carbon, platinum) and enzymes (alcohol dehydrogenase, ADH; lactate dehydrogenase, LDH; xanthine oxidase, XOx; glucose oxidase, GOx). The limit of detection for the ADH, LDH, XOx, and GOx was equal to 0.18, 0.14, 0.0031, and 0.11 U L^{-1} (or 4.2, 0.72, 89, and 6.0 pM), respectively. The simplicity, reliability, and short analysis time make the ICECEA competitive with the optical enzyme assays currently in use.

Invention Description

The majority of existing enzyme assays rely on changes in the optical properties of enzyme solution. However, such assays often require auxiliary enzymes and/or toxic chromogenic agents involve a large number of liquid-handling steps require a time-consuming incubation have a limited utility in turbid solutions Researchers at UT San Antonio have developed a simple, reliable and cost effective, electrochemical assay for fast quantification of enzyme activity. The assay requires only a small amount of enzyme to quickly determine its activity with no need for the enzyme-based external calibration or the reactivation of electrode surface. This assay is amenable to automation and miniaturization and is well suited for the fast analysis of commercial batches of enzymes, quantification of enzymes. The assay is performed in the constant-potential amperometric mode in a stirred solution of enzyme’s substrate. It requires three solutions only to quantify the enzyme activity:

1. A solution of enzyme’s substrate.
2. A solution of redox active species participating in enzymatic reaction.
3. A solution of assayed enzyme.

The assay involves only three simple steps:

1. Record a baseline current in solution A.
2. Known aliquots of solution B are added to solution A to record current steps.
3. Add Solution C and trigger enzymatic reaction and record an angled current-time segment, which is calculate the enzyme activity.
Typically such "current steps-current slope" amperometric trace is recorded in a short 5 minute experiment.

Application area

Potential commercial applications include companies that need fast analysis of commercial batches of enzymes, quantification of enzyme biomarkers for various diseases, and optimization of assays for newly discovered enzymes and high-throughput enzyme assays.

Advantages

Low Detection Limit:Very low limit of detection: picomolar level.
Cost Effective:Does not use expensive enzymes to calibrate.
Simplistic:Eliminates the need for transferring the working electrode between the calibration and assay solutions and does not need extra reagents such as auxiliary enzymes or toxic chromogenic agents.

Institution

University of Texas, San Antonio

Inventors

联系我们

叶先生

电 话：021-65679356

手 机：13414935137

邮 箱：yeyingsheng@zf-ym.com