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A protocol study of serotonin determination using a validated electrochemical sensing method in human blood serum

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Abstract

Serotonin (5-hydroxytryptamine, 5-HT) is a pivotal neurotransmitter involved in a myriad of physiological processes, including the regulation of mood, appetite, and sleep cycles. Accurate quantification of 5-HT levels in human serum is fundamental to elucidating its pathophysiology in various clinical conditions and disorders. Over the recent years, electrochemical sensing techniques have emerged as highly reliable and sensitive modalities for 5-HT detection. This article details a protocol study designed to determine 5-HT concentrations in human serum using a validated electrochemical sensing platform. The protocol encompasses a comprehensive overview of electrochemical sensing techniques, methodology validation, sample collection and processing, experimental procedures for 5-HT determination, and subsequent data analysis. The outcomes of this study hold significant potential for advancing the current understanding of 5-HT-associated disorders and may facilitate the development of novel, targeted therapeutic interventions.

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Introduction

Serotonin (5-HT), an essential neurotransmitter, plays a pivotal role in modulating mood, emotional states, and various physiological processes within the human body (1). Assessing 5-HT levels in the bloodstream can provide critical insights into mental health disorders, such as depression and anxiety, while also contributing to advancements in pharmacology and drug development (2). Furthermore, 5-HT levels are increasingly being investigated as a potent biomarker for detecting complications associated with diabetes mellitus. It is hypothesized that beyond the gastrointestinal and central nervous systems, 5-HT interacts with pancreatic beta cells; consequently, researchers have been striving to fully elucidate this underlying mechanism (3).

The present study aimed to quantify 5-HT levels in serum utilizing a well-established electrochemical sensing methodology. The primary objective was to develop a standardized detection protocol for analyzing serum 5-HT concentrations via electrochemical sensing. By establishing this protocol, we intend to provide researchers and healthcare professionals with a validated framework for 5-HT analysis, thereby facilitating more accurate diagnosis and management of disorders related to 5-HT imbalances. Moreover, this study evaluates the reliability, sensitivity (Responsiveness), and precision of the sensing technique in 5-HT detection.

Overview of the electrochemical sensing method

Electrochemical sensing is a technique that leverages the intrinsic electrical properties of an analyte to measure its concentration within a sample. In the context of 5-HT determination, electrochemical sensors utilize the redox (Reduction-oxidation) reactions inherent to 5-HT molecules to quantify their levels (4). By applying a specific voltage to the sensor, the resulting electrical current can be measured and correlated with the 5-HT concentration present in the sample. Various architectures of electrochemical sensors can be utilized for 5-HT detection, including glassy carbon electrodes (GCE), screen-printed electrodes (SPEs), carbon nanotube-based electrodes, and microfabricated electrodes (Figure 1). These engineered sensors provide high sensitivity, superior selectivity, and rapid response times, rendering them optimal candidates for 5-HT investigations (5). The selection of a specific sensor is dictated by factors such as cost-effectiveness, ease of operation, and the specific requirements of the intended application.

Comparison with conventional methods

High-performance liquid chromatography (HPLC) is a widely established and reliable gold standard for 5-HT detection. However, it can be time-consuming and necessitates specialized instrumentation alongside high technical proficiency. Mass spectrometry (MS) is another frequently utilized modality for 5-HT quantification. While offering high precision and sensitivity, MS is prohibitively expensive and requires sophisticated equipment and specialized technical expertise (6,7). In contrast, electrochemical sensing provides a cost-effective and user-friendly alternative that circumvents the need for complex instrumentation, while yielding results comparable to those obtained via HPLC.

Validation of the electrochemical sensing method

To ensure the analytical rigor and clinical reliability of the electrochemical sensing method for 5-HT determination, a stringent validation process is mandatory (8). This protocol involves a comprehensive assessment of the method's precision, accuracy, linearity, limit of detection (LOD), and stability. By performing repetitive experimental trials and benchmarking the results against a gold standard, the electrochemical sensing platform can be clinicaly validated for routine 5-HT analysis (9). For qualitative investigation, cyclic voltammetry (CV) is typically employed to characterize the electrochemical properties of an analyte in solution as it adsorbs onto the electrode surface. For quantitative analysis, differential pulse voltammetry (DPV), square-wave voltammetry (SWV), and electrochemical impedance spectroscopy (EIS) are primarily utilized to investigate the analyte interactions at the electrode interface (Figure 2).

During the validation process, various analytical parameters and criteria are meticulously evaluated. These include intra-day and inter-day precision, which serve to assess the method's repeatability and reproducibility, respectively. Accuracy is determined by benchmarking experimental results against a known reference standard. Linearity is established by analyzing the correlation between the electrochemical response and 5-HT concentrations across a predefined range. Additionally, the limit of detection (LOD) and stability are evaluated to ascertain the method's sensitivity and its robustness against minor fluctuations in experimental conditions (8).

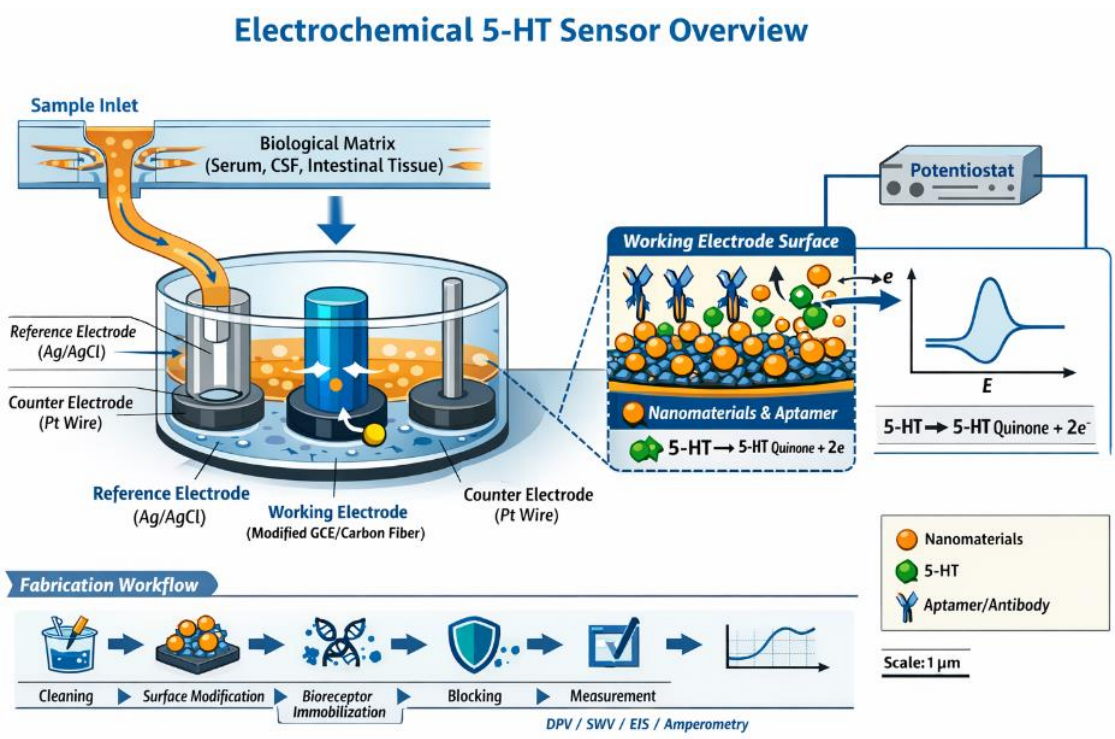

Figure 1. Overview schematic of an electrochemical 5-HT sensing platform.
Center: A three-electrode configuration comprising the Working Electrode (WE), Reference Electrode (RE), and Counter Electrode (CE).
Inset: A nanostructured working electrode with immobilized aptamers/antibodies designed to capture 5-HT, subsequently undergoing electrochemical oxidation.
Right: Potentiostat readout illustrating analytical parameters (voltammogram, calibration, and selectivity).
Bottom: Sequential illustration of the fabrication workflow.

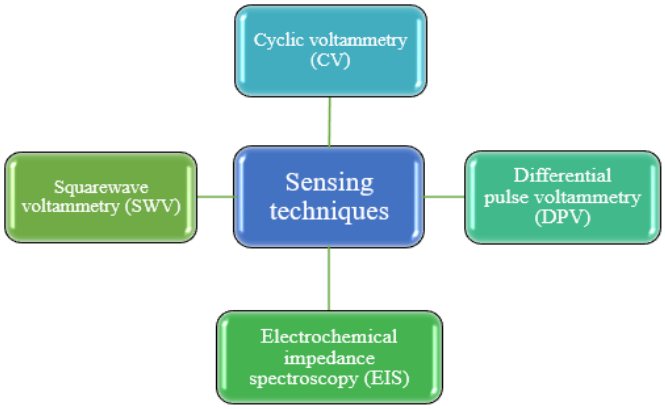

Figure 2. Electrochemical sensing techniques for the analyte determination

Electrode stability, storage conditions, and operational longevity are critical parameters governing sensor performance. Factors such as exposure to humidity, temperature fluctuations, and extended use can induce degradation, alter electrochemical behavior, and compromise long-term reliability (10).

Methods

Serotonin hydrochloride (5-HT), reagents, and salts should be procured from reputable commercial sources. All reagents and salts must be of analytical grade unless otherwise specified.

For this study, human serum samples are selected to ensure a representative range of 5-HT concentrations. Samples may be obtained from healthy volunteers or collected from clinical settings, strictly adhering to established ethical guidelines and obtaining informed consent. Figure 3 illustrates the systematic methodology for 5-HT detection using the proposed electrochemical sensing platform.

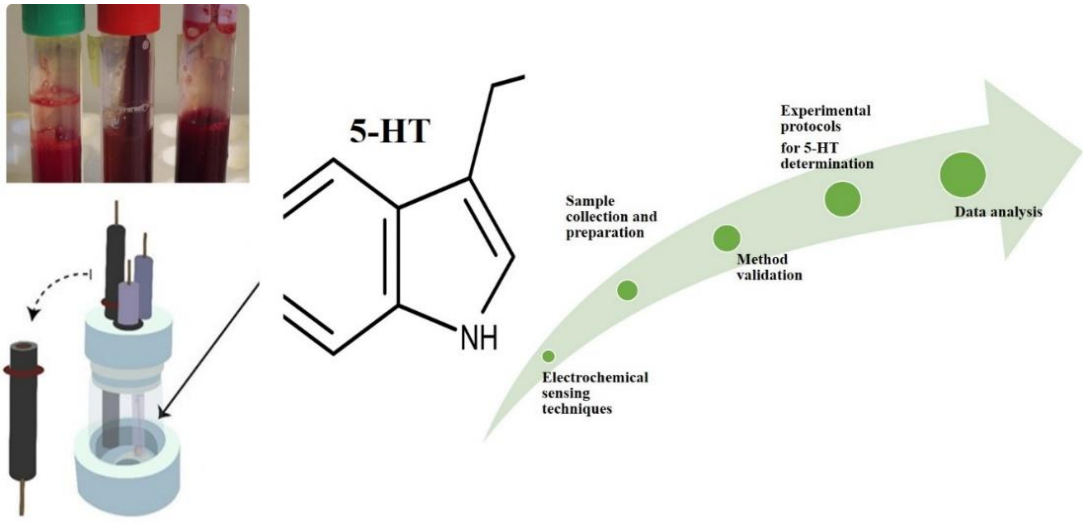

Figure 3. Schematic of the 5-HT detection procedure utilizing the electrochemical sensing method

To preserve sample integrity, stringent storage and handling protocols were implemented. Serum samples are typically stored at ultra-low temperatures (-20°C or below) to mitigate enzymatic activity and prevent degradation. Furthermore, samples must be shielded from light to avoid photochemical reactions that could alter 5-HT concentrations. Homogenization procedures were meticulously performed to ensure sample uniformity and to preclude potential analyte loss or contamination.

Prior to analysis, serum samples undergo specific preparation steps to eliminate interfering constituents and concentrate the target analyte. Conventional techniques include protein precipitation, solid-phase extraction, and derivatization (11). These preparatory steps aim to enhance the sensitivity and accuracy of the electrochemical sensing method by minimizing matrix effects and optimizing 5-HT detection in the serum (4). Interference studies were conducted to assess the selectivity of serotonin detection in the presence of structurally and electrochemically analogous biomolecules, such as dopamine, uric acid, and ascorbic acid, which are prevalent in serum and may otherwise compromise analytical precision (12).

A calibration curve was established to determine 5-HT levels in human serum using the validated electrochemical sensing method. This curve enables the correlation of measured electrochemical responses with standardized concentrations of 5-HT. The protocol for generating the calibration curve involves the preparation of a series of standard solutions with known 5-HT concentrations.

These standard solutions span a broad concentration range to ensure analytical accuracy and reliability. The electrochemical response for each standard solution is then recorded using the proposed sensing method. The calibration curve is generated by plotting the measured electrochemical signals against the corresponding 5-HT concentrations. This curve facilitates the determination of 5-HT concentrations in unknown samples by interpolating their electrochemical responses. Once the calibration curve is validated, 5-HT quantification in human serum can be performed. The analytical procedure comprises the following steps:

Preparation of electrode

I. Thoroughly clean the working electrode using an appropriate solvent, ensuring the surface is polished and free of contaminants.
II. Assemble the electrochemical cell, ensuring the proper positioning of the reference and counter electrodes.

Preparation of solutions

I. Prepare 5-HT standards of known concentrations by performing serial dilutions of a stock solution.
II. Prepare the electrolyte and buffer solutions in accordance with the specific requirements of the electrochemical setup.

Calibration

I. Immerse the working electrode into the 5-HT standard solution.
II. Utilize the potentiostat/galvanostat to apply a controlled potential to the working electrode.
III. Record the subsequent current response.
IV. Replicate this procedure for the series of standard solutions to construct a calibration curve correlating current intensity to 5-HT concentration.

Sample analysis

I. Immerse the electrode into the 5-HT serum sample.
II. Apply the designated potential and record the resulting current response.
III. Employ the validated calibration curve to determine the 5-HT concentration within the sample.

Data analysis and interpretation

Following the quantification of 5-HT concentrations in the serum samples, rigorous statistical analysis is performed to derive clinically meaningful insights. Descriptive statistics, including mean, standard deviation (SD), and confidence intervals (CI), are utilized to summarize the dataset. Furthermore, appropriate hypothesis testing, such as independent t-tests or analysis of variance (ANOVA), is employed to compare 5-HT concentrations across different study cohorts or experimental conditions. This approach facilitates the identification of statistically significant differences and the formulation of robust conclusions.

The Limit of Detection (LOD) and Limit of Quantification (LOQ) are calculated using the 3σ and 10σ criteria, respectively. These values are derived from the standard deviation of blank measurements (Or low-concentration signals) and the slope of the calibration curve.

1. Measure multiple blank samples or low-concentration replicates.
2. Calculate the standard deviation (σ).
3. Determine the slope of the calibration curve (S).
4. Apply the following equations for LOD and LOQ determination:

LOD= $3.3\sigma/S$ and LOQ= $10\sigma/S$

To validate the accuracy and clinical reliability of the proposed electrochemical sensing method, results were cross-referenced with those obtained through established reference methods for 5-HT determination. This comparative analysis serves to evaluate the degree of agreement between the two modalities and assesses the clinical suitability of our electrochemical platform for monitoring 5-HT levels in human serum. Briefly, the raw data acquired from the electrochemical workstation are processed to calculate the absolute 5-HT concentration in each sample based on the validated calibration curve.

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Ethical Statement

This study does not require an ethics review as it does not involve the collection of personal data; instead, it synthesizes and summarizes information from publicly available literature and studies.

Conflicts of Interest

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author Contributions

Sole author.

Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Use of Artificial Intelligence

The author used a generative AI tool for language editing and structural improvement of the manuscript. All scientific content, data analysis, interpretation, and conclusions were conducted and verified by the author.

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