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Classic and Modern Methods for Detection of Serotonin

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Abstract: The detection of serotonin and other neurotransmitters from different biological samples (serum, plasma, platelets, urine, saliva, cerebrospinal liquid) are very important in clinical diagnosis. For this reason, several classic and modern methods for detection of serotonin were presented in this paper. The advantages and disadvantages of classic methods (colorimetric, fluorometric, chromatographic methods and immunoassay) were presented.
The new methods (sensors, biosensors, imaging techniques - PET) are also indicated. Electrochemical detection of serotonin is indicated in the scientific literature, using different types of electrodes (glassy carbon electrode, screen printed electrode).

Keywords: Serotonin, Dopamine, Colorimetric method, Fluorometric assay, Chromatography, ELISA, PET, Electrochemical sensors, Glassy carbon electrode, Screen printed electrode.

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Introduction

Serotonin (5-hydroxytryptophan - 5-HT) is a monoamine neurotransmitter of high biological importance widely distributed in the human body, especially in the gastrointestinal tract, platelets and in the central nervous system. Biochemically derived from tryptophan, it plays important functions in normal physiology, including developmental, cardiovascular, gastrointestinal and endocrine function, sensory perception, behaviors such as aggression, appetite, sex, sleep, mood, cognition, learning and memory. Also serotonin is involved in a number of pathological states (psychiatric disorders, depression, mental retardation, infantile

autism, etc.). Modulation of serotonin at synapses is thought to be a major action of several classes of pharmacological antidepressants [1].

Serotonin determination has found a major importance in diagnosis, control and surveillance of diseases like depression, migraines, ADHD, autism, inflammatory syndromes, in monitoring changes in neurotransmitter secretion and balance as a result of therapeutic and pharmacokinetic studies. The increase of 5-HIAA (5-hydroxyindoleacetic acid), a serotonin metabolite, in urine is a marker for diseases such as Whipple, celiac disease.

Blood serotonin determinations have already been applied to the studies of non-carcinoid malignancy, carcinoid syndrome, Down's syndrome, Hermansky-Pudlak syndrome, postcibal concentrations involving a possible hormonal role in gastrointestinal physiology and to various blood disorders [2].

Methods for Detection of Serotonin

Since the discovery of serotonin in the gastro-intestinal tract in 1930's, various methods have been developed in order to determinate the presence and the concentration of this neurotransmitter. The 5-HT determination methods can be divided into two major categories: (i) classic methods (e.g. RIA, ELISA) and (ii) modern methods (e.g. with sensors, PET).

Classic Methods for Detection of Serotonin

Colorimetric method involves conversion of the aldehyde, produced as a result of serotonin deamination by monoamine oxidase, to a stable derivative (2,4 –dinitrophenylhydrazone) which have a bright yellow color in alkaline solution and can be measured colorimetric [3].

The colorimetric determination is no longer in use because is expensive, time consuming and, most importantly, is neither sensible nor specific. It can detect the presence of 5-HT, but not its quantity.

Fluorometric Tests (**FIA**) require to extract serotonin onto an ascorbic acid solution by freezing, thawing and sonication. Ascorbic acid stabilizes the serotonin in the extract, and added ethanol enhances the final fluorescence of serotonin, which is measured in a concentrated hydrochloric acid; sample used being platelets -0.67(SE0.03) $\mu g/10^9$ [4].

Immunoenzymatic tests **(ELISA, EIA)** is also recommended by several scientific references for serotonin detection.

There is a competition between a biotinylated and a non-biotinylated antigen for a fixed number of antibody binding sites. The amount of biotinylated antigen bound to the antibody is inversely proportional to the analyte concentration of the sample. When the system is in equilibrium, the free biotinylated antigen is removed by a washing step and the antibody bound biotinylated antigen is determined by use of anti-biotin alkaline phosphatase as marker and p-nitrophenyl phosphate as substrate.

Quantification of the target analytes ("unknowns") is achieved by comparing the enzymatic activity of unknowns with a response obtained by preparing and measuring the known (defined) standards [5,6].

Radioimmunoassay (RIA) ... This approach is based on the antigen-antibody reaction in witch tracer amounts of the radio-labeled antigen (Ag) competes with endogenous Ag for limited binding sites of the specific antibody (Ab) against the same Ag. This is called the double antibody technique. Most used radio-isotope is iodine attached to tyrosine. The same preparation was recommended for any type of sample. Acylation process was performed in liquid phase. There were used 6 standards and 2 controls (ready for use) [7].

Chromatographic Tests (LC, HPLC, GC) ... Liquid chromatography (LC) and High Pressure Liquid Chromatography (HPLC) are common used techniques for serotonin determination. The principle of the methods is the same in all chromatography, a separation technique where the mobile phase is a liquid. HPLC relies on the pressure of mechanical pumps on a liquid solvent to load a sample mixture onto a specific column, in which separation occurs [8,9]. Some methods are briefly presented (see Table I, overleaf) with the possibility of seeing which one is most fast, reliable and recommended method.

Modern Methods for Detection of Serotonin

Sensors and Biosensors. Because 5-HT is an electrochemically active compound it can also be determined by electrochemical methods [10], using different types of electrodes (glassy carbon electrodes, screen printed electrodes).

The researches in this area are incorporating also the determination of dopamine (DA), another important hormone and neurotransmitter. DA and 5-HT influence each other in their respective releasing and most of the electrochemically methods developed till now are offering the possibility of simultaneous determination of both substances.

Table I: *Classic methods for detection of serotonin*

Parameter	ELISA	RIA	HPLC
Sample Types	1 - 100 µL, all biological fluids	25 µl serum, urine, platelets, 100 µL LCR, PFP	10 mL urine
Sensitivity	5 pg/mL (depending on the sample volume)	6.7 ng/mL serum, urine, 0.3 ng/mL LCR, PFP	
Total Assay Time	Sample preparation - 30 min and ELISA overnight	Acylation 30 min and RIA 2 h	2-12 min
Standard Range	6.7 to 425 µg	0 / 15 – 2500 ng/mL	50-250 µg/24 h
Storage	2 - 8 °C	2 - 8 °C	2 - 8 °C
Advantages	• Test any biological fluid; • High sensitivity; • Small sample volumes (low cost); • Fast determination of antigen and antibody; • No radiation	• Total assay time approx. 2.5 hours; • No interference by medical drugs known; • Wide standard ranges. convenient measurement of pathological samples without predilution; • 15 week shelf-life	• Very fast and efficient technique; • Sensitive method; • It offers the possibility of testing multiple samples with small differences in results (screening test)
Ref(s).	[5,6]	[7]	[8,9]

However, a major problem encountered in the detection is interference from ascorbic acid (AA), which can be oxidized at potential close to that of 5-HT and DA at bare electrode, resulting in an overlapping voltammetric response. After further researches, various methods were developed in order to separate the anodic peaks of 5-HT, DA and AA, all consisting of a modified electrode creating new sensors and biosensors [2,11].

Glassy Carbon Electrodes. Carbon materials are widely used in both analytical and industrial electrochemistry due to their low cost, relatively inert electrochemistry, electrocatalytic activity for a variety of redox reactions and wide potential window. By combining the advantages of carbon materials with those of nanostructured materials, carbon-based nanoscaled materials have been widely used in preparation of modified electrodes including carbon nanotubes, carbon nanofibers, highly ordered mesoporous carbons, etc. [12,13,14].

Table II (overleaf) surveys the methods employing chemically and biologically modified glassy carbon electrodes used to detect the single serotonin or serotonin with dopamine, together with specification on how those electrodes have been modified.

Screen Printed Electrodes. Glassy carbon electrode has been widely used in research for 5-HT determination because it possesses high sensitivity and selectivity. Still, the reaction surface of the electrode needs to be remade after each test, which results in limited application for pharmacological study. Recently, screen-printed electrode has attracted an increasing interest because of the various advantages including simple fabrication, low-cost, small size, disposability and ease of mass production [16].

Liu and his collaborators [2] prepared a sensor for 5-HT detection based on screen-printed electrode and applied it to a rat depression model caused by chronic unpredictable mild stress (CUMS). Colloidal gold-modified graphite ink was included in the counter electrode and the working electrode. Nafion (2.0 μL, 1%) added to the surface of the SPEs was evaporated at room temperature. The advantages that recommend this method are: very good separation peak from AA, DA, and UA (uric acid), low cost, and short time needed.

Imaging Techniques (*Positron Emission Tomography-PET*). Molecular imaging can be used to visualize, characterize and quantify biological processes at the cellular and sub cellular levels within an intact living organism.

Positron emission tomography as one successful molecular imaging technique is based on the principle of labeling the ligand of interest with a positron-emitting isotope that after annihilation with an electron produces two γ-rays which can then be detected. The most commonly used isotopes are ^{15}O, ^{13}N, ^{11}C and ^{18}F. An advantage of PET oversingle-photon emission computed tomography (SPECT), another molecular imaging technique, is its significantly higher sensitivity and resolution, making PET interesting and promising for studying the molecular processes in human brain [18].

The PET shows the distribution of serotonin receptor (proteins) in different brain regions and quantifies these proteins with receptor function to visualize alterations in psychiatric and neurological disorders.

The main advantage of this non-invasive method is the possibility to analyze the entire serotonin system, including receptors, the distribution in the body, the synthesis, secretion, transport and degradation of serotonin.

Table II: *Modified glassy carbon electrodes (GCEs) used to detect serotonin*

Modified CGE	Modification method	Advantages/ Disadvantages	Ref.
1. Multi-wall-nanotubes-ionic liquid gel/modified glassy carbon electrode. Simultaneous determination of DA and 5-HT;	• CNTs-ionic liquids composite gel; • modified glassy carbon electrode (GCE), was fabricated by mixing 1-butyl-3-methylimidazoliumhexafluoro phosphate (OMIMPF6) and multi-wall carbon nanotubes (MWNTs)	• high electrochemical performance; • good adsorption ability and catalytic effect of carbon nanotubes; • good electron conductivity of ionic liquid; • large peak separations between DA, 5-HT and AA	[13]
2. 5-hydroxitriptofan-modified glassy carbon electrode. Simultaneous determination of DA and 5-HT;	• biosensor fabricated by covalent modification of 5-(5-HTP) on the surface of glassy carbon electrode; • 5- hydroxy-tryptophan (HTP) electrochemically deposited on the GCE support by CV	• 5-HTP/GCE was more suitable for DA analysis for the higher linear detection limit (0.31 μM) in the low concentration.	[15]
3. Gold nanoparticle-modified glassy carbon electrode. Selectively 5-HT determination.	• gold nanoparticles (GNPs) were allowed to self-assemble onto a glassy carbon electrode (GCE) that was prior modified by L-cysteine.	• detection of serotonin unaffected by the presence of epinephrine, dopamine, ascorbic acid and folic acid.	[16]
4. Meso-tetrakis (2-aminophenyl) porphyrin(TAPP)-single walled carbon nanotubes (SWNT) on glassy carbon electrode. Selectively 5-HT determination.	• electropolymerization of meso-tetrakis (2-aminophenyl) porphyrin (TAPP)-single walled carbon nanotubes (SWNT) on the surface of a glassycarbonelectrode (GCE)	• very effective to determined 5-HT in a mixture	[17]
5. PEDOP/MWCNTs-Pd nanoparticle modified glassy carbon electrode Determination of serotonin	• the GCE was modified by palladium-functionalized, multi-walled carbon nanotubes (MWCNTs-Pd) with electrochemical deposition of poly 3,4-ethylenedioxy pyrrole (PEDOP), denoted as PEDOP/MWCNTs-Pd/GCE	• determination at physiological pH; • was best in response compared to other modified electrodes made in the same lab	[12]

Samples Used for Detection

The most used biological samples are from serum [8], plasma [8] and urine [10], but the concentration of 5-HT can be determined also from platelets [7, 9], cerebrospinal fluid [19, 25] and saliva [27].

Because most blood serotonin is stored in the platelets and because the release reaction of serotonin occurs easily, platelet and plasma measurements are complicated by artifacts caused by sample conservation and (or) preparation. Thus, many investigators tend to prefer whole-blood assay. In tissues and body fluids, radioimmunoassay (RIA) [19, 20, 21], enzyme immunoassay [22], and fluorometric methods for determining serotonin [23] exhibit a lack of sensitivity or specificity or are time consuming.

Various liquid-chromatographic methods that incorporate electrochemical [24, 25, 26] and fluorometric [23, 27] detection can determine serotonin concentrations in platelet-rich plasma and in serum after a single deproteinization step. In whole blood, the presence of the hemoglobin iron and of oxygen alters the recovery of serotonin during deproteinization with concentrated perchloric acid. Ascorbic acid can be added before deproteinization when fluorometric detection is used. However, ascorbic acid interferes with amperometric detection of serotonin. Conversion of the free oxyhemoglobin to carboxyhemoglobin by bubbling carbon monoxide through the blood sample before precipitating the proteins effectively prevents serotonin oxidation [26].

Serotonin has been measured in urine by radioimmunoassay [7] and liquid chromatography with fluorometric or electrochemical detection. These methods have been combined into a cation-exchange purification step to increase selectivity. However, the serotonin peak was not always completely separated from interfering compounds. Direct injection of diluted and filtered urine samples was developed to profile serotonin and related indols by HPLC with fluorometric detection [27]. Nevertheless, the quaternary gradient elution required was time consuming and too complicated for daily routine use.

Conclusions

The serotonin determination has a major importance in clinical medicine. Nowadays, the used screening methods are based on the antigen-antibody reaction (ELISA, RIA) or HPLC. Still, those methods are presenting fewer advantages as those based on sensors and biosensors. The presented sensors and biosensors have good stability, sensitivity and selectivity for serotonin.

As shown and discussed above, the interference effects from other substances like ascorbic acid, dopamine, and uric acid can effectively be eliminated, which makes the respective procedures particularly attractive.

In conclusion, the electrochemical methods are a very promising tool for analysis; nevertheless, more research in this area still must be done in order to develop faster and easier ways to detect serotonin and related substances in the body.

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