



Study on detection methods for tryptophan in food and biological samples

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Abstract

Tryptophan (Trp) is an essential amino acid to humans. It is a vital constituent of proteins and indispensable in human nutrition for establishing and maintaining a positive nitrogen balance. Also, Trp is an important amino acid for brain functions and neuronal regulatory mechanisms. Thus, the establishment of a simple and rapid method for the determination of Trp with high selectivity and sensitivity is of great significance to people's health. In this article the studies of detection methods for Trp in recent years are reviewed.

Keywords: Tryptophan; Trp; determination; detection; sensor.

1. Introduction

Tryptophan (2-amino-3-(1H-indol-3-yl)-propionic acid) (Trp) is one of the eight essential amino acids for the human body and animal life activities, which plays a vital role in human and animal growth and metabolism. Metabolic disorder of Trp may induce a waste product in the brain and cause hallucinations and delusions. Moreover, Trp deficiency has also been associated with Alzheimer Disease (AD), since it is shown that the increased Trp intake would decrease pathological plaques in AD [1-3]. In order to correct possible dietary deficiencies, the intake of Trp is necessary as food products and pharmaceutical preparations, because it is not synthesized in our body [4-6]. Therefore, simple, rapid and sensitive determination of Trp in body fluids, pharmaceuticals, and food samples is of great importance. In this paper, the attributes of different analytical technique for the determination of Trp in recent years are reviewed.

2. Analytical Methods

2.1. Colorimetric method. Colorimetry has commonly been used for routine analysis due to its simplicity, low-

cost and practicability. It does not require any expensive or sophisticated instruments and the color changes can be even directly observed by the naked eye. Recently, some colorimetric systems have been set up to detect various kinds of substances such as DNA, biologically relevant molecules, metal ions, viruses and micro-organisms and so on [7-9].

Rawat *et al.* [10] described a simple, sensitive and reliable colorimetric method for assay of arginine, histidine, methionine and Trp in biological samples using 4-amino nicotinic acid assembled gold nanoparticles (ANA-Au NPs) as a colorimetric reader. 4-Amino nicotinic acid acted as a multifunctional agent to reduce Au^{3+} and to functionalize the synthesized Au NPs surfaces with an average size 13.1 nm. The aggregation of ANA-Au NPs induced by the above amino acids via electrostatic interactions and hydrogen bonding between ANA-Au NPs and amino acids resulted in a color change from dark pink to blue. Under optimum conditions, good linear relationships existed between the absorption ratios and the concentrations of Arg, His, Met and Trp in the

range of 0.001–100 μ M, with detection limits of 7.2, 4.2, 5.1 and 6.5 nM for Arg, His, Met and Trp, respectively. Chakrapani *et al.* [11] developed a simple method for the synthesis of copper nanoparticles (CuNPs) with relatively high concentration by using casein, hydrazine, and ascorbic acid as stabilizing, reducing and antioxidant agents, respectively. The synthesized CuNPs exhibited good antibacterial activity against Gram positive and Gram negative bacterial strains. Additionally, the optical properties of CuNPs for the first time have been utilized for the selective colorimetric sensing of Trp amino acid and Hg²⁺ metal cations in aqueous solution at ppm and ppb level, respectively.

2.2.HPLC method. High-performance liquid chromatography (HPLC) is a powerful tool that enables the separation of complex mixtures into individual components, and is a highly sensitive and reproducible analytical technique. In recent years, HPLC has been combined with many sensitive detection techniques and has experienced continuous improvement of stationary phases, which have improved its sensitivity and specificity. HPLC is currently widely used for the analysis of drugs and dosage forms with respect to quality control, quantitative determination of active ingredients and impurities, monitoring drug blood concentration in patients, and bioequivalence assessment [12,13].

Liu *et al.* [14] developed a novel method for the simultaneous determination of kynurenine and Trp by HPLC with electrochemical detection at multi-wall carbon nanotube-modified glassy carbon electrode. The typical HPLC experiments were conducted by using a reversed-phase ODS column with a mobile phase consisting of stock acetate buffer–methanol using an isocratic elution at the flow rate of 1.0 mL/min. The obtained LODs for kynurenine and Trp were 0.5 and 0.4 mmol/L, respectively. The analytical method for human plasma samples was validated and confirmed by LC-UV and LC-MS.

Zhao *et al.* [15] developed a HPLC with ultraviolet detection method for the simultaneous determination of a set of reliable markers of renal function, including creatinine, uric acid, kynurenine and Trp in plasma. Separation was achieved by an Agilent HC-C18 (2) analytical column. The total run time was 25min with all peaks of interest being eluted within 13min. Good linear responses were found with correlation coefficient >0.999 for all analytes within the concentration range of the relevant levels. The limit of detection for Trp was 1 μ mol/L. The developed method could be employed as a useful tool for the detection of chronic kidney disease, even at an early stage.

2.3. Chemiluminescence method. Chemiluminescence (CL) is a well-known and popular analytical method because of its high sensitivity, low detection limit, wide linear working range, and its rapidity, as well as the fact

that it can be performed with relatively simple and inexpensive instrumentation, as an excitation source and optical filters are not needed. It has been widely applied in various fields, including clinical diagnosis, biotechnology, pharmacology, food safety, and environmental chemistry [16].

Li *et al.* [17] developed a highly sensitive flow-injection CL method to determine Trp based on the CL reaction of galangin–KMnO₄–Trp in polyphosphoric acid (PPA) media. The presence of galangin greatly increased the luminous intensity of Trp in KMnO₄–PPA systems. Under optimized conditions, Trp was determined in the 0.05–10 μ g/mL range, with a detection limit of 5.0 \times 10⁻³ μ g/mL.

Li *et al.* [18] synthesized water-soluble ZnS quantum dots (QDs) modified with 3-mercaptopropionic acid, L-cysteine and thioglycolic acid. Through the study of the ZnS QDs effects on the CL system of luminol, they found that ZnS QDs could obviously enhance the CL of the luminol–N-bromosuccinimide (NBS) system in an alkaline medium. At the optimal experimental conditions, they researched the impact of 17 kinds of amino acids on this system. It was found that Trp and L-tyrosine (Tyr) had significantly inhibitory effects on luminol–NBS–ZnS QDs CL. Based on the inhibitory effects, they developed a novel CL method with a wider linear range and lower detection limit for determination of Trp and Tyr. The detection limits were 1.5 \times 10⁻¹¹ g mL⁻¹ and 2.0 \times 10⁻¹⁰ g mL⁻¹, respectively.

2.4. Electrochemical method. Since the early 70s electrochemistry has been used as a powerful analytical technique for monitoring electroactive species in living organisms. Trp being an electroactive compound, electroanalytical techniques provide an alternate way to analyze Trp with certain advantages such as quick response, high sensitivity, high selectivity, inexpensiveness, amenability to miniaturization, low power consumption and wide linear dynamic range. However, the electrochemical detection of Trp faces some problems. At traditional working electrodes, Trp follows a sluggish kinetics and has very high oxidation overpotential. The other electroactive biomolecules which coexist with Trp in biological matrices interfere with the determination of Trp due to their similar oxidation peak potentials [19-21]. In order to eliminate the effect of interferences and to enhance the selectivity of the biosensor, much effort for Trp detection has been devoted to design the modified electrodes to improve the catalytic properties, sensitivity, and selectivity of electrochemical sensors. Numerous materials, such as metal nanoparticles, polymers, carbon nanotubes, fullerenes, graphenes, and enzymes, have been used as modifiers to construct highly sensitive and selective Trp biosensors [22-24].

Peng *et al.* [25] utilized a metal–organic framework MIL-101(Fe) and silver nanoparticles composite (AgNPs/MIL-101) to act as a novel electrode modified material for the

detection of Trp. The AgNPs/MIL-101 modified glassy carbon electrode (AgNPs/MIL-101/GCE) produced an increase in the oxidation current of Trp compared with the bare electrode. Additionally, the electrochemical behaviours of Trp on AgNPs/MIL-101/GCE were investigated by cyclic voltammetry (CV) and differential pulse voltammetry (DPV). Under the optimum experimental conditions, the oxidation peak currents were proportional to the concentrations of Trp over the ranges of 1 μM to 50 μM and 50 μM to 150 μM , respectively. The detection limit was 0.14 μM . Moreover, the presented method was successfully applied to the determination of Trp in urine samples with good recovery.

Wang *et al.* [26] fabricated a glassy carbon electrode modified with poly(L-methionine) and graphene composite film (PLME/GR/GCE) by electropolymerization for determination of Trp in the presence of dopamine (DA). DPV was utilized to investigate the electrocatalytic oxidation of Trp from the potentially interfering species on the PLME/GR/GCE. Under optimum conditions, the proposed method exhibited a wide linear dynamic range, a much lower detection limit, good reproducibility and high selectivity. Moreover, the proposed modified electrode has been successfully applied to determine Trp in milk and human serum samples.

2.5. Capillary electrophoresis method. In recent decades, capillary electrophoresis (CE) has been developed for trace analysis because of its small sample size of only nanoliters to femtoliters, short analysis time, and biocompatible environments. In addition, rapid separations are feasible with CE because high voltages can be applied to short capillaries and separation efficiency is not dependent on column length. To identify biological and pharmaceutical analysis, CE is coupled to a variety of detectors, including fluorescence, mass spectrometry, and electrochemical detection [27,28]. Zhao *et al.* [29] prepared a modified carbon fiber microdisk electrode (CFME) via the one-step electrochemical reduction of graphene oxide (GO) in a film on the surface of the electrode. This one-step electrochemically reduced GO-modified electrode (ERGO/CFME) displayed improved voltammetric responses to Trp, uric acid (UA), and glutathione (GSH). The ERGO/CFME was used as the detector in capillary electrophoresis–electrochemical detection (CE-ECD) to separate and determine these species, and the linear concentration range of Trp was 0.6–600 $\mu\text{mol L}^{-1}$. The detection limit of Trp was 0.1 $\mu\text{mol L}^{-1}$. The ERGO/CFME showed good reproducibility, stability, and durability, and was successfully used to determine Trp, UA, and GSH in human whole-blood extracts.

Zinellu *et al.* [30] described an easy CE method with UV detection for the separation and detection of kynurenine (Kyn) and Trp in human plasma using methyltryptophan as internal standard. The obtained limits of detection for

Kyn and Trp were 0.15 and 0.40 $\mu\text{mol/L}$, respectively. The method suitability was tested by measuring analyte levels both in healthy volunteers, acute myocardial infarction and chronic kidney disease patients.

2.6. Other methods. In addition to these main approaches mentioned above for Trp detection, still a few special techniques with high sensitivity have been applied. Sutariya *et al.* [31] developed a unique fluorescence biosensor for selective detection of Trp and histidine. Rang *et al.* [32] reported the determination of kynurenine and Trp in human plasma by stacking-micellar electrokinetic chromatography. Huang *et al.* [33] designed a metal ion-assisted infrared optical sensor for selective determination of Trp in urine samples.

3. Conclusion

Trp is an essential amino acid for humans and a precursor for serotonin, melatonin, and niacin. It has been implicated as a possible cause of schizophrenia in people who cannot metabolize it properly. Therefore, the determination of Trp in food, pharmaceutical products and living bodies especially for human is very important [34,35]. This review has highlighted the significant developments in rapid and alternative techniques for the detection of Trp in recent years. We believe the development of Trp sensors with better sensitivity and specificity, lower cost, simplicity, along with *in vivo* analytical technique is still the future effort.

Acknowledgments


The work was supported by the Hebei Provincial Natural Science Foundation of China (No. B2015201161), Medical Engineering Cross Foundation of Hebei University (No. BM201108) and Medical Discipline Construction Foundation of Hebei University (No. 2012A1003).

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Quick Response Code	
DOI: 10.22192/ijcrops.2016.03.09.004	

How to cite this article:

Siyue Jia, Xiaodong Dong. (2016). Study on detection methods for tryptophan in food and biological samples. *Int. J. Curr. Res. Chem. Pharm. Sci.* 3(9): 27-31. DOI: <http://dx.doi.org/10.22192/ijcrops.2016.03.09.004>