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Recent advances in analytical techniques for the determination of dopamine

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Abstract
Dopamine (DA) is one of the most important catecholamine neurotransmitters in the human central nervous system in the brain and plays a key role in the functioning of the renal, hormonal, and cardiovascular systems. Abnormal release of DA will contribute to some diseases such as Alzheimer's and Parkinson's disease. Therefore, the sensitive determination of DA becomes increasingly significant in the field of clinical disease diagnosis and the research of physiological functions. A number of analytical methods have been developed to provide fast, sensitive, selective and reliable quantification in complex biological samples. In this article the studies of detection methods for DA in recent years are reviewed.

Keywords: dopamine; neurotransmitter; Parkinson's disease; detection; sensor

1. Introduction
Dopamine (DA), 2-(3,4-dihydroxyphenyl)ethylamine, is a kind of neurotransmitter which plays an important role in the function of mammalian central nervous, renal, hormonal and cardiovascular systems. It is one of the most crucial catecholamines and belongs to the family of excitatory chemical neurotransmitters. It is derived from tyrosine and is the precursor to norepinephrine and epinephrine. The hydrochloride salt of DA can be supplied as a medication in the treatment of shock, which may be caused by trauma, heart attack, open heart failure, kidney failure and severe bacterial infections of the blood. Excess amounts of DA in the brain often cause pleasurable, rewarding feelings, and sometimes even euphoria, while the deficiency of DA in the brain could cause a few central nervous system disorders, such as Alzheimer's and Parkinson's disease [1-5]. Therefore, the control and fluctuations of the amount of DA are extremely important in monitoring with analytical systems in the human brain. Many researchers have focused on developing analytical methods with a high sensitivity and good selectivity for detecting DA in biological samples and many strategies have been reported for the determination of DA. In this paper, the attributes of different analytical technique for the determination of DA 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 [6-8]. 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. The colorimetric detection of DA has also been reported, but with some limitations [9].
Over the last few decades, the gold nanoparticles (AuNPs) have been widely investigated and applied in molecular catalysis and biosensors due to their unique electrical and optical properties. Typically, the synthesized AuNPs with a size of 13 nm exhibit a specific absorption band around 520 nm while dispersed in liquid media. With the continual aggregation of AuNPs, the increased particle size will cause a red shift in the absorption spectrum which is easy to observe and analyze. Because of the high extinction coefficients and the unique size dependent optical property of AuNPs, AuNP-based colorimetric assays have been widely applied for detecting DNA, proteins, metal ions and small molecules. The reasonable designs of the surface chemistry of AuNPs promote specific interactions between receptors and analytes, leading to a highly selective detection [10, 11]. These methods require a very simple sample preparation process and minimal apparatus investment and can be conducted in the field with portable devices. Therefore, they are very promising in the field of sensor.

Liu *et al.* [12] reported a simple, sensitive and selective colorimetric assay for DA using dithiobis (succinimidylpropionate) (DSP)-modified AuNPs as probes and ferric ions as cross-linkers. By the standard amine coupling reaction between the amino groups of DA and activated carboxyl groups of DSP, DA molecules could be assembled onto the surface of DSP-AuNPs. Accordingly, Fe^{3+} ions induced a change of DSP-AuNPs in color and UV-vis absorbance by coordinating to the catechol groups of the anchored DA. The detection limit could reach 2 nM and the work demonstrated the feasibility for the detection of DA in artificial cerebrospinal fluid.

Chen *et al.* [13] presented an approach for the colorimetric detection of DA based on the modification of melamine on the surface of AuNPs through its exocyclic amino groups. The reaction of AuNPs with the free exocyclic amines of melamine leaded to aggregation of AuNPs. But the aggregated AuNPs induced by melamine could be separated by the melamine–DA conjugate through hydrogen-bonding interactions. Therefore, the blue color of AuNPs changed to blue-purple. With the optimized conditions, melamine–AuNPs were highly specific for DA and the detection limit could reach 33 nM. Furthermore, they demonstrate the application of the approach in human urine samples, which suggested its great potential for diagnostic purposes.

Su *et al.* [14] reported a facile, economic and eco-friendly colorimetric method for DA detection. The method was based on the interaction between Cu^{2+} ions with amino group and hydroxyl groups of the DA adsorbed on the surface of AuNPs. Cu^{2+} ions served as the selective ''discriminator and linker'' for DA detection. At high ionic strength, introduction of Cu^{2+} ions to AuNPs solution including DA aroused the aggregation of AuNPs, the color of the solution changed from wine red to blue, and the red shift of ultraviolet absorption peaks. The detection limit could reach 200nM. Furthermore, this probe was successfully applied to detect DA in human serum with high sensitivity, and this strategy may provide a selective sensing approach for measuring DA under physiological condition.

Lee *et al.* [15] developed a novel and high performance colorimetric probe for DA detection. They used aqueous-phase AuNPs extracted with 4-(dimethylamino)pyridine (DMAP) from toluene solvent as the reaction probes. The original AuNPs of diameter around 13 nm were separated into 2–5 nm sizes when DA was added, resulting in the color change of the AuNP solution from red to blackish green. The results confirmed that the DMAP capped AuNPs are etched by the DA molecules due to the strong affinity between DA and AuNPs, thus causing a blue shift in the absorption spectrum. The detection limit could reach 5 nM. The developed AuNP etching protocol for DA detection provided a novel and versatile approach for rapid biosensing applications.

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 [16-18].

De Benedetto *et al.* [19] described a fast and simple isocratic HPLC method for the determination of 3,4-dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE), DA, and serotonin (5-HT) in homogenate samples of mouse striatum employing the direct fluorescence of the neurotransmitters. The analytes were separated in 15 min on a reversed-phase column (C18) with acetate buffer–methanol as mobile phase. The fluorescence measurements were carried out at 320 nm with excitation at 279 nm. Due to its simplicity, rapidity and adequate working range, the method can be used for the determination of DOPAC, DA, NE and 5-HT in animal tissues. An experimental 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson-like disease has been used to demonstrate the method is fit-for-purpose.

Lin *et al.* [20] studied the variation in the concentration of monoamine neurotransmitters and their metabolites in an experimental Parkinsonian animal model established by unilateral 6-hydroxydopamine administration. In order to detect monoamine neurotransmitters and their metabolites more sensitively, they fabricated an acetylene black nanoparticles modified electrode and used it as the working electrode for an electrochemical detector in HPLC. The results indicated that the modified electrode exhibited efficiently electrocatalytic oxidation for monoamine neurotransmitters and their metabolites with relatively high sensitivity, long life, and stability. The detect ability was on the level of 0.1 nmol L^{-1}. Coupled with in vivo microdialysis sampling, the validated method was successfully applied to measure monoamine neurotransmitters and their metabolites in both sides of the striatum of conscious and freely moving Parkinsonian rats, and the extracellular monoamine neurotransmitters and their metabolites in the lesioned-side striatum of unilateral 6-hydroxydopamine-lesioned rats were lower than that in the intact side striatum or in the striatum of control rats.

Park *et al.* [21] established an effective derivatization method followed by high-performance liquid chromatography (HPLC) coupled to electrochemical ionization mass spectrometry to measure the levels of serotonin (5-hydroxyltryptamine, 5-HT), DA, 3,4-hydroxyphenylacetic acid (DOPAC), 3-methoxytyramine (3-MT) and homovanillic acid (HVA) simultaneously. The derivatization reaction of biological samples with ethyl chloroformate occurred rapidly at room temperature in aqueous conditions, and the resulting derivatives were isocratically separated with good selectivity using a C18 reversed-phase column within 30 min. The study results showed that the new derivatization procedure offers an excellent means of simultaneous determination of 5-HT, DA and their metabolites in mouse brain homogenates, which are important in a number of physiological and behavioral functions.

2.3. ECL method. Electrochemiluminescence (ECL) is a light emission that arises from the high-energy electron transfer reaction between electrogenerated species. As a valuable detection method, ECL has attracted great interest in analytical chemistry owing to its distinct advantages of simplicity, rapidity, sensitivity, controllability and low background, and has been extensively used for different analytical purposes such as squamous cell carcinoma antigen, glucose, cell tumor and so on. ECL is also a commendable model for investigating the mechanism of electron transfer [22-24].

Liu *et al.* [25] proposed a promising ECL sensing strategy with dual-stabilizers-capped CdSe quantum dots (QDs) as ECL emitters. The dual-stabilizers-capped CdSe QDs were covalently immobilized onto p-aminobenzoic acid modified glass carbon electrode with ethylenediamine as a link molecule. This strategy can preserve the completely passivated surface states of dual-stabilizers capped CdSe QDs, so that the sensor demonstrated eye-visible greenish, band gap engineering and monochromatic ECL emission at 546 nm with a fwhm of 35 nm. Moreover, the proposed sensor could accurately quantify DA from 10.0 nM to 3.0 μM with a detection limit of 3.0 nM in practical drug, human urine, and cerebrospinal fluid samples without any signal amplification techniques. This strategy is promising for developing ECL sensors with high sensitivity and spectral selectivity.

Huang *et al.* [26] reported the ECL behavior of the bioinspired peptide nanovesicles (PNVs) for the first time. The PNVs modified glassy carbon electrodes have shown a stable and efficient cathodic ECL signal with $K_2S_2O_8$ as coreactant in aqueous solution. They chose DA as a model analyte to study the potential of the PNVs in the ECL analytical application. It was found that the ECL intensity of the PNVs was effectively increased by trace amounts of DA. The limit of detection was estimated to be 3.15pM. These results suggest that the PNVs could be a new class of promising materials for the ECL design and bioassays in the future due to their fascinating features, such as excellent biocompatibility, tunable composition as well as capability of molecular recognition.

Li *et al.* [27] observed the ECL emission from the water-soluble, bovine serum albumin (BSA)-stabilized Au nanoclusters for the first time, which was ascribed to the effective electron transfer from the conduction-band of excited indium tin oxide (ITO) to Au nanoclusters (NCs). Futhermore, they developed a simple label-free method for the detection of DA based on the Au NCs ECL in aqueous media. The study showed the Au NCs could be an effective candidate for new types of ECL biosensors in the future due to their fascinating features, such as good water solubility, low toxicity, ease of labeling, and excellent stability.

Yan *et al.* [28] reported a novel strategy to amplify the ECL signal of peroxydisulfate solution based on the Au nanoparticle decorated reduced graphene oxide (Au NP–RGO). Due to the synergistic amplification of Au NPs and RGO, the ECL signal of peroxydisulfate solution on the Au NP–RGO modified electrode was about 5-fold enhanced compared to that of the bare electrode with the ECL onset potential positively shifted from -1.2 V to -0.9 V. Because the ECL intensity of peroxydisulfate solution increased with the increase of DA concentration, they fabricated an ECL biosensor for DA determination. The as-prepared solid-state ECL DA sensor showed a wide linear response of 0.02–40 mM with a detection limit of 6.7 nM. Moreover, this work would open up a new field in the application of peroxydisulfate solution ECL for highly sensitive bioassays.

2.4. Fluorescence methods. In recent years, fluorescence measurements have received more attention owing to their operational simplicity, high sensitivity, good reproducibility and real-time detection. A series of fluorescence probes have been designed for the detection of biomolecules and metal ions. For example, gold nanoclusters (AuNCs), which exhibit molecule-like properties including discrete electronic states and size-dependent luminescence have received great attention. Fluorescent silicon nanoparticles (SiNPs), which have a zero-dimensional silicon-based nanostructure, have been widely used in biology, owing to their good biocompatibility, low cytotoxicity, and antiphotobleaching capability. Colloidal quantum dots (QDs) which exhibit broad absorption profiles and narrow emission with high quantum yields and allow the chemical modification of functional groups on their surfaces make QDs naturally suitable for serving as fluorescent platforms for sensing and imaging in biology [29-31].

Ban *et al.* [32] designed a novel luminescence probe based on mono-6-amino-β-cyclodextrin (NH_2-β-CD) functionalized gold nanoclusters (β-CD-AuNC) for DA detection. The NH_2-β-CD molecules were conjugated onto the surface of 11-mercaptoundecanoic acid capped AuNCs via a carbodiimide coupling reaction. The integrity of the β-CD cavities was preserved on the surface of AuNCs and they retained their capability for molecular DA host–guest recognition. DA could be captured by the β-CD cavities to form an inclusion complex in which the oxidised DA could quench the fluorescence of the β-CD-AuNC probe by electron transfer. The probe could be used to quantify DA in the range of 5–1000 nM with a detection limit of 2 nM. This strategy was successfully applied to a DA assay in spiked human serum samples and it exhibited remarkable accuracy, sensitivity and selectivity.

Zhang *et al.* [33] synthesized highly fluorescent water-soluble SiNPs with a simple, one-pot method, and the SiNPs were used as a fluorescent probe for DA detection. It was found that the fluorescence of SiNPs can be linearly quenched by DA in a broad DA concentration range from 0.005 to 10.0 μM. The detection limit can reach 0.3 nM. In addition, it was found that other common molecules in the living system did not interfere with the DA detection, making it possible to use SiNPs for DA detection in a living system.

Mu *et al.* [34] reported an effective fluorescent sensor based on adenosine capped CdSe/ZnS quantum dots (A-QDs) for highly sensitive detection of DA in human urine samples. With the work, adenosine served as a capping ligand or stabilizer for QDs to render high-quality QDs dispersed in water, and as a receptor for DA to attach DA onto the surface of A-QDs. DA molecules can bind to A-QDs via non-covalent bonding, leading to the fluorescence quenching of A-QDs due to electron transfer. The A-QDs based fluorescence probe showed a limit of detection of ca. 29.3 nM for DA detection. This facile method exhibited high selectivity and anti-interference in the presence of amino acid, ascorbic acid (AA), uric acid (UA) and glucide with 100-fold higher concentration in PBS solution.

Zhao *et al.* [35] prepared water-soluble and luminescent ZnO QDs capped by (3-aminopropyl) triethoxysilane through two-step procedure and found the fluorescence of ZnO QDs could be selectively quenched by DA directly. Accordingly, they developed a novel label-free ZnO QDs based fluorescent probe for sensitive and selective detection of DA in biological fluids. With optimum conditions, the relative fluorescence intensity was linearly proportional to the concentration of DA within the range from 0.05 to 10 mM, and the detection limit could reach 12nM. The work has shown that the ZnO QDs based fluorescent probe has a potential for the practical application in clinical analysis.

2.5. Electrochemical method. Since the early 70s electrochemistry has been used as a powerful analytical technique for monitoring electroactive species in living organisms. Because DA is an electroactive compound that can

be easily oxidized on the electrode, electroanalysis of DA based on its electro-oxidation has been widely studied. Since the bare electrodes have poor sensitivity and selectivity, the oxidation peak potential of DA appears more positive over potential at the bare electrodes, much effort for DA 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 DA biosensors [36-38].

Wang *et al.* [39] fabricated a DNA and graphene (GR) bi-layer modified carbon ionic liquid electrode (CILE) by an electrodeposition method. GR nanosheets were electrodeposited on the surface of CILE at the potential of -1.3V and then DNA was further deposited at the potential of +0.5V on GR modified CILE. Then they studied the electrochemical behaviors of DA on the modified electrode with the calculated electrochemical parameters. Under the optimized conditions, a linear relationship between the oxidation peak current and the concentration of DA was obtained in the range from 0.1 μmol/L to 1.0 mmol/L with a detection limit of 0.027 μmol/L. The modified electrode exhibited excellent reproducibility, repeatability, stability, validation and robustness for the electrochemical detection of DA. The proposed method was further applied to the DA injection solution and human urine samples determination with satisfactory results.

Li *et al.* [40] synthesized the tremella-like graphene–Au (t-GN–Au) composite by a one-step hydrothermal method for selective detection of DA. The t-GN–Au composites were directly used for the determination of DA via cyclic voltammetry (CV) and the chronoamperometry (CA) technique. CA measurement gave a wide linear range from 0.8 to 2000 μM, and the detection limit of 57 nM for DA. Moreover, the modified electrode was applied to the determination of DA in human urine and serum samples.

Li *et al.* [41] proposed a novel electrochemical sensor for the determination of DA based on the molecularly imprinted electropolymers (MIPs)/copper oxide (CuO) nanoparticles modified electrode. MIPs were firstly prepared by using nicotinamide as an environment-friendly monomer to selectively recognize the template molecules. CuO nanoparticles were used to enhance the number of imprinted sites per unit surface area of the electrode and then improve the selectivity and sensitivity of the electrochemical sensor. Thus, the obtained electrochemical sensor could effectively minimize the interferences caused by ascorbic acid (AA), uric acid (UA) and sample matrix. The line arrange for the detection of DA was changed from 0.02 μmol L^{-1} to 25 μmol L^{-1} with the detection limit of 8nmol L^{-1}. It was shown that the proposed sensor exhibited significant promise as a reliable technique for the detection of DA in human serum samples.

2.6. 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 neurotransmitters, CE is coupled to a variety of detectors, including fluorescence, mass spectrometry, and electrochemical detection [42-44].

Wang *et al.* [45] described a simple method for the direct detection of DA in single rat pheochromocytoma cells (PC12 cells) by capillary electrophoresis with a palladium nanoparticles modified carbon fiber microdisk electrode (PdNPsCFME). The PdNPsCFME provided an enhanced effective electrode surface and high catalytic activity toward DA compared to traditional CFME. Under optimized detection conditions, DA responded linearly from the range of 0.2 mM to 2 mM and the concentration detection limit was 0.1 mM. This method had been successfully applied to determine DA in single PC12 cells and it is convenient, sensitive, and will become an attractive alternative method for single-cell analysis.

Fang *et al.* [46] developed capillary electrophoresis with fast-scan cyclic voltammetry detection (CE-FSCV) for analysis of DA, serotonin, and adenosine content in tissue punches from rat brain slices. Using field-amplified sample stacking, the limit of detection was 5 nM for DA, 10 nM for serotonin, and 50 nM for adenosine. Neurotransmitters could be measured from a tissue punch as small as 7 mg (7 nL) of tissue, three orders of magnitude smaller than a typical HPLC sample. CE-FSCV should facilitate measurements of tissue content in nanoliter samples, leading to a better understanding of how diseases or drugs affect DA, serotonin, and adenosine content.

Zhao *et al.* [47] developed a sensitive method based on quantum dot (QD)-enhanced capillary electrophoresis–chemiluminescence (CE–CL) detection for simultaneous determination of DA and epinephrine (E). In this work, CdTe QD was added into the running buffer of CE to catalyze the post-column CL reaction between luminol and hydrogen peroxide, achieving higher CL emission. Negative peaks were produced due to the inhibitory effects on CL emission from DA and E eluted from the electrophoretic capillary. The decrease in CL intensity was proportional to the concentration of DA and E in the range of 80nM–5μM and 40nM –5μM, respectively. Detection limits for DA and E were 23nM and 9.3nM, respectively. With this method, the levels of DA and E in human urine from healthy donors were determined.

2.7. Other methods. In addition to these main approaches mentioned above for DA detection, still a few special techniques with high sensitivity have been applied. Van Staden *et al.* [48] reviewed the flow-injection analysis systems with different detection devices and other related techniques for the in vitro and in vivo determination of DA as neurotransmitter. Ranc *et al.* [49] presented a method for a fast analysis of DA levels in samples of cerebrospinal fluid and mouse striatum. The method was based on a nanocomposite composed of magnetite and silver nanoparticles, whose surface was modified with iron nitriloacetic acid. The magnetic properties of this nanocomposite enabled simple separation of targeted molecules from a complex matrix while the silver acted as a platform for surface-enhanced Raman scattering. Silver and magnetite nanoparticles were joined by carboxymethyl chitosan, useful in biological environments and enhancing the sensitivity due to the presence of carboxyl groups. An *et al.* [50] used surface-enhanced raman spectroscopy to detect DA by DNA targeting amplification assay in Parkisons's model.

3. Conclusions

The dopaminergic neural system is a crucial part of the brain responsible for many of its functions including mood, arousal, and other roles. DA is the key neurotransmitter of this system, and a determination of its level presents a demanding task

needed for a deeper understanding of the processes, even pathological, involving this brain part. Thus, a fast, facile, low-cost, sensitive, and selective method for the detection of DA is quite essential [51-53]. This review has highlighted the significant developments in rapid and alternative techniques for the detection of DA in recent years. On the whole, newly developed methods have focused on better sensitivity and specificity, along with more simplicity and lower cost. Although numerous designs for DA detection have been reported, developing more efficient and practicable methods still remains necessary. Currently, multiplexed detection and in situ detection are especially important, because DA never work alone, but instead function within a network of several components in vivo. For diagnosis, in situ detection, especially in blood plasma, is of clinical significance. Therefore, it is likely that future effort will be focused on the development of in vitro and in vivo analytical technique for the determination of DA.

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