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Study on detection methods for ascorbic acid in medicine and food

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Abstract
Ascorbic acid (AA) is an essential nutrient for health maintenance. It plays an important role in many biological processes, such as collagen formation, amino acid metabolism, ion absorption and so on. The deficiency of AA will lead to the symptoms of scurvy and gingival bleeding, while excessive intake of AA will result in diarrhea, hyperacidity and kidney calculi. Therefore, the rapid, sensitive, and selective detection of AA level is significant for food and pharmaceutical analysis, clinical diagnosis applications. In this article the studies of detection methods for AA in recent years are reviewed.

Keywords: ascorbic acid; vitamin C; AA; determination; detection; sensor.

1. Introduction
Ascorbic acid (AA), more commonly known as vitamin C, is an essential nutrient which is found in many biological systems and foodstuffs. It is essential for many metabolic functions and redox processes. It plays a key role in the formation and maintenance of collagen, and is a powerful antioxidant, which reacts with reactive oxygen species or free radicals. It strengthens and protects the immune system, enhances iron bioavailability and is believed to help to reduce cholesterol levels. AA is also widely used in foods and drinks as an antioxidant and essential additive in the food processing industry. Despite the necessity and importance of AA for the human body, excessive intake of AA can result in urinary stone, diarrhea, and stomach convulsion [1, 2]. Therefore, the highly selective detection of AA is of great importance in pharmaceutical analysis, clinical diagnosis, and food industries. Many strategies have been reported for the determination of AA [3-5]. In this paper, the attributes of different analytical technique for the determination of AA in recent years are reviewed.

2. Analytical Methods
2.1. Spectrophotometric method: High sensitivity, sufficient accuracy, simplicity, speed and the necessity of less expensive apparatus make spectrophotometric method as an attractive method for the determination of AA in samples with different matrices such as foods, biological and pharmaceutical samples. Currently, the combination of a flow-based technique such as flow injection analysis, with spectrophotometric detection methods increase the automation degrees and sample throughput for analysis of AA in various samples [6, 7].
Liamas *et al.* [8] reported a flow-injection spectrophotometric determination with a photodegradation step to determine AA and total sugars. The flow-injection system included a simple ultraviolet photoreactor for the on-line photodegradation. The method was based on the determination of AA at 300 nm before the photodegradation step, followed by UV irradiation and measurement of total sugars at 268 nm. The proposed method was used to determine AA and total sugars in commercial and natural fruit juice samples.
Kukoc-Modun *et al.* [9] proposed a flow-injection indirect spectrophotometric method for the determination of AA in pharmaceutical preparation. The method was based on the reduction of iron (III) to iron (II) by the AA, and by the subsequent reaction of the produced iron (II) with 2, 4, 6-tripyridyl-s-triazine in buffered medium to form a colored complex. The linear range of the method is from 0.08 to 10μM of AA, with the detection limit 24 nM of AA. The proposed method could be applied for the determination of AA in pharmaceutical preparations, down to picomolar quantity.

2.2. 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 [10-12].

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 [13, 14]. 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.

Bahram *et al.* [15] applied a new synthetic route to the fabrication of AuNPs. In addition, they studied the interactions between synthesized AuNPs with acetaminophen (ACET), AA and folic acid (FA). The results showed that the synthesized AuNPs could be used as a new and highly efficient spectrophotometric sensor for the selective recognition and monitoring of three drugs in real samples such as serum samples. The detection limit of ACET, AA and FA were obtained as 1.8μM, 2.7μM, 2μM for ACET, AA and FA, respectively. The proposed method was successfully used to the determination of ACET, AA and FA in various serum samples.

Zhang *et al.* [16] presented an approach for visual sensing of AA. They prepared the terminal azide- and alkyne-functionalized AuNPs probes. In the presence of Cu^{2+}, AA could rapidly induce the aggregation of the AuNPs, thereby resulting in a red-to-purple (or pink) color change. AA could be quantified visually or using a UV-vis spectrometer. The present limit of detection for AA was 3 nM. This method exhibited excellent selectivity over other common organic reducing compounds. This method provided a potentially useful tool for the on-site detection of AA.

2.3. 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 [17, 18].

Khan *et al.* [19] reported a new, simple, economical and validated HPLC linked with electrochemical detector (HPLC–ECD) method for the determination of aminothiols and AA in human plasma and erythrocytes. The dopamine was used as internal standard. Complete separation of all the targets analytes at 35 ℃ on Discovery HS C18 RP column was achieved using 0.05% TFA: methanol as a mobile phase pumped at the rate of 0.6 ml min^{-1} using electrochemical detector in DC mode at the detector potential of 900 mV. The suggested HPLC–ECD method was not only applicable for routine analyses, but would also be suitable for understanding the aminothiols metabolism abnormalities, improvement of diagnoses, and interpretation of nutritional intervention.

Koblova *et al.* [20] reported a fast and simple HPLC method with UV spectrophotometric detection for determination of AA, phenylephrine, paracetamol and caffeine. Salicylic acid was used as internal standard. The analytes were successfully separated in less than 5 min by isocratic elution using monolithic column, Onyx Monolithic C18, with mobile phase composed of acetonitrile and phosphate buffer at a flow rate of 1.0 mL min^{-1} and 25 ℃, sample volume was 10 mL. Detection was observed at two wavelengths 210 nm and 235 nm. The optimized method was applied for the determination of the analytes in pharmaceutical formulation Coldrex tablets commonly used in virosis treatment.

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 [21, 22].

Li *et al.* [23] used ovalbumin stabilized AuNCs as sensing probes for AA detection. The fluorescence of AuNCs was found to be quenched effectively through the change in the oxidation state of AuNCs by AA. Upon the addition of AA under optimal conditions, the fluorescence intensity quenched linearly with the logarithm of AA concentration over the range of 1.0 nM to 100 mM with a detection limit of 0.5 nM. Results indicated that the proposed method gave a good sensitivity and simple operation for detecting AA. This work also had promising potential for protein-modified AuNCs.

Li *et al.* [24] developed a novel and highly sensitive fluorescence probe for determination of AA based on the distinct fluorescence quenching of Mn-doped CdTe fluorescence emission in the presence of AA. Under the optimum conditions, the response was linearly proportional to the concentration of AA in the range of 0.4 to 10 nM with an extremely low detection limit of about 0.081 nM. The newly developed method was successfully applied for the determination of AA in pharmaceutical tablets with good recoveries.

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 AA is an electroactive compound that can be easily oxidized on the electrode, electroanalysis of AA based on its electro-oxidation has been widely studied. Since the bare electrodes have poor sensitivity and selectivity, the oxidation peak potential of AA appears more positive over potential at the bare electrodes, much effort for AA 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 AA biosensors [25, 26].

Zhao *et al.* [27] fabricated a promising electrochemical biosensor for simultaneous detection of AA, dopamine (DA) and uric acid (UA) by electrochemical deposition of MgO nanobelts on a graphene-modified tantalum wire electrode. In the threefold co-existence system, the linear calibration plots for AA, DA and UA were obtained over the concentration range of 5.0–350 mM, 0.1–7 mM and 1–70 mM with detection limits of 0.03 mM, 0.15 mM and 0.12 mM, respectively. The modified electrode showed excellent selectivity, good sensitivity and good stability, making it attractive as a sensor for simultaneous detection of AA, DA and UA in biological fluids.

Ouyang *et al.* [28] prepared a glassy carbon electrode modified with poly (bromocresol green) by electropolymerization process for simultaneous determination of UA, DA and AA. The simultaneous determination of UA, DA and AA in 0.1 M phosphate buffer solution was carried out by differential pulse voltammetric technique. Under optimum conditions, the results showed that the peaks of three species were well separated. The proposed sensor exhibited linear responses to UA, DA and AA in the ranges of 0.5–200 μM, 0.05–10 μM and 0.5–1000 μM, respectively. Moreover, the modified electrode had been successfully applied to determine UA, DA and AA in human serum samples and vitamin C tablets.

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 [29, 30].

Wang *et al.* [31] prepared a platinum nanoparticles modified carbon fiber micro-disk electrode and exploited it as an amperometric detector for CE. The prepared sensor displayed rapid and sensitive response towards AA. Under optimized detection conditions, AA responded linearly from the range of 1.0 μM to 8.0 μM and 8.0 μM to 1 mM with correlation coefficients of 0.9981 and 0.9993. The concentration detection limits was 0.5 μM. Compared with carbon fiber micro-disk electrode, the sensitivity was enhanced nearly four times. Several merits of the novel electrochemical sensor coupled with CE, such as comparative repeatability, easy fabrication and high sensitivity, hold great potential for the single-cell analysis.

Tao *et al.* [32] applied CE for the separation of three charged compounds, cysteine (Cys), AA and UA. These compounds were then simultaneously determined using an electrochemiluminescence (ECL) system. The ECL intensities were linear with the concentrations of three compounds over the ranges from 2.0 μM to 100 μM. The detection limits for Cys, AA, and UA were 0.5 μM, 0.4 μM, and 0.02 μM, respectively. The method was applied successfully for monitoring three compounds in the human serum and urine samples.

2.7. Other methods: In addition to these main approaches mentioned above for AA detection, still a few special techniques with high sensitivity have been applied. Malashikhina *et al.* [33] designed an assay for rapid detection of AA with a DNA zyme cleaving its DNA substrate in the presence of Cu^{2+} and AA. Employment of gold nanoparticles decorated with fluorescein-modified DNA was allowed to improve the detection limit of AA by two orders of magnitude due to enhanced cleavage of DNA catalysed by Au clusters. With good selectivity, the method was applied for rapid and sensitive analysis of AA in vitamin C tablets, urine and orange juice. Dong *et al.* [34] proposed a novel flow-injection chemiluminescence (CL) method for the determination of AA, based upon its enhancing effect on the CL reaction of luminal with ferricyanide catalyzed by gold nanoparticles in alkaline solution. This method had been successfully applied in the determination of AA in several real samples. Vakh *et al.* [35] developed a simple and easy performed flow system based on sandwich technique for the simultaneous separate determination of iron (II) and AA in pharmaceuticals. The implementation of sandwich technique assumed the injection of sample solution between two selective reagents and allowed the carrying out in reaction coil two chemical reactions simultaneously. The suggested approach was validated according to the following parameters: linearity, sensitivity, precision, recoveries and accuracy.

3. Conclusions

The design and development of high-performance methods and sensors for AA determination is vital, given the constant importance and presence of this key analyte in foodstuffs, pharmaceutical and biological fluids, with implications in redox processes, human health and food quality [36]. This review has highlighted the significant developments in rapid and alternative techniques for the detection of AA in recent years. We believe the development of AA sensors with better sensitivity and specificity, lower cost, simplicity, along with in vivo analytical technique is still the future effort.

4. Acknowledgments

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5. References

1. Yang SS, Ren CL, Zhang ZY, Hao JJ, Hu Q, Chen XG. Aqueous synthesis of CdTe/CdSe core/shell quantum dots as pH-sensitive fluorescence probe for the determination of ascorbic acid, J Fluoresc. 2011; 21(3):1123-1129.
2. Zhu XH, Zhao TB, Nie Z, Liu Y, Yao SZ. Non-redox modulated fluorescence strategy for sensitive and

selective ascorbic acid detection with highly photoluminescent nitrogen-doped carbon nanoparticles via solid-state synthesis, Anal Chem 2015; 87(16):8524-8530.
3. Szpikowska-Sroka B, Poledniok J. Spectrophotometric determination of *L*-ascorbic acid in pharmaceuticals, J Anal Chem. 2011; 66(10):941-945.
4. Heli H, Sattarahmady N. Amperometric determination of ascorbic acid in pharmaceutical formulations by a reduced graphene oxide-cobalt hexacyanoferrate nanocomposite, Iran J Pharm Res. 2015; 14(2):453-463.
5. Stojanovic Z, Svarc-Gajic J, Vitas J, Malbasa R, Loncar E. Direct chronopotentiometric method for ascorbic acid determination in fermented milk products, J Food Compos Anal. 2013; 32(1):44-50.
6. Shishehbore MR, Aghamiri Z. A highly sensitive kinetic spectrophotometric method for the determination of ascorbic acid in pharmaceutical samples, Iran J Pharm Res. 2014; 13(2):373-382.
7. Salkic M, Selimovic A, Pasalic H, Keran H. Peroxydisulfate oxidation of L-ascorbic acid for its direct spectrophotometric determination in dietary supplements, J Appl Spectrosc. 2014; 81(1):134-139.
8. Llamas NE, Nezio Di MS, Band BSF. Flow-injection spectrophotometric method with on-line photodegradation for determination of ascorbic acid and total sugars in fruit juices, J Food Compos Anal. 2011; 24(1):127-130.
9. Kukoc-Modun L, Biocic M, Radic N. Indirect method for spectrophotometric determination of ascorbic acid in pharmaceutical preparations with 2,4,6-tripyridyl-s-triazine by flow-injection analysis, Talanta 2012; 96(SI):174-179.
10. Hu SL, Song JJ, Zhao F, Meng XG, Wu GY. Highly sensitive and selective colorimetric naked-eye detection of Cu^{2+} in aqueous medium using a hydrazone chemosensor, Sens Actuator B: Chem 2015; 215:241-248.
11. Deng HM, Shen W, Gao ZQ. Colorimetric detection of single nucleotide polymorphisms in the presence of 10^3-fold excess of a wild-type gene, Biosens Bioelectron 2015; 68:310-315.
12. Wang FF, Liu SZ, Lin MX, Chen X, Lin SR, Du XZ *et al.* Colorimetric detection of microcystin-LR based on disassembly of orient-aggregated gold nanoparticle dimmers, Biosens Bioelectron 2015; 68:475-480.
13. Wang Q, Yang XH, Yang XH, Liu F, Wang KM. Visual detection of myoglobin via G-quadruplex DNAzyme functionalized gold nanoparticles-based colorimetric biosensor, Sens Actuator B: Chem 2015; 212:440-445.
14. Leng YM, Xie K, Ye LQ, Li GQ, Lu ZW, He JB. Gold-nanoparticle-based colorimetric array for detection of dopamine in urine and serum, Talanta 2015; 139:89-95.
15. Bahram M, Hoseinzadeh F, Farhadi K, Saadat M, Najafi-Moghaddam P, Afkhami A. Synthesis of gold nanoparticles using pH-sensitive hydrogel and its application for colorimetric determination of acetaminophen, ascorbic acid and folic acid, Colloid Surf A-Physicochem Eng Asp 2014; 441:517-524.
16. Zhang YF, Li BX, Xu CL. Visual detection of ascorbic acid via alkyne-azide click reaction using gold nanoparticles as a colorimetric probe, Analyst 2010; 135(7):1579-1584.
17. Ye NS, Gao T, Li J. Hollow fiber-supported graphene oxide molecularly imprinted polymers for the determination of dopamine using HPLC-PDA, Anal Methods 2014; 6(18):7518-7524.
18. Capone DL, Ristic R, Pardon KH, Jeffery DW. Simple quantitative determination of potent thiols at ultratrace levels in wine by derivatization and high-performance liquid chromatography−tandem mass spectrometry (HPLC-MS/MS) analysis, Anal Chem 2015; 87(2):1226-1231.
19. Khan A, Khan MI, Iqbal Z, Shah Y, Ahmad L, Nazir S *et al*. A new HPLC method for the simultaneous determination of ascorbic acid and aminothiols in human plasma and erythrocytes using electrochemical detection, Talanta 2011; 84(3):789-801.
20. Koblova P, Sklenarova H, Brabcova I, Solich P. Development and validation of a rapid HPLC method for the determination of ascorbic acid, phenylephrine, paracetamol and caffeine using a monolithic column, Anal Methods 2012; 4(6):1588-1591.
21. Zhou X, Ma PP, Wang AQ, Yu CF, Qian T, Wu SS *et al*. Dopamine fluorescent sensors based on polypyrrole/graphene quantum dots core/shell hybrids, Biosens Bioelectron 2015; 64:404-410.
22. Yildirim A, Bayindir M. Turn-on fluorescent dopamine sensing based on in situ formation of visible light emitting polydopamine nanoparticles, Anal Chem 2014; 86(11):5508-5512.
23. Li YX, Chen YT, Huang L, Ma L, Lin Q, Chen GN. A fluorescent sensor based on ovalbumin-modified Au nanoclusters for sensitive detection of ascorbic acid, Anal Methods 2015; 7(10):4123-4129.
24. Li L, Cai XY, Ding YP, Gu SQ, Zhang QL. Synthesis of Mn-doped CdTe quantum dots and their application as a fluorescence probe for ascorbic acid determination, Anal Methods 2013; 5(23):6748-6754.
25. Rageh HM, Abou-Krisha MM, Abo-Bakr AM, Abd-Elsabour M. Electrochemical behavior and the detection limit of ascorbic acid on a Pt modified electrode, Int J Electrochem Sci 2015; 10(5):4105-4115.
26. Sun JY, Li L, Zhang XP, Liu D, Lv SM, Zhu DR *et al*. Simultaneous determination of ascorbic acid, dopamine and uric acid at a nitrogen-doped carbon nanofiber modified electrode, RSC Adv 2015; 5(16):11925-11932.
27. Zhao LW, Li HJ, Gao SM, Li MJ, Xu S, Li CP *et al*. MgO nanobelt-modified graphene-tantalum wire electrode for the simultaneous determination of ascorbic acid, dopamine and uric acid, Electrochim Acta 2015; 168:191-198
28. Ouyang XQ, Luo LQ, Ding YP, Liu BD, Xu D, Huang AQ. Simultaneous determination of uric acid, dopamine and ascorbic acid based on poly(bromocresol green) modified glassy carbon electrode, J Electroanal Chem. 2015; 748:1-7.
29. Tortajada-Genaro LA. Determination of L-ascorbic acid in tomato by capillary electrophoresis, J Chem Educ. 2012; 8(9):1194-1197.
30. Falkova MT, Bulatov AV, Pushina MO, Ekimov AA, Alekseeva GM, Moskvin LN. Multicommutated stepwise injection determination of ascorbic acid in medicinal plants and food samples by capillary zone electrophoresis ultraviolet detection, Talanta 2015; 133(SI):82-87.
31. Wang XL, Li LJ, Li ZY, Wang J, Fu HY, Chen ZZ. Determination of ascorbic acid in individual liver cancer cells by capillary electrophoresis with a platinum nanoparticles modified electrode, J Electroanal Chem. 2014; 712:139-145.

32. Tao YW, Zhang XJ, Wang JW, Wang XX, Yang NJ. Simultaneous determination of cysteine, ascorbic acid and uric acid by capillary electrophoresis with electrochemiluminescence, J Electroanal Chem. 2012; 674:65-70.
33. Malashikhina N, Pavlov V. DNA-decorated nanoparticles as nanosensors for rapid detection of ascorbic acid, Biosens Bioelectron 2012; 33(1):241-246.
34. Dong YP, Gao TT, Chu XF, Chen J, Wang CM. Flow injection-chemiluminescence determination of ascorbic acid based on luminol-ferricyanide-gold nanoparticles system, J Lumines. 2014; 154:350-355.
35. Vakh C, Freze E, Pochivalov A, Evdokimova E, Kamencev M, Moskvin L *et al*. Simultaneous determination of iron (II) and ascorbic acid in pharmaceuticas based on flow sandwich technique, J Pharmacol Toxicol Methods. 2015; 73:56-62.
36. Pisoschi AM, Pop A, Serban AI, Fafaneata C. Electrochemical methods for ascorbic acid determination, Electrochim Acta 2014; 121:443-460.