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RESEARCH PAPER

Simultaneous and Sensitive Determination of Levodopa and Carbidopa in Pharmaceutical Formulation and Human Serum by High Performance Liquid Chromatography with On-Line Gold Nanoparticles-Catalyzed Luminol Chemiluminescence Detection

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Abstract: Levodopa, the metabolic precursor of dopamine, is usually administrated in combination with carbidopa to control dopamine levels in an appropriate manner and reduce side effects in the treatment of Parkinson's disease. In this study, a selective and sensitive high performance liquid chromatography coupled with on-line gold nanoparticles-catalyzed luminol chemiluminescence method for simultaneous determination of levodopa and carbidopa was developed. This method was based on the strongly enhanced chemiluminescence signal of on-line gold nanoparticles-catalyzed luminol-H_2O_2 system by levodopa and carbidopa. The possible enhancement mechanism was attributed to that levodopa and carbidopa could promote the on-line formation of a large number of gold nanoparticles, which catalyzed the luminol-H_2O_2 chemiluminescence reaction. The good separation of levodopa and carbidopa was achieved with isocratic elution using a mixture of methanol and 0.2% aqueous phosphoric acid (5:95, *V/V*) within 10.5 min. Under the optimal conditions, the linear ranges of levodopa and carbidopa were 2.24–448 ng mL^{-1} and 4.32–1080 ng mL^{-1} with the detection limits of 0.89 and 1.08 ng mL^{-1} (*S/N* = 3), corresponding to 17.92 and 21.60 pg for 20 μL sample injection, respectively. The validated method was successfully applied to simultaneous quantification of levodopa and carbidopa in controlled-release tablets (Sinemet®) and human plasma. The average recoveries of levodopa and carbidopa in the tablets were 100.5% and 103.1% with the precisions (RSDs) of 2.4% and 4.0%. The recoveries of levodopa and carbidopa in human plasma ranged from 97.0% to 103.5% with RSDs of no more than 3.3%.

Key Words: Levodopa; Carbidopa; Chemiluminescence; On-line; Gold nanoparticles; Luminol

1 Introduction

Parkinson's disease is a neurodegenerative disorder of the central nervous system. The loss of neurons in the substantia nigra, a region of the midbrain, leads to a deficiency of dopamine neurotransmitter in the brain, which may lead to Parkinson's disease[1]. Dopamine is not effectively used for the treatment of Parkinson's disease, because it could not penetrate the blood-brain barrier (BBB)[2]. Levodopa (LD, Fig.1A), the metabolic precursor of dopamine, has been

Fig.1 Structures of (A) levodopa and (B) carbidopa

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regarded as the standard for treating Parkinson's disease, which is a condition precipitated by dopamine depletion in the central nervous system[3]. Unlike dopamine, LD can cross the BBB via a saturable transporter and is converted to dopamine by l-aromatic amino acid decarboxylase in the brain[4]. For the better therapeutic effect and lower toxicity, carbidopa (CD, Fig.1B) is administered in association with LD in the pharmaceutical formulations containing 10%–25% of CD. CD is a competitive peripheral l-aromatic amino acid decarboxylase inhibitor with little or no pharmacological activity when given alone in usual dosage to Parkinson's disease patients[5]. CD does not cross the BBB and contributes to the production of effective brain concentrations of dopamine from lower doses of LD by inhibiting the peripheral decarboxylation of LD to dopamine. In addition, the reduced peripheral formation of dopamine decreases the peripheral side effects such as nausea, vomiting and cardiac arrhythmia[6]. To release the fluctuating symptoms in clinical, the most commonly used method is to administrate with controlled-release LD/CD tablet (Sinemet®, specifications 200/50 mg), which is developed to prolong the therapeutic plasma level of LD[7,8]. Therefore, it is very important to establish a selective and sensitive method for simultaneous detection of LD and CD in pharmaceutical formulations and human serum, because of the coexistence of LD and CD in pharmaceutical formulations and the rapid fluctuations of plasma drug concentration of LD. However, the accurate and sensitive analysis of LD and CD is challenging due to their poor stability, low molecular mass, and high polarity[9].

Many analytical methods including chemiluminescence (CL)[10,11], spectrophotometry[12–14], amperometric and voltammetric determination[2,15] have been reported for determination of LD or CD alone in various pharmaceutical preparations and biological samples. Simultaneous analysis of LD and CD was commonly achieved by high performance liquid chromatography (HPLC), because of the merits of high resolution and short analysis time. Fluorescent[16], mass spectrometric[17–19] and electrochemical[20,21] detection have been used to couple with HPLC method for sensitive quantification of LD and CD. Among them, electrochemical detection method had the limitation of bad reproducibility, and mass spectrometry had the disadvantage of high instrument cost.

The combination of HPLC method with CL detector is a high-efficiency technique and has many advantages such as high sensitivity, rapidity and superb reproducibility. To our knowledge, there is only one published literature that reported the luminol-potassium ferricyanide CL system coupled with HPLC method for determination of LD[22]. Luminol-H_2O_2 CL reaction, a popular CL system, has been widely applied to the detection of various substances[23–25]. Gold nanoparticles (AuNPs) have been widely applied in CL reactions as catalysts because of their excellent catalytic activities and facile synthesis. Zhang *et al*[26] first applied AuNPs to enhance the CL of luminol-H_2O_2 system, and the enhancement was supposed to originate from the catalysis of AuNPs, which facilitated the radical generation and electron-transfer processes taking place on the surface of AuNPs. In our previous work, a novel on-line AuNPs-catalyzed luminol-H_2O_2 CL detector for HPLC method was established to simultaneously determine eight phenolic compounds in red wine and catecholamines (norepinephrine, epinephrine and dopamine) at trace levels in rat brain[27,28].

In this work, it was found that LD and CD could strongly enhance the CL intensity of on-line AuNPs-catalyzed luminal-H_2O_2 reaction. This phenomenon allowed us to couple the highly sensitive on-line AuNPs-catalyzed luminol-H_2O_2 CL detection with HPLC (HPLC-nanoCL) for simultaneous quantification of LD and CD for the first time. This method exhibited the advantages such as low cost, short time and simple sample handling process. The experimental conditions for the good HPLC separation and maximal and stable CL intensities of LD and CD were systematically optimized. The possible mechanism of on-line AuNPs-catalyzed luminol-H_2O_2 CL enhanced by LD and CD was studied by UV-visible absorption sepctroscopy and transmission electron microscopy. The proposed HPLC-nanoCL method was applied to detect LD and CD in human serum and pharmaceutical formulation with satisfactory results.

2 Experimental

2.1 Materials and reagents

A stock solution of luminol (10 mM) was prepared by dissolving luminol (Merck, Darmstadt, Germany) in sodium hydroxide solution (0.1 M) and stored at least 7 days before dilution. The buffer solutions of $NaHCO_3$-Na_2CO_3 were prepared by mixing 0.1 M $NaHCO_3$ and Na_2CO_3 aqueous solution. $NaHCO_3$ and Na_2CO_3 were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). The fresh H_2O_2 working solution was prepared daily from 30% (*w/w*) H_2O_2 (Suzhou Chemical Reagent Company, China). A stock solution (1%, *w/w*) of $HAuCl_4$ was prepared by dissolving $HAuCl_4 \cdot 4H_2O$ (Shanghai Chemical Reagent Company, China) in water. The reference compounds of LD and CD were purchased from the Chinese National Institute for the Control of Pharmaceutical and Biological Products. Stock solutions of LD and CD were individually prepared in water containing H_3PO_4 (0.2%, *V/V*, Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) at final concentration of 0.1 mg mL^{-1}, respectively. All stock solutions and working solutions were stored in the dark at 4 ºC before use. Methanol was of HPLC grade, and all other chemicals were of analytical-reagent grade. Ultrapure water (18.3 MΩ cm,