Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 126 (2014) 178–183

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Label-free silver nanoparticles for the naked eye detection of entecavir

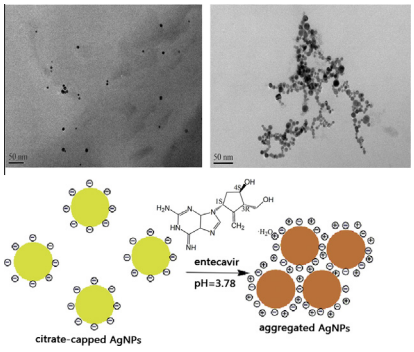
Mengmeng Gao [a,1], Rui Lin [b,1], Lili Li [e], Li Jiang [c], Baofen Ye [a,*], Hua He [a,d,*], Lanlan Qiu [b]

[a] Division of Analytical Chemistry, China Pharmaceutical University, Nanjing 210009, China
[b] Yancheng Health Vocational and Technical College, Yancheng 224005, China
[c] Department of Pharmacy, Jiangxi Cancer Hospital, 519 Beijing East Road, Nanchang 330029, China
[d] Key Laboratory of Drug Quality Control and Pharmacovigilance (China Pharmaceutical University), Ministry of Education, China
[e] Medical School, Pingdingshan University, Pingdingshan 467000, China

HIGHLIGHTS

- A new platform for the visual detection of entecavir was realized through their interaction with citrate-capped AgNPs.
- The concentration of entecavir could be determined with naked eye or UV–vis spectrometer.
- This method was simple and fast, with good selectivity and sensitivity.
- The proposed method was used to on-site screening of entecavir in human urine samples.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 4 July 2013
Received in revised form 13 January 2014
Accepted 2 February 2014
Available online 15 February 2014

Keywords:
Entecavir
Naked eye colorimetric detection
Human urine
Label-free
Silver nanoparticles

ABSTRACT

A simple, rapid, field-portable colorimetric method for the detection of entecavir was proposed based on the color change caused by the aggregation of silver nanoparticles. Neutralization of the electrostatic repulsion from each silver nanoparticle resulted in the aggregation of AgNPs and a consequent color change of AgNPs from yellow to wine-red, which provided a platform for rapid and field-portable colorimetric detection of entecavir. The concentration of entecavir could be determined with naked eye or UV–vis spectrometer. The proposed method can be used to detect entecavir in human urine with a detection limit of 1.51 μg mL^{-1}, within 25 min by naked eye observation without the aid of any advanced instrument or complex pretreatment. Results from UV–vis spectra showed that the absorption ratio was linear with the concentration of entecavir in the range of 5.04–25.2 μg mL^{-1} and 1.01–5.04 μg mL^{-1} with linear coefficients of 0.9907 and 0.9955, respectively. The selectivity of AgNPs detection system for entecavir is excellent comparing with other ions and analytes. Due to its rapid, visible color changes, and excellent selectivity, the AgNPs synthesized in this study are suitable to be applied to on-site screening of entecavir in human urine.

Introduction

Hepatitis B virus (HBV) is a deoxyribonucleic acid (DNA) virus that produces both acute and chronic infection of the liver in humans, HBV infection is a major global health concern [1,2]. Entecavir which is a novel antiviral agent used in the treatment of HBV, is a guanosine nucleoside analog with selective activity against the

* Corresponding authors. Address: China Pharmaceutical University, 24 Tongjia Lane, Nanjing 210009, Jiangsu province, China. Tel./fax: +86 025 83271505.
E-mail addresses: baofenye@yahoo.com.cn (B. Ye), dochehua@163.com (H. He).
[1] These authors equally contributed to this work and should be considered co-first authors.

http://dx.doi.org/10.1016/j.saa.2014.02.009
1386-1425/

HBV. It selectively inhibits the HBV, by blocking all three steps in the replication process. Moreover, entecavir suppresses HBV replication more rapidly and effectively than lamivudine or adefovir in compensated patients and has less resistance in chronic therapy [3,4]. The quantification of entecavir is usually required in clinic medical assay.

In recent years, analytical methods for the detection of entecavir have been established including reversed phase high performance liquid chromatography (RP-HPLC), liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI-MS/MS) [5], liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) [6,7], hydrophilic interaction chromatography-ultra-high-performance liquid chromatography tandem mass spectrometry (HILIC-UHPLC-MS/MS) [8]. These methods have advantages of high sensitivity and selectivity, but most of them demand expensive instruments and time-consuming purification, preconcentration, derivatization of the analyte by tedious traditional isolation procedures, making it difficult for on-site and real-time determination of entecavir. Therefore, it is desirable to develop a simple, rapid, and field-portable method for the detection of entecavir.

Recently, many colorimetric assay methods for detection of heavy metal ions based on gold or silver nanoparticles have been reported [9–11]. The major advantage of Au/AgNP-based assays is that the molecular recognition events can be transformed into color changes, which can be observed by the naked eye, therefore sophisticated instruments are not required. However, AgNPs have shown some unique characters and advantages over AuNPs to a certain degree since they possess much higher extinction coefficients than AuNPs of the same size. When the silver nanoparticles approach each other and aggregate, the color of the AgNPs changes from yellow to red [12,13]. A lot of colorimetric sensors have been established for detection of metal ions [9,14], small molecular [15–19], proteins [20–22], chiral compounds [13,23,24] and biological applications [7,21,25]. AgNPs have attracted great interests as a colorimetric probe, which can directly detect analytes by monitoring the color change, using UV–vis spectroscopy, or even with naked eyes. Apparently, nearly no complicated instruments are involved in the detection procedures. A large number of researchers have started to explore small molecular detection method based on gold or silver nanoparticles.

In this study, a simple, economical and field-portable visual method for the detection of entacavir was developed. The method is based on a rapid color change from yellow to wine-red when label-free AgNPs is mixed with entecavir. Citrate-capped AgNPs have electronegative charged surface exhibiting yellow color for the plasmon resonance absorption. However, the presence of positive charged entecavir would induce the aggregation of AgNPs owing to the electrostatic attraction, causing a color change of AgNPs suspension. Therefore, the selective colorimetric detection of entecavir can be established by visualizing the color of AgNPs suspension by naked eye without any equipment. To our knowledge, although there are some reports of quantification of entecavir in human plasma or human urine, this work represents the first attempt of on-site screening of entecavir in human urine.

Materials and methods

Chemical and reagents

A 29.5 μg mL^{-1} stock solution of the entecavir was prepared by directly dissolving the commercially purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) product into super-purified water (18.2 MΩ). Silver nitrate ($AgNO_3$ ⩾99.8%) was obtained from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Sodium borohydride ($NaBH_4$ ⩾96.0%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sodium citrate was purchased from Shanghai Rongrun Chemical Reagent Co., Ltd. (Shanghai, China). All chemicals used were of analytical reagent grade without further purification, and solutions were prepared with Milli-Q-purified distilled water.

Apparatus

Surface Plasmon resonance (SPR) absorption data of Ultraviolet and visible spectrophotometer (UV–vis) were performed using a Shimadzu UV-1800 spectrophotometer (Shimadzu, Japan) equipped with 1.0 cm quartz cells. All pH measurements were handled with a pHS-25 digital pH-meter (Shanghai Wei Ye Instrument Factory, China). Transmission electron microscope (TEM) was used to characterize AgNPs.

Preparation of citrate-stabilized AgNPs

All the glassware used in the experiment was soaked in aqua regia and rinsed thoroughly with Milli-Q water and dried in air prior to use. AgNPs were synthesized according to previous method [26]. Briefly, 25 mL $AgNO_3$ solution (1.0 mM) was added dropwise into 75 mL $NaBH_4$ solution (2.0 mM) under vigorous stirring. Ten minutes later, 5 mL 1% (w/w) sodium citrate aqueous solution was added to stabilize the colloid. The colloid was stirred for another 20 min and then left for 2 days at 4 °C. Finally, AgNPs were washed by deionized water and centrifuged for three times to remove the excess sodium citrate. At last, AgNPs were dispersed in 100 mL water for further investigation. The size of AgNPs was about 22 nm and the concentration was 1.15 nM. AgNPs were also quantified using a molar extinction coefficient of $\varepsilon = 3.35 \times 10^9\ M^{-1}cm^{-1}$.

Detection of entecavir

For detection of entecavir using AgNPs suspension, typically, 0.4 mL AgNPs dispersion was added into a mixture of 1 mL entecavir aqueous solution and 0.5 mL of Britton–Robinson buffer solution (pH ≈ 3.78). Then the total volume of the mixture was 1.9 mL. Finally, the mixture was maintained at room temperature for five minutes, and then the color changed from yellow to wine-red gradually. Then the solution was transferred into a 1 cm optical quartz cell for the measurements of the UV–vis absorption on UV–vis spectrophotometer. The photographs were captured with a digital camera 25 min later.

Results and discussion

Mechanism of the colorimetric sensor

Typically, colloidal solution of sliver nanoparticles is yellow owing to its SPR absorption band extended from 400 to 600 nm. The SPR band is not only dependent on the size of the particle and the refractive index of the surrounding media, but also gets significant changes with shape, and inter-particle distance. If aggregation of AgNPs gets accrued, the SPR absorption band would change obviously for the decreasing of inter-particles distance, causing color change of AgNPs solution. It was reported that the AgNPs in aqueous solution can be stabilized by coating with negative-charged citrate ions [27].

The reasons for the aggregation of AgNPs were also investigated in this paper. It was found that the citrate-capped AgNPs prepared in this study are electronegative charged, and dispersed from each other symmetrically by the electrostatic repulsion of each