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Original Article

Gold nanoparticles grafted modified silica gel as a new stationary phase for separation and determination of steroid hormones by thin layer chromatography

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ARTICLE INFO

Article history:
Received 24 August 2014
Received in revised form
22 November 2014
Accepted 24 November 2014
Available online 24 February 2015

Keywords:
nanoparticles
progesterone
testosterone
thin layer chromatography

ABSTRACT

A new thin layer chromatographic layer using gold nanoparticles grafted 3-triethoxysilyl propylamine modified silica gel (Au NPs-APTS modified silica gel) was developed as a stationary phase for separation and determination of two steroid hormones, namely progesterone and testosterone. Acetone–n-hexane 25:75 (v/v) was used as the mobile phase, and the results were compared with those obtained using plain (i.e., unmodified) silica gel plates. Some chromatographic parameters used for separation of the two steroids on an Au NPs-APTS modified silica gel plate as well as on a plain silica gel plate, including ΔR_F, separation factor (α), and resolution (R_S), were evaluated and compared. The reproducibility of R_F values was also determined by analysis of the two steroids in 7 consecutive days on both plates. Validity of the method was investigated, and a wide linear range of 1–200 ng per spot, and low detection limits of 0.16 ng and 0.13 ng per spot, low quantification limits of 0.51 ng and 0.40 ng per spot, and good precision (expressed as percent relative standard deviation) lower than 3.1% and 2.7% were obtained for progesterone and testosterone, respectively. As the results revealed, the proposed method is rapid and sensitive, and it is applicable to separation and determination of progesterone and testosterone in biological matrices such as urine samples.

1. Introduction

Steroid hormones are widely used to improve feed conversion efficiency and growth rates [1]. Many governmental regulatory agencies have dedicated much effort to develop analytical methods for monitoring hormones. However, assessment of natural steroids has been an analytical challenge due to their extremely low concentration and presence of interferences in biological matrices [2].

Recently, the role of planar chromatography, especially thin layer chromatography (TLC), has systematically increased [3].

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http://dx.doi.org/10.1016/j.jfda.2014.11.005

The main advantages of TLC over other specific liquid–mobile phase separation techniques, including high-performance liquid chromatography and capillary electrophoresis (CE), are the use of inexpensive equipment, lower purity of consuming solvents, saving a significant amount of consumables, and better waste removal [4]. Although unmodified silica gel or C_{18} functionalized silica are typically used as stationary phases for many TLC methods, some drawbacks impede their use in certain applications, such as peak tailing and dewetting in highly aqueous environment [5]. During the past few decades, many efforts have been made focusing on the improvement of the chromatographic properties of silica-based stationary phases. Furthermore, several new stationary phases have been developed, providing users alternative selectivity for more difficult chromatographic separations [6–10].

Recently, nanotechnology has led to innovations in various fields of separation science. Specially, nanostructured materials with a large surface-to-volume ratio and specific chemical properties attracted more and more attention for their potential application in chromatographic separations [11]. For example, the use of gold nanoparticles (Au NPs) has gradually been increased due to their ease of preparation, controllable particle size, narrow size distribution, good solubility, and convenient modification [11]. Significant advances have been achieved using Au NPs in gas chromatography, TLC, and CE methods. Gross et al [12] reported the first application of Au NPs in gas chromatography. They prepared monolayer-protected Au NPs by covalent immobilization of dodecanethiol on gold surface. The gold modified stationary phase provided fast mass transfer, and it can be used for specific applications based on the known gold chemistry. Shapovalova et al [13] reported the first use of these NPs for immobilization of L-cysteine on TLC plates. The first article describing the application of Au NPs in CE was published by Neiman et al [14] in 2001. Two different Au NPs (citrate and thiol stabilized) were used to generate a pseudostationary phase. The authors ascertained that although both modified Au NPs provided efficient and repeatable CE separations, the thiol-stabilized Au NPs resulted in better CE systems. In 2006 Yu et al [15] utilized surfactant modified Au NPs for CE separation of acidic and basic proteins. Although all the above-mentioned methods are sensitive, they are rather complex and have complex, tedious, and very time-consuming sorbent preparation steps, along with laborious column packing and pretreatments. Recently, three steroids (progesterone, testosterone, and testosterone propionate) were successfully separated on CE columns prepared through the alkanethiol self-assembly and dithiol layer-by-layer self-assembly processes onto Au NPs by Liu [16,17]. It was found that silica gel, which is coated by Au NPs self-assembled layer by layer with alkanethiols, is a good solid-phase extraction material for CE determination of natural steroids in urine samples. In fact, unreacted Au NP surface can eliminate urinary proteins. Thus, we suspected that the use of Au NPs in the structure of silica stationary phase could minimize baseline interferences of biological fluids and improve the sensitivity of TLC determination. Therefore, the suggested method enables quick, efficient, and sensitive measurements of the steroids with minimal baseline interferences.

This study mainly focused on the development of a simple, cost-effective, and sensitive TLC method based on the use of Au NPs grafted 3-triethoxysilyl propylamine (APTS) modified silica gel (Au NPs-APTS modified silica gel) as an efficient stationary phase to separate steroid hormones. Some chromatographic parameters such as ΔR_F, separation factor (α), and resolution (R_S) for separation of the two steroids were evaluated, and the method was applied for the separation and determination of progesterone and testosterone (Fig. 1) from urine samples. Although, Au NPs were used previously to modify conventional stationary phases, to the best of our knowledge, this is the first report dealing with the use of a new Au NP-based modified silica gel as a stationary phase in TLC for the separation of the steroids.

2. Materials and methods

2.1. Materials and reagents

All chemicals and reagents were of analytical grade and used without further purification. Hydrogen tetrachloroaurate ($HAuCl_4$), silica gel, zinc sulfide, calcium sulfate, APTS, trisodium citrate, nitric acid, hydrochloric acid, methanol, ethanol, acetone, and n-hexane were purchased from Merck Company (Darmstadt, Germany). Progesterone and testosterone standards were obtained from the Center of Quality Control of Drug, Tehran, Iran, and deionized water was used throughout the experiments.

Stock solutions of progesterone and testosterone (1000 μg/mL) were prepared by dissolving 100 mg of each analyte in 100 mL methanol. The solutions were stored at 4°C in dark and were stable for at least 2 weeks. The working standard solutions were prepared in the concentration range of 1–200 μg/mL for construction of a calibration curve, and all dilutions were made with methanol.

2.2. Preparation of colloidal Au NPs

Colloidal Au NPs were prepared by citrate reduction of gold ions in water following the method introduced by Turkevich et al [18] in 1951, with slight modifications. Briefly, a solution of 5mM $HAuCl_4$ (2 mL) was added to deionized water (45 mL) in a conical flask and allowed to boil with constant stirring under reflux. The reaction was followed by the addition of 25mM of trisodium citrate (3 mL) under continuous stirring. The color of

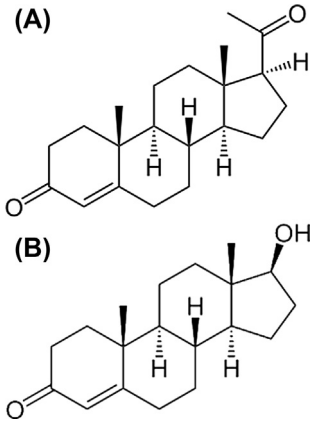

Fig. 1 – Structure of (A) progesterone and (B) testosterone.