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Chemiluminescence lateral flow immunoassay based on Pt nanoparticle with peroxidase activity

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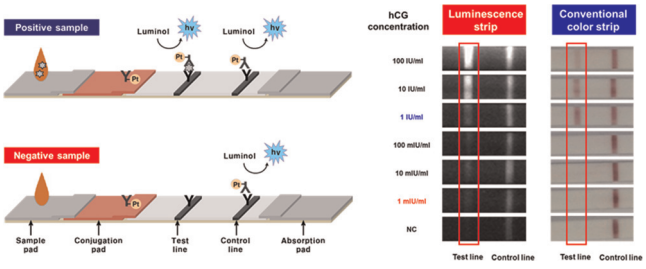
HIGHLIGHTS

- A lateral flow immunoassay (LFI) was developed with a chemiluminescent signal band.
- Pt nanoparticles were synthesized for the chemiluminescence reaction on the test strip.
- The peroxidase activity of Pt nanoparticles was optimized for the reaction conditions.
- The sensitivity of developed LFI was compared with the conventional colorimetric test.

GRAPHICAL ABSTRACT



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ABSTRACT

A lateral flow immunoassay (LF-immunoassay) with an enhanced sensitivity and thermostability was developed by using Pt nanoparticles with a peroxidase activity. The Pt nanoparticles were synthesized by citrate reduction method, and the peroxidase activity of Pt nanoparticles was optimized by adjusting reaction conditions. The peroxidase activity was estimated by using Michaelis–Menten kinetics model with TMB as a chromogenic substrate. The kinetics parameters of K_M and V_{max} were calculated and compared with horseradish peroxidase (HRP). The thermal stability of the Pt nanoparticles was compared with horseradish peroxidase (HRP) according to the storage temperature and long-term storage period. The feasibility of lateral flow immunoassay with a chemiluminescent signal band was demonstrated by the detection of human chorionic gonadotropin (hCG) as a model analyte, and the sensitivity was determined to be improved by as much as 1000-fold compared to the conventional rapid test based on colored gold-colloids.

1. Introduction

The lateral flow immunoassay (LF-immunoassay) has been widely used for point-of-care (POC) tests during medical diagnosis. This type of immunoassay has been also called a “strip test” because the entire immunoassay process, including washing and signal reporting, can be carried out simply by dropping a sample onto a test strip [1–4]. During capillary flow through the test strip, the colored signal bands are produced by antigen–antibody interactions. Recently, highly sensitive strip tests with fluorescent and chemiluminescent signal bands have been reported by using an antibody labeled with an enzyme called horseradish peroxidase (HRP). The chemiluminescence reaction of luminol has been used

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for highly sensitive immunoassays utilizing HRP for the oxidation of luminol [5–9]. Specifically, such strip tests with chemiluminescence signal bands showed a far higher sensitivity in comparison with conventional immunoassays [10–17].

However, HRP is known to be sensitive to the reaction temperature and storage conditions. In this work, thermostable Pt particles were developed for the chemiluminescence reaction with luminol. Inorganic nanoparticles made of gold or platinum have also been reported to be used in the chemiluminescence reaction of luminol instead of HRP [18–21]. Such inorganic nanoparticles usually have a far higher thermostability in comparison with enzymes, which require the maintenance of a three-dimensional structure for their functionality. Additionally, inorganic particles were reported to be applied in immunoassays after conjugation with antibodies [20–22].

Here, a LF-immunoassay with enhanced sensitivity and thermostability was developed by Pt nanoparticles with the peroxidase activity. As the antibody labeled with Pt nanoparticles should flow through the cellulose membrane by capillary forces, the Pt nanoparticles were developed to have a comparable size to antibodies (<10 nm) by citrate reduction. Additionally, the Pt nanoparticles were optimized to be well-dispersed in aqueous solution at physiological pH. The thermal stability of Pt nanoparticles was compared with horseradish peroxidase (HRP) according to the storage temperature and long-term storage period. The feasibility of the lateral flow immunoassay with a chemiluminescent signal band was demonstrated by the detection of human chorionic gonadotropin (hCG) as a model analyte.

2. Materials and methods

2.1. Materials

Human chorionic gonadotropin (hCG), horseradish peroxidase (HRP), anti-HRP antibodies, and platinum chloride ($H_2PtCl_6{\cdot}6H_2O$) were purchased from Sigma–Aldrich Korea (Seoul, Korea). Anti-hCG antibodies and HRP-labeled anti-hCG antibodies were purchased from Abcam Co. (Cambridge, UK). Luminol was purchased from Pierce Co. (Rockford, USA). Nitrocellulose membranes, glass fiber conjugation pads, and absorption pads were purchased from HBI Diagnostic Co. (Gyeonggi, Korea).

2.2. Synthesis of Pt nanoparticles

Pt nanoparticles were prepared by reduction of platinum chloride ($H_2PtCl_6{\cdot}6H_2O$) via a citrate reduction method [23–25]. The platinum chloride (0.3 mM) was heated for 1 h at 100 °C, and

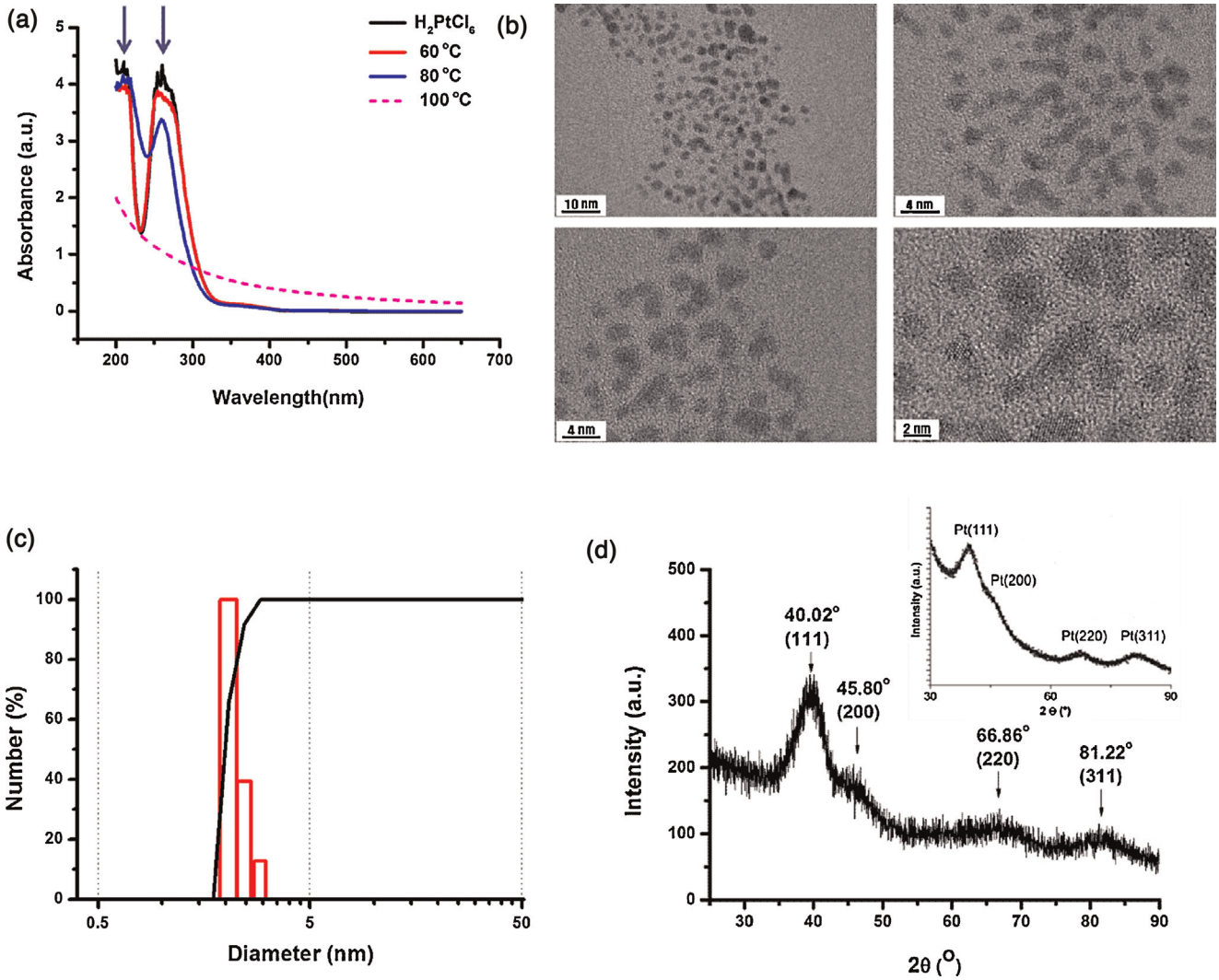

Fig. 1. Synthesis and characterization of Pt nanoparticles. (a) UV–vis spectra during the synthesis of Pt nanoparticles. (b) TEM analysis of Pt nanoparticles. (c) Size distribution of Pt nanoparticles by laser scattering. (d) XRD analysis of Pt nanoparticles.