NOVEL OPTICAL IMMUNOASSAY BASED ON MACROPOROUS SILICON WAVEGUIDE FOR DETERMINING HYDROXYSAFFLOR YELLOW A

X. Y. LÜ†, Z. H. JIA‡, J. W. LI§ and F. C. ZHANG§

† *Postdoctoral Station of Computer Science and Technology, Xinjiang University, Urumqi, PR China.*
‡ *College of Information Science & Engineering, Xinjiang University, Urumqi, PR China. xiaoz813@yahoo.com.cn*
§ *Key Laboratory of Xinjiang Biological Resources and Gene Engineering, College of Life Sciences and Technology, Xinjiang University, Urumqi, PR China*

***Abstract*— Porous silicon (PSi) has attracted much attention for biosensing due to its large surface area and easy preparation. In this paper, an optical immunoassay method based on a macro-PSi silicon resonant waveguide, with pore size much larger than that of large molecules such as bovine serum albumin, has been developed for the detection of the antigen-antibody reaction between hydroxysafflor yellow A (HSYA, i.e., the component of *Carthamus tinctorius L.*) and the specificity of the polyclonal anti-HSYA antibodies. HSYA antibodies were immobilized into the macro-PSi silicon waveguide using standard amino-silane and glutaraldehyde chemistry. The waveguide resonance angle was increased by binding HSYA onto the immobilized antibodies. The label-free immunosensor is simple and exhibits high sensitivity to HSYA. Therefore, this research is expected to have applications for quick and accurate determination of HSYAand can also be used for various immunoassays with other antigens..**

***Keywords*— Porous silicon, waveguide, label-free biosensor, HydroxySafflor yellow A.**

I. INTRODUCTION

Hydroxysafflor yellow A (HSYA, MW: 432 Da) is the main chemical component of the Chinese herb *Carthamus tinctorius L*., which is extensively used in traditional Chinese medicine for treatment of cerebrovascular and cardiovascular diseases (Wei *et al.*, 2005). The levels of HSYA content are the key criterion to evaluate the quality of *Carthamus tinctorius L.* and the corresponding traditional Chinese medicine. Thus, it is significant to explore a quick and accurate determination of such small molecules for judging the quality of medicines, monitoring the quality of pharmaceutical process and conducting pharmacokinetic analysis. Traditionally, the method for determination of HSYA is high-performance liquid chromatography (HPLC), a sensitive but cumbersome technique which requires expensive equipment. As an important analytical method, the immunoassay can be used as a tool for determining HSYA, however, traditional immunoassays such as enzyme-linked immunosorbent assay (ELISA) involve labeling and a longer detection time.

An optical biosensor combined with immunoassay technology offers the advantage of being highly selective and sensitive while remaining simple and label-free. Porous silicon (PSi) is an ideal optical biosensor material because of its high surface area, low cost, wide availability (Meskini *et al.*, 2007; Lin *et al.*, 1997), and compatibility with standard IC processes. In previous work, we investigated single and multilayered PSi as a biosensor platform for determining the artificial immunogen of HSYA (Lü *et al.*, 2009). Saarinen and coworkers (Rong *et al.*, 2008; Saarinen *et al.*, 2005) reported that using a PSi waveguide for detecting DNA fragments has many advantages over other thin resonant or PSi-based biosensors, such as high sensitivity and fast-response; however, the surface pore diameters of the PSi waveguide they reported are only about 20 nm, which is too small for easy detection of large size biomolecules such as the anti-HSYA antibodies.

To enable large size molecules to infiltrate the PSi waveguide easily thus allowing detection of HSYA, in this experiment, an immunoassay based on macro-PSi resonant waveguide (pore size > 60 nm), was developed, building on the published work of Rong *et al.* (2008) who described the nanoscale PSi waveguide. We have successfully used a crosslink method linker HSYA antibody to the macro-PSi waveguide and then measured the shift of the waveguide resonance angle before and after the antigen-antibody reaction. The results show that the shift of the waveguide resonance angle increases with HSYA concentration. Thus, the macro-PSi waveguide is highly sensitive, a finding which lays a foundation for the development of simple and label-free immunosensors.

II. METHODS

A. Principle

As shown in Fig. 1, the schematic of the macro-PSi waveguide builds on the published work of Rong *et al.* (2008) who describe the same PSi waveguide structure. Two layers of PSi were formed on the silicon substrate: a top layer of low porosity and high refractive index, and a bottom layer of high porosity and low refractive index. An air gap separated the PSi waveguide from the prism, which had a refractive index of 1.811. PSi played the same role as metal in a surface plasmon resonance (SPR sensor) (Saarinen *et al.,* 2005). There is a resonance dip in the spectrum when the incidence angle is at the vicinity of the resonance angle (Rong *et al.*, 2008). Before and after the antigenantibody reaction, the refractive index of the PSi waveguide changed, resulting in the shift of the waveguide resonance angle.