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Fabrication of surface plasmon resonance nanosensor for the selective determination of erythromycin via molecular imprinted nanoparticles

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ABSTRACT

The main objective of this study was to develop a novel surface plasmon resonance (SPR) nanosensor method based on a more rapid and selective determination of erythromycin (ERY) in the aqueous solution. This study is a combination of three techniques, which are miniemulsion polymerization, molecular imprinting and surface plasmon resonance techniques. In the first part, nanoparticles prepared with methacryl groups of functional monomer at surface acted as reactive sites for erythromycin as a template molecule. The molecularly imprinted nanoparticles were characterized by FTIR, SEM and zetasizer. After immobilization of nanoparticles on gold surface of SPR chip, nanosensor was characterized with contact angle measurements. This nanosensor was then used for selective determination of erythromycin. The linearity range and detection limit were obtained as 0.99 (r^2) and 0.29 ppm, respectively. Association kinetic analysis, Scatchard, Langmuir, Freundlich and Freundlich–Langmuir isotherms were applied data. The selectivity of the SPR nanosensor was determined by using competitor agents (kanamycin sulfate, neomycin sulfate, spiramycin). The non-imprinted nanosensor was also used to evaluate the selectivity of ERY imprinted nanosensor. Finally, the nanosensor was tested for repeatability and it gave satisfactory response. These results demonstrate a method which is of low cost, rapid and provide reliable results in order to be used in detection of erythromycin from aqueous solution.

1. Introduction

Pharmaceuticals have brought great benefit due to their usage in diagnosis, treatment or prevention of disease or other abnormal conditions [1]. Nevertheless, they can have dramatic effects on the hormonal system of organisms at trace concentrations in the aquatic environment. Therefore, pharmaceuticals have become a new concern for public health. There is a critical need for the analysis and monitoring of pharmaceuticals that provide the benefits of that compounds, while eliminating their adverse effects [2,3]. The recent study is focused on analyzing and monitoring pharmaceutical erythromycin from aqueous solutions as its residues may lead to direct toxic effects on the public health [4,5]. Erythromycin, produced by *Saccharopolyspora erythreas* (formely known as *Streptomyces erythraesus*) during fermentation process, is a broad-spectrum macrolide antibiotic consisting of 14 membered lactone ring with ten asymmetric centers and two sugar molecules (L-cladinose and D-desoamine). In fermentation process several related substances of erythromycin can be formed. Even though erythromycin A is the main component of commercially available erythromycin, some structurally and chemically similar analogs, especially erythromycin B and erythromycin C, are presented in small amount [6–9]. The structures of erythromycin A and its related substances are shown in Fig. 1. Up to now, there have been various methods reported for the determination of erythromycin in different sources by several methods including high-performance liquid chromatography (HPLC) [10–12], liquid chromatography–mass spectroscopy (LC–MS) [13–15], liquid chromatography–tandem mass spectroscopy (LC–MS/MS) [16–19], capillary electrophoresis chromatography (CEC) [20,21], near infrared reflectance spectroscopy (NIR) [22], ultraviolet (UV) [23] and electrochemical detection [4,24,25]. Although these analysis methods are successfully applicable for erythromycin, some of these methods are also complicated, expensive, time consuming and laborious [4,8]. UV and electrochemical techniques are simple than these methods but they are not sensitive and selective. Therefore, it is necessary for us to find a simple, low cost, selective and quick response method which serves as an alternative detection method for erythromycin.

Nowadays, many Molecular imprinted polymers of nanosensors have been reported for detection of biomolecules and

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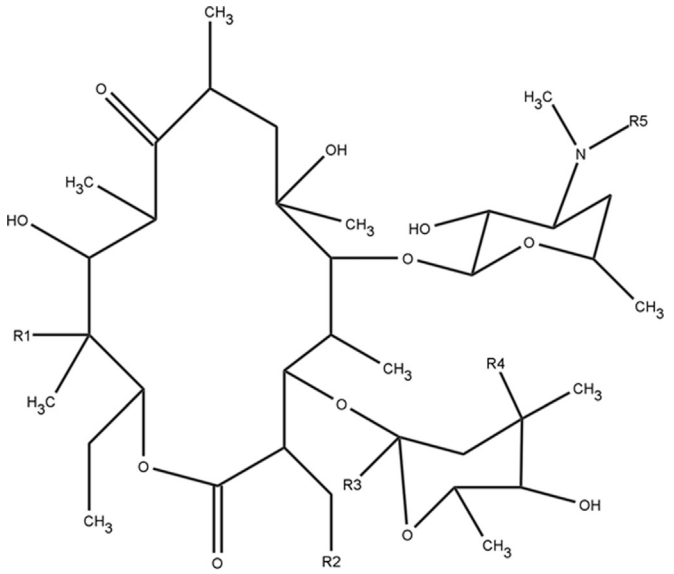

Structures name	R1	R2	R3	R4	R5
Erythromycin A	OH	H	H	OCH_3	CH_3
Erythromycin B	H	H	H	OCH_3	CH_3
Erythromycin C	OH	H	H	OH	CH_3
Erythromycin D	H	H	H	OH	CH_3
Erythromycin E	OH	O	O	OCH_3	CH_3
Erythromycin F	OH	OH	H	OCH_3	CH_3

Fig. 1. The structures of erythromycin and related substances.

drugs [26–29]. Surface plasmon resonance (SPR) sensors have been known as sensing analytical devices which, by optical means, take advantage of the sensitivity of a special type of electromagnetic field, a surface plasmon, to changes in refractive index. SPR biosensor has attracted great interest in a wide range of fields by reason of compact design, low cost and sensitive properties [30–33].

Molecular imprinting is an artificial (non-biological) molecular recognition approach based on the "molecular key and lock" principle. One of the most attractive features of molecular imprinting is the potential to adapt this technology to a wide range of polymer type such as cryogels [34–36], monolith [37], microparticles [38], nanoparticles [26,39,40] etc. owing to their desired selectivity, physical robustness, thermal stability, as well as low cost and easy preparation. Molecular imprinted polymers have been extensively utilized in sensor system; on the other hand, recently imprinted nanoparticles have been chosen due to the inherent advantages in terms of sensitivity, selectivity, low cost and ease of use mode [41].

In this study, we combined three techniques, which are nanotechnology, molecular imprinting and surface plasmon resonance. The ERY imprinted SPR nanosensor was developed for the first time in our study and applied for detection of ERY in aqueous solution. ERY imprinted nanoparticles were prepared by miniemulsion polymerization technique. The molecularly imprinted nanoparticles were characterized by FTIR, SEM and zetasizer. After characterization of nanoparticles, gold surface of SPR chip was modified with nanoparticle and then this nanosensor was characterized with contact angle measurements. Kinetic experiments of the nanosensor were performed for selective determination of erythromycin. Then kinetic and isotherm parameters were calculated to analyze the interaction kinetics between SPR nanosensor and the ERY molecule. Therefore, detection of ERY was successfully achieved by this newly developed nanosensor with high selectivity and sensitivity.

2. Experimental

2.1. Materials

Gold SPR chips were taken from Horiba (UK). Analytical grade reagents and solvents were used in all experiments. Erythromycin, kanamycin sulfate, neomycin sulfate and spiramycin used in this study were obtained from Sigma (St. Louis, MO, USA). Erythromycin is almost insoluble in water and soluble in alcohol and diluted hydrochloric acid. Moreover stability of erythromycin is pH dependent and the instability of erythromycin in the alkaline media at pH $\geq$ 9 has already been reported [4,9,42,43]. The stock solution of ERY (135 μM) was prepared by dissolving it in 20 mL of 2 M HCl and then diluting it with ultra-pure quality water to 80 mL. The stock solution was stored in the fridge at 4 °C. Methylacrylic acid (MAA), 2-hydroxyethyl methacrylate (HEMA) and ethylene glycol dimethacrylate (EDMA) were purchased from Sigma (St. Louis, MO, USA). Solvents used were hydrochloric acid (HCl), methanol and acetic acid that were purchased from Fluka (St. Gallen, Switzerland).

2.2. Preparation of erythromycin imprinted nanoparticles

Erythromycin imprinted nanoparticles were prepared via two-phase mini-emulsion polymerization method according to the literature procedure with minor modifications as reported elsewhere [44]. Two-phase miniemulsion polymerization method was used to prepare the ERY imprinted nanoparticles. Before polymerization two different aqueous phases were prepared. Prior to polymerization, the first aqueous phase was prepared by dissolving PVA (93.75 mg), SDS (14.425 mg) and sodium bicarbonate (11.725 mg) in 5 mL deionized water. The second phase was prepared by dissolving PVA (50 mg) and SDS (50 mg) in 100 mL of deionized water. HEMA (0.225 mL), EGDMA (1.05 mL) and methacrylic acid (0.190 mL) were then mixed to form the organic phase. The template molecule [ERY, 100 mg ($\approx$ 135 μmol)] was added to organic phase to establish the ratio between monomer and template as 1:16 in mole basis. Then, the organic phase was mixed with first aqueous phase. The mixture was homogenized at 25,000 rpm (Homogenizer, T10, 1ka, Labortechnik, Germany) to get a miniemulsion. After homogenization, the mixture was then added into the second aqueous phase and was stirred magnetically at 600 rpm (Radleys Carousel 6, UK). The polymerization mixture was slowly heated to 40 °C, polymerization temperature. Finally, the initiators, sodium bisulfite (125 mg) and ammonium persulfate (125 mg), were injected into the mixture, and the polymerization process was allowed to proceed for 24 h. The larger particles were removed through centrifugation at 5000 rpm. The solution consisting of nanoparticles was centrifuged at 26,500 rpm for 1.5 h (Allegra-64R Beckman Coulter, USA). Upon completion, the polymer was washed five times each with a solution of DI water: ethanol (1:1 v/v), to remove the surfactant or any unreacted monomer and initiator, and finally three times with deionized water. The washed imprinted nanoparticles were dispersed in deionized water and were kept at 4 °C until use. Non-imprinted nanoparticles were prepared in a similar manner as above, except without the addition of the template, ERY.

2.3. Characterization of methods

Characterization of the nanoparticles was done by zeta-sizer measurement. The size distribution of nanoparticles was performed by Nano Zetasizer (NanoS, Malvern Instruments, London, UK). The solution (3.0 mL) consisting of nanoparticles was placed in sample holder and light scattering was performed at an incidence angle of 90° and at 25 °C. The density and the refractive