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Early diagnosis of fungal infections using piezomicrogravimetric and electric chemosensors based on polymers molecularly imprinted with D-arabitol

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ABSTRACT

An elevated concentration of D-arabitol in urine, especially compared to that of L-arabitol or creatinine, is indicative of a fungal infection. For that purpose, we devised, fabricated, and tested chemical sensors determining D-arabitol. These chemosensors comprised the quartz crystal resonator (QCR) or extended-gate field-effect transistor (EG-FET) transducers integrated with molecularly imprinted polymer (MIP) film recognition units. To this end, we successfully applied a covalent approach to molecular imprinting, which involved formation of weak reversible covalent bonds between vicinal hydroxyl groups of arabitol and boronic acid substituents of the bithiophene functional monomer used. The MIP films were synthesized and simultaneously deposited on gold electrodes of quartz crystal resonators (Au-QCRs) or Au-glass slides by oxidative potentiodynamic electropolymerization. With the QCR and EG-FET chemosensors, the D-arabitol concentration was determined under flow-injection analysis and stagnant-solution binding conditions, respectively. Selectivity with respect to common interferences, and L-arabitol in particular, of the devised chemosensors was superior. Limits of detection and linear dynamic concentration ranges of the QCR and EG-FET chemosensors were 0.15 mM and 0.15 to 1.25 mM as well as 0.12 mM and 0.12 to 1.00 mM, respectively, being lower than the D-arabitol concentrations in urine of patients with invasive candidiasis (>220 μM). Therefore, the devised chemosensors are suitable for early diagnosis of fungal infections caused by *Candida* sp. yeasts.

1. Introduction

Clinical statistics shows that the number of invasive fungal infections has rapidly been growing for recent years (Alcazar-Fuoli and Mellado, 2014) with the most common pathogens responsible for these infections being *Candida* yeasts (Guery et al., 2009a; Johnson and Bundle, 2013). Presumably, this trend will grow in the nearest future (Alcazar-Fuoli and Mellado, 2014). Despite novel antifungal therapeutic strategies developed, high mortality caused by fungal infections still remains as a serious problem (Alcazar-Fuoli and Mellado, 2014). Fast and unambiguous diagnosis of these infections at their very early stage of development is crucial for successful therapy (Guery et al., 2009b). One of the most advantageous and convenient methods of diagnosis of infections caused by *Candida* sp. involves determination of concentration of D-arabitol or (D-arabitol)-to-creatinine ratio, or (D-)-to-(L-arabitol) ratio (Scheme 1) in body fluids, i.e., urine, blood plasma, cerebrospinal fluid (CSF), etc. Human cells produce D- and L-arabitol as natural metabolites in almost equal amounts. However, fungi of the *Candida* family can produce D-arabitol only. Therefore, an elevated concentration of D-arabitol in body fluids is a useful diagnostic indication of invasive candidiasis. Concentration of D-arabitol exceeding 1.0 μg/mL (6.6 μM) in blood plasma (Kiehn et al., 1979), the (D-arabitol)-to-creatinine ratio above 4.0 μM per mg/dL in blood plasma (Switchenko et al., 1994; Walsh et al., 1995; Yeo et al., 2006) or (D-)-to-(L-arabitol) molar ratios above 4.0 in blood plasma or urine (Arendrup et al., 2010; Christensson et al., 1999) all are indicative of progressive fungal infection requiring treatment. Undoubtedly, the major advantage of this

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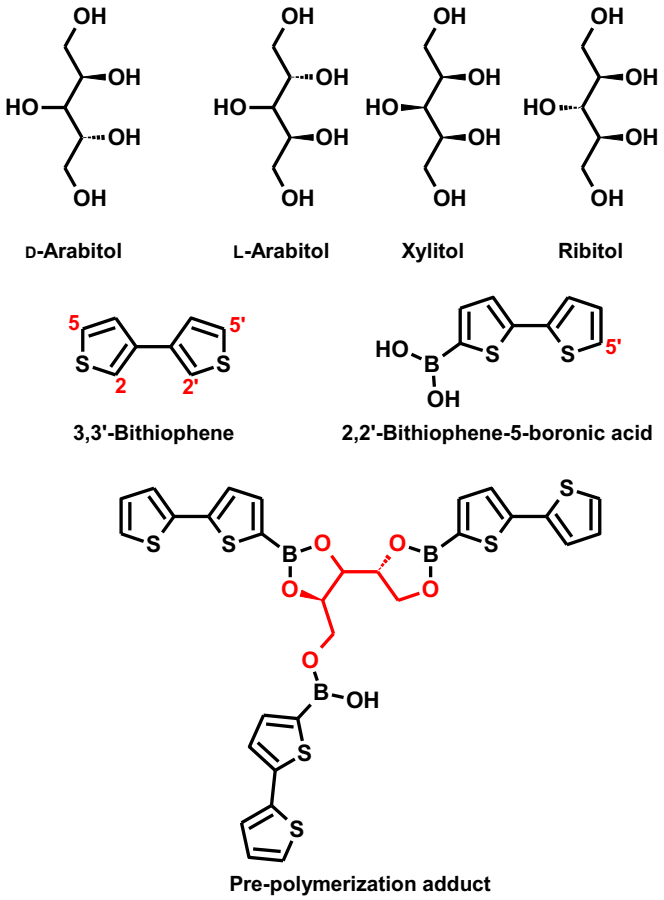

Scheme 1. Structural formulas of the D-arabitol template, the L-arabitol, xylitol, and ribitol interferences as well as the 2,2′-bithiophene-5-boronic acid functional, 3,3′-bithiophene cross-linking monomers, and D-arabitol esterificated with three molecules of 2,2′-bithiophene-5-boronic acid. Carbon atoms 2, 5, 2', and 5' in thiophene rings capable of polymer formation are indicated with red numbers.

concentration determination is the ability of early diagnosis, thus enabling immediate initiation of treatment and patient protection from unnecessary complications (Alcazar-Fuoli and Mellado, 2014; Bensadoun et al., 2011). Additionally, monitoring the status of patients receiving antifungal medication is then relatively straightforward (Kiehn et al., 1979).

Physiological concentrations of D-arabitol in blood plasma, CSF, and urine are in the range of 0–5 μM (Huck et al., 2004), 12.7–25.3 μM (Lentner et al., 1981–1984) and 55 to 950 μM (Bouatra et al., 2013; Christensson et al., 1999), respectively. Most of currently used protocols of the D-arabitol determination employ GC–MS. Although accurate, these technique require relatively expensive laboratory instrumentation and trained personnel for its operation (Hui et al., 2004). Therefore, diagnostic methods using them are not accessible for many hospitals, physicians and, most importantly, for patients at home. To remediate this deficiency, several fluorometric or spectrophotometric enzyme assays using recombinant protein D-arabitol dehydrogenase were developed (Switchenko et al., 1994; Walsh et al., 1995; Wong and Brauer, 1988; Yeo et al., 2006; Yeo et al., 2000). The main drawback of these analytical procedures is application of unstable and tedious in preparation biological recognition materials. Another method involves electrochemical determination of D-arabitol (Wang et al., 2010). Unfortunately, selectivity, especially to stereoisomers, of this chemosensor is very low. Therefore, there is a clear need to develop an inexpensive and straightforward method for enantioselective determination of D-arabitol. For that, we explore herein the use of molecular imprinting, a relatively simple and inexpensive procedure for preparation of synthetic polymer receptors with appreciable affinity, selectivity, and robustness (McCluskey et al., 2007). Moreover, MIPs can be used as recognition units in chemosensors (Huynh et al., 2015). In several aspects, molecularly imprinted polymers (MIPs) are more advantageous than their bio-recognition counterparts. That is, their stability with respect to harsh environmental conditions, such as extreme values of temperature, ionic strength, or pH, or the presence of aggressive solvents, is superior to those of the bioreceptors. Moreover, MIP materials can readily be prepared in bulk quantities and at high purity. Because of these advantages, MIPs are very attractive recognition materials for fabrication of chemosensors for D-arabitol. To our best knowledge, all of analytical methods and sensors for D-arabitol are using either costly equipment or unreliable and expensive enzymatic tests. To our best knowledge, there is no attempt in the literature to develop artificial D-arabitol recognition material by using the MIP procedure. Therefore, in the present research, we devised, fabricated, and tested an MIP film enantioselective with respect to D-arabitol and used it as a recognition unit of selective chemosensors.

Quartz crystal resonators (QCRs) are widely explored for analytical signal transduction in chemical sensors using MIPs (Suriyanarayanan et al., 2010). For this transduction platform, simultaneous changes of mass and visco-elasticity of an MIP film used as the recognition unit of the chemosensor are monitored. Therefore, QCRs are suitable for precise determination of binding characteristic of the MIP films. Moreover, they enable critical evaluation of investigated MIP films for application in devising chemosensors. Despite of undoubted advantages, QCRs are very sensitive to disturbance, therefore practical application of QCRs is often inconvenient. An electric technique using an extended-gate field-effect transistor (EG-FET) is from practical point of view a very useful extension of the field-effect transistor transduction (Bergveld, 1970). It is based on measurement of changes of the source-drain current at constant gate voltage. This current is modulated by the gate voltage changes incurred by sorption of charged species within the gate region of the transistor. This effect can readily be used as a transduction method in the chemosensor (Jimenez-Jorquera et al., 2010; Lee et al., 2009). The classical FET construction is sometimes difficult to modify with a recognition film, as “wet” chemical methods are not always compatible with classical semiconductor technology. However, the gate region can electrically be “extended” as an external electrode, thus greatly facilitating its chemical modification for sensing. This technique has successfully been used for fabricating different sensors (Batista et al., 2006; Chen et al., 2011; Chi et al., 2000; Yin et al., 2000) including that based on an MIP (Iskierko et al., 2015).

Herein, we used both QCR and EG-FET transducers for our MIP-(D-arabitol) chemosensors. In order to prepare a (D-arabitol)-templated an MIP film, a covalent approach was adopted to bind D-arabitol in MIP reversibly. This strategy is more successful in comparison to the non-covalent imprinting approach due to formation of a pre-polymerization adduct with well-defined geometry and, therefore, formation of a well-defined molecular cavity. This ultimately leads to higher selectivity of the MIP. Within the present research, 2,2′-bithiophene-5-boronic acid was used, for the first time, as the functional monomer. Moreover, its electropolymerization can be easily initiated because of its relatively low potential of electro-oxidation. Analytical performance of the QCR chemosensors was evaluated with an electrochemical quartz crystal microbalance (EQCM) under flow-injection analysis (FIA) conditions. Our EG-FET chemosensors with MIP-coated extended gates were tested under stagnant-solution conditions. Sensitivity, the limit of detection (LOD) of the target D-arabitol analyte, and selectivity with respect to most common interferences were determined. The LOD of the chemosensors was critically evaluated