Biosensors and Bioelectronics 40 (2013) 323–328

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Real-time monitoring of ischemia inside stomach

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ARTICLE INFO

Available online 8 August 2012

Keywords:
All-solid state ion selective sensor (ISE)
Low pH detection
Implantable sensor
Ischemia
Stomach tissue
Endoscopy

ABSTRACT

The low pH in the gastric juice of the stomach makes it difficult to fabricate stable and functional all-solid-state pH ISE sensors to sense ischemia, mainly because of anion interference and adhesion problem between the ISE membrane and the electrode surface. In this work, the adhesion of ISE membrane on solid surface at low pH was improved by modifying the surface with a conductive substrate containing hydrophilic and hydrophobic groups. This creates a stable and robust candidate for low pH applications. Moreover, anion interference problem at low pH was solved by integration of all-solid-state ISE and internal reference electrodes on an array. So, the same tendencies of anion interferences for all-solid-state ISE and all-solid-state reference electrodes cancel each other in differential potentiometric detection. The developed sensor presents a novel all-solid-state potentiometric, miniaturized and mass producible pH ISE sensor for detecting ischemia on the stomach tissue on an array designed for endoscopic applications.

1. Introduction

The field of electrochemical sensors based on microelectronic devices has increased its important role in the last decade (Grieshaber et al., 2008). It has been largely focused on all-solid-state electrochemical ion selective electrode (ISE) sensors, in which all the ISE compounds are integrated into a polymeric matrix and attached in direct contact with the metal electrode. This interest arises from certain advantages of all-solid-state ISEs over conventional ISE such as the solid nature, miniaturizability, and the possibility of multi-sensing and mass fabrication (Kwon et al., 2005). All-solid-state ISE with its advantageous properties can be used to sense gases, electrolytes and metabolites in vivo and in vitro for different kinds of applications. Medical diagnosis is one of the field that exploits the resources of the ISE, since the changes in these parameters are directly related with disease occurrence such as cancer (Keller et al., 2011), diabetics (Lin and Sun, 2010), neurological disorders (Mattson, 2004), and ischemia (Ammann et al., 1985), among others.

Ischemia is a shortage of the blood supply to an organ. Real time monitoring of ischemia is very important, since a prolonged ischemia condition causes severe tissue damage and failure of organs. Under these conditions, oxygen and glucose levels decrease and ion pumps of these ischemic cells cannot work properly, creating a difference between intracellular and extracellular ions concentrations, such as hydrogen (pH), sodium, potassium, and chloride (Ammann et al., 1985). However, clinical results have proven that detection of ischemic organs by measuring the changes in blood pH was not sufficient (Gonullu et al., 2007). In situ detection methods on the organ tissue are necessary for early detection of ischemia. Stomach is one of the foremost organs for ischemia detection, because the diagnosis is delayed and misled by losing time with the same symptoms of different diseases, making it harder to diagnose (Quentin et al., 2006). However, the low pH in the gastric juice of the stomach makes it difficult to fabricate stable and functional all-solid-state pH ISE sensors for use under these conditions.

The most common plasticizer used in all-solid-state pH ISE sensors is poly(vinyl chloride) (PVC). However, this membrane has poor adhesion to the electrode surface, which inhibits its applications in implantable sensors (Piao et al., 2003). There are a lot of scientific works focused in solving the adhesion problem, like anchoring chemically PVC membranes containing OH^- groups to an oxide surface (Harrison et al., 1988), attachment of the membrane mechanically by suspended mesh fabrication (Blackburn and Janata, 1982), chemical modification of PVC by modifying its structure with carboxylic acid groups (PVC–COOH) (Cosofret et al., 1995), and photocuring techniques by UV light (Abramova and Bratov, 2009). However, the hard analytical conditions and low pH of the stomach complicate the adhesion of PVC to the electrode surface. Ionophore protonation is another

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http://dx.doi.org/10.1016/j.bios.2012.07.071

limiting factor for the usage of ISE electrodes at low pH. Sensors based on ISE membranes have selective ionophores for trapping hydrogen ions, which create a concentration difference between hydrogen ions within the ISE membrane and solution. At low pH, ionophores start to get affected by anion interference due to the high protonation. This issue was partially solved by using neutral carrier based membranes for conventional ISE electrodes (Oesch et al., 1986), where the membrane glass base is in electrical contact through an inner electrolyte solution with the reference electrode (Bobacka et al., 1995), but these conventional ISE electrodes are fragile and hard to miniaturize. However, anion interference is still inevitable for all-solid-state ISE sensors at low pH.

Other sensors devoted to low pH detection were developed by Zine et al. (2006) and Won-Sik Han et al. (2001). The first was based on PPy[3,3′-Co(1,2-C2B9H11)2], reaching a working range of 3.5–11 while the second was developed with tribenzylamine neutral carrier in a poly(vinyl chloride) membrane with a poly(aniline) solid contact electrode, obtaining a lower detectable pH of 2.48. But, a work that reports lower pH detection is the one reported by Anastasova-Ivanova et al. where 2–9 range of pH was observed in a miniaturized all-solid-state potentiometric sensor. However, this sensor cannot be used at pH below 2, since the signal starts to go down below this pH (Anastasova-Ivanova et al., 2010).

Anion interference is also a drawback in all-solid-state reference electrodes (REs). These electrodes based on Ag/AgCl paste are not in contact to an internal electrolyte, which could cause a voltage change due to the variations of anion concentration (Simonis et al., 2004). The anion interference problem was solved for commercial glass REs by keeping them in saturated KCl solutions to minimize liquid-junction potential. However, solution based systems have a lot of drawbacks as introduced with the conventional glass based ISEs. Some approaches have been undertaken to fabricate such miniaturized REs with inner electrolyte in a micro-channel liquid-junction (Hassel et al., 1999), liquid-junction with thin-film techniques (Suzuki et al., 1999) and liquid-junction-free RE (Cranny and Atkinson, 1998; Lee et al., 1998), in which KCl is immobilized in a carrier material. The problems of these systems are of insufficient stability when the inner electrolyte and the Ag/AgCl layer are placed in contact and their potential instability at pH below 2 (Blaz et al., 2005; Kisiel et al., 2005; Tymecki et al., 2004; Rius-Ruiz et al., 2011). Due to these reasons, no implantable all-solid-state ISE for in situ detection of ischemia inside the stomach has still been reported.

This work presents an approach for an all-solid-state potentiometric, miniaturized, and mass producible pH all-solid-state ISE sensor integrated in an array for detecting ischemia at low pH (0.7–2.5) on stomach tissue. In this platform, the problem of anion interference on ISE sensors has been solved by using an all-solid-state RE, which is affected by anions, migrated to the electrode surface, in the same tendency as that of the ISE, canceling the anion interference in the differential potentiometric measurement. Also, the sub-nernstian behavior of the pH sensor was fixed by increasing the concentration of lipophilic anions in ISE membrane. Moreover, the adhesions of ISE membrane on different solid surfaces were studied and improved, creating a stable and robust candidate for implantable sensors.

This sensor will be inserted into the stomach in a scarless way, using the natural orifice transluminal endoscopy (NOTE). For this purpose a round shape 7 mm diameter array design was performed. The all solid state pH ISEs and reference electrode (RE) reported in this work were integrated in the array. The diameter of each electrode is 600 μm and each electrode has 3 mm length. Electrodes were distanced enough from each other to avoid crosstalks between the electrodes (Fig. 1).

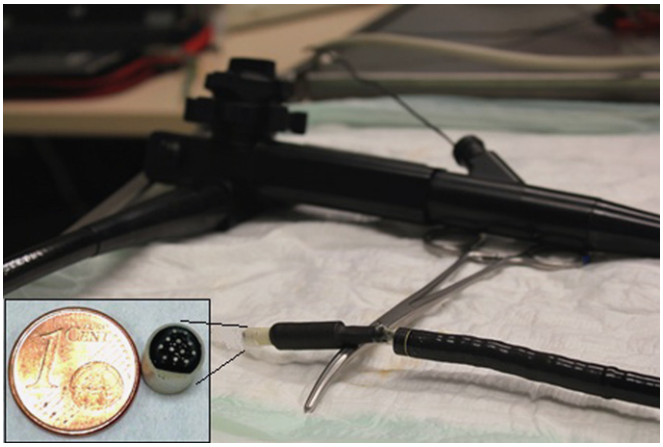

Fig. 1. Picture of the developed sensor array inserted in a gastroendoscope. The inlet picture shows the size of the array.

2. Experimental

2.1. Reagents and materials

Hydrogen ionophore IV (Octadecyl isonicotinate), poly(vinyl chloride) (PVC) high molecular weight, 2-nitrophenyl octyl ether and potassium tetrakis (4-chlorophenyl) borate (KTClPB) were purchased from Fluka. Perfluorinated ion-exchange resin (Nafion) was obtained from Sigma. Tetrahydrofurane (THF), tris(hydroxymethyl) aminomethane, KCl, NaCl, and HCl were received from Panreac. Ag/AgCl, gold and carbon inks were supplied by Dupont. MCS 5 and 12 series electrode arrays were obtained from Omnetics Connector Corporation and resin hardener complex (EPOTEK 301-2) was provided by Epoxy Technology.

2.2. Functionalization of electrodes on the array

The electrochemical sensor array was composed of 12 electrode pins of beryllium copper alloys. Prior to the modification, they were washed with double deionized (MilliQ) water and dried under nitrogen atmosphere. The sides of the electrodes pins were insulated with a commercial biocompatible resin mixed with a hardener complex. Electrodes were completely covered with this mixture and cured at 80 °C for 3 h. This insulation reduces the background noise signal and brings high biocompatibility and chemicals resistance to the electrodes. 600 μm of beryllium copper diameter electrode area was delimited after polishing the electrodes tips. Afterwards, they were cleaned by sonication inside pure ethanol for 2 min and remaining contaminants were removed under nitrogen gas. Beryllium copper surface was first covered by carbon ink by soaking the tip of the electrodes in a homogeneous thin ink layer and left to dry at 130 °C for 6 min. Once carbon surface was dried, a layer of Ag/AgCl ink was deposited in the same manner. As the last step, the Ag/AgCl layer used as reference electrode (RE) was covered by Nafion to increase the stability of the signal and to prevent a reaction between Ag/AgCl surface and acidic solution. After the coverage with Nafion membrane, the electrodes were left for 48 h under vacuum and then left to dry under 100 °C for 1 h (Kwon et al., 2007). The electrode used as WE was covered with ISE membrane and left to dry overnight.

ISE membrane was prepared with a mixture of 1.0 wt% hydrogen ionophore IV, 1.33 wt% KTClPB, 68.0 wt% 2-nitrophenyl octyl ether, and 29.67 wt% PVC of high molecular weight. 300 mg in total of these chemicals was dissolved in 3 mL of freshly distilled THF (Oesch et al., 1986).