Carbohydrate Polymers 99 (2014) 783–790

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Reduction of thrombogenicity of PVC-based sodium selective membrane electrodes using heparin-modified chitosan

Ibrahim H.A. Badr[a,*], M. Gouda[b,1], R. Abdel-Sattar[a,1], Hossam E.M. Sayour[c]

[a] Chemistry Department, Faculty of Science, Ain-Shams University, Cairo 11566, Egypt
[b] National Research Centre, Textile Research Division, Cairo 12311, Egypt
[c] Animal Health Research Institute, Department of Chemistry and Nutritional Deficiency Diseases, Cairo, Egypt

ARTICLE INFO

Article history:
Received 6 May 2013
Received in revised form 24 August 2013
Accepted 27 August 2013
Available online 2 September 2013

Keywords:
Membrane electrodes
Chitosan
Heparin-modified chitosan
Ion-selective electrodes
Biocompatibility

ABSTRACT

Heparin-modified chitosan (H-chitosan) membrane was utilized to enhance biocompatibility of sodium selective membrane electrode based on the highly thrombogenic polyvinyl chloride (PVC). Sodium ion sensing film was prepared using PVC, sodium ionophore-X, potassium tetrakis(chlorophenyl)-borate, and *o*-nitrophenyloctylether. The PVC-based sensing film was sandwiched to chitosan or H-chitosan to prevent platelet adhesion on the surface of PVC. Potentiometric response characteristics of PVC-chitosan and PVC-H-chitosan membrane electrodes were found to be comparable to that of a control PVC based sodium-selective electrode. This indicates that chitosan and H-chitosan layers do not alter the response behaviour of the PVC-based sensing film. Biocompatibility of H-chitosan was confirmed by in vitro platelet adhesion study. The platelet adhesion investigations indicated that H-chitosan film is less thrombogenic compared to PVC, which could result in enhancement of biocompatibility of sodium selective membrane electrodes based on PVC, while maintaining the overall electrochemical performance of the PVC-based sensing film.

1. Introduction

Ion selective electrodes (ISEs) are among the most commonly used electrochemical devices because of their simplicity, sensitivity and high selectivity. Furthermore, they can be used for direct and rapid measurement of various cations, anions, some gases and polyions (Bakker, Diamond, Lewenstam, & Pretsch, 1999; Bühlmann, Pretsch, & Bakker, 1998; Johnson & Bachas, 2003). By far, the most important application of ISEs is in clinical and physiological analyses. The availability of implantable sensors suitable for the continuous in vivo monitoring of clinically important analytes in physiological fluids would provide better understanding of physiological processes and would be a great analytical tool in emergency room and intensive care units. Particularly the effective management of critically ill patients often requires the frequent measurement of blood electrolytes (Na^+, K^+, Ca^{2+}) and gases (pO_2, pCO_2 and pH). Sensor-based technologies capable of providing such measurements in real time are receiving increased interest (Wang, Y. et al., 2008). Clotting on the surface of ISE membranes, however, presents the major difficulty to overcome to make ISEs suitable for in vivo application.

Any device introduced into a physiological medium induces a biological reaction (Wisniewski, Moussy, & Richard, 2000). For example, upon introduction of an ion selective electrode into blood or plasma, proteins readily adsorb onto the surface. This protein adsorption is the first step leading to several biological events, including activation of coagulation cascade, cell adhesion, activation of platelets and thrombus formation. Several strategies have been developed to enhance the biocompatibility of membrane electrodes to make them suitable for in vivo applications. For instance, new materials, such as substituted PVC (Cosofret, Lindner, Buck, Kusy, & Whitley, 1993; Cosofret, Buck, & Erdosy, 1994), cellulose triacetate (Berrocal, Badr, Gao, & Bachas, 2001; Cha & Meyerhoff, 1989; Cha et al., 1995), polyurethanes (Cosofret et al., 1996; Lindner et al., 1995; Liu, Meyerhoff, Goldberg, & Brown, 1993; Yun et al., 1997), polymethacrylates (Ambrose & Meyerhoff, 1996; Bratov et al., 1995; Heng & Hall, 1996; Heng & Hall, 2000), and silicone rubber (Högg, Lutze, Cammann, 1996; Poplawski et al., 1997; Shin, Sakong, Nam, & Cha, 1996; Tsujimura, Sunagawa, Yokohoma, & Kimura, 1996), are among the polymers that have been explored as alternative to PVC in the fabrication of ISE membranes. These polymers were proven to present several advantages over PVC.

Moreover polymeric coatings, such as a biocompatible hydrogel containing phosphorylcholine groups, poly(2-methacryloyloxyethyl phosphorylcholine-*co*-butyl-methacrylate) or poly(MPC-*co*-BMA), were used to enhance the biocompatibility

* Corresponding author. Present address: Taibah University, Faculty Science, Department of Chemistry, Al Madenah Al Monwarah, Saudi Arabia.
Tel.: +966 500213248, fax: +966 48401743.
E-mail addresses: ibadr1@yahoo.com, ibadr1@gmail.com (I.H.A. Badr).
[1] Present address: Chemistry Department, Collage of Science, King Faisal University, Al-Hasah, Saudi Arabia.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.08.087

of amperometric and potentiometric sensors (Berrocal et al., 2000; Zhang, Benmakroha, Rolfe, Tanaka, & Ishihara, 1996). Modification of surface of the membrane with covalently attached biomolecules (e.g., anticoagulants such as heparin), has also been employed to obtain sensors with better blood compatibility (Badr, Feiler, & Bachas, 2005). Further, membrane electrode prepared by blending poly (vinyl chloride) with hydrophilic polymers such as poly(ethylene oxide), was proven to enhance blood compatibility of such sensors (Espadas-Torre & Meyerhoff, 1995). Another successful approach to enhance the biocompatibility of sensors was introduced by Meyerhoff's group and is based on doping the polymeric matrix with nitric oxide (NO) releasing compounds (Espadas-Torre, Oklejas, Mowery, & Meyerhoff, 1997; Frost et al., 2003; Kim et al., 2011; Schenfisch et al., 2000). The released NO inhibits platelet aggregation. Different classes of NO donors (diazeniumdiolates and nitrosothiols) dipped within or grafted to polymers were used to create materials that release NO for a wide range of time period (Espadas-Torre et al., 1997; Schenfisch et al., 2000). NO could be also released locally utilizing catalytic polymers possessing immobilized Cu(II) or Se(IV) complexes that can generate NO at the polymer/blood interface from endogenous NO precursors (e.g., nitrite and nitrosothiols) in the presence of physiological reducing agents (e.g., ascorbate and thiolates) (Cha & Meyerhoff, 2006; Frost Reynold, & Meyerhoff, 2005).

Chitosan is a polysaccharide composed mainly of β-(1,4) linked 2-deoxy-2-amino-Dglucopyranose and partially of β-(1,4) linked 2-deoxy-2-acetamido-D-glucopyranose. Because of several unique and interesting chemical and biological properties of chitosan such as biocompatibility, biodegradability, and nontoxic properties, chitosan has been considered for several biomedical and pharmaceutical applications such as artificial skin and wound dressing, as well as scaffolds for tissue engineering and vehicles for drug and gene delivery (Francis & Matthew, 2000; Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004; Kurita, 1998; Kumar, 2000; Muzzarelli, 2009; Muzzarelli et al., 2012; Xu, McCarthy, & Gross, 1996). Moreover, the existence of reactive primary amino and hydroxyl groups present in chitosan makes it easily chemically modified to improve its hemocompatibility. A great deal of research work proves that chitosan's blood compatibility could be dramatically improved by simple chemical modifications (Bannan, Danby, Cowan, Ashraf, & Martin 1997; Blair, Guthrie, Law, & Turkington, 1987; Chandy, Rao, Wilson, & Das, 2002; Don, King, &Chiu, 2002). For instance, heparin-modified chitosan showed dramatic improvement of blood compatibility (Bannan et al., 1997; Chandy et al., 2002). Furthermore, due to the high mechanical strength, excellent film forming ability, good adhesion, good biocompatibility, nontoxicity, remarkable affinity to proteins, and excellent gel-forming ability of chitosan biopolymers, they have been extensively applied in biosensors (Karim & Fakhruddin, 2012; Liang, Peng, & Qin, 2008; Qiu et al., 2009; Zou, Xiang, Sun, & Xu, 2008). In such cases, chitosan serves as a matrix for the assembly of biomolecules, cells, nanoparticles, and other substances. Chitosan film can be also integrated in devices by several methods and such methods could be adapted to microfabrication technology, including solution casting, spin casting, electrodeposition, electrospary, screen printing and nanoimprinting (Koev et al., 2010; Sun & Li, 2011; Yi et al., 2005). Such previous investigations indicated that modified chitosan is a good candidate for construction of membrane electrodes and in particular for improving the biocompatibility of ISEs based on highly thromogenic PVC.

The biocompatibility of membrane electrodes is related strongly to the nature of the electrode's surface that comes in direct contact with physiological fluids. Utilization of more biocompatible polymers in the fabrication of sensing film (Ambrose & Meyerhoff, 1996; Berrocal et al., 2001; Bratov et al., 1995; Cha & Meyerhoff, 1989; Cha et al., 1995; Cosofret et al., 1994, 1996; Lindner et al., 1995; Liu et al., 1993; Yun et al., 1997), addition of substances that release anticoagulants at the membrane surface (Espadas-Torre et al., 1997; Frost et al., 2003; Kim et al., 2011; Schenfisch et al., 2000), coating the membrane surface with a biocompatible polymer (Berrocal et al., 2000; Zhang et al., 1996), or immobilization of anticoagulants to the membrane surface (Badr et al., 2005), have been found successful in the enhancement of the biocompatibility of the membrane electrodes. Our approach to improve the biocompatibility of membrane electrodes based on PVC is to sandwich chitosan or H-chitosan layer to the highly thrombogenic PVC sensing film. It is worth mentioning that, other techniques could be also utilized to integrate chitosan film to different platforms of PVC-based membrane electrodes. Possible techniques include solution casting, spin casting, electrodeposition, electrospary, and screen printing (Koev et al., 2010; Sun & Li, 2011; Yi et al., 2005).

In this study we investigated the utility of chitosan and heparin-modified chitosan biopolymers to improve the blood compatibility of ion selective electrodes based on highly thrombogenic polymers (e.g., PVC). Chitosan or H-chitosan biopolymer was sandwiched to the sensing PVC film to prevent the contact between the highly thrombogenic PVC and the sample solution, and therefore enhance the biocompatibility of PVC-based membrane electrodes. To the best of our knowledge, this is the first report on the utilization of chitosan or H-chitosan to improve the biocompatibility of potentiometric sensors.

2. Experimental

2.1. Materials

Sodium ionophore-X, porcine intestinal mucosal heparin, *o*-nitrophenyl octylether (NPOE), potassium tetrakis(chlorophenyl)borate (KTClPB) and carbonyldiimidazole (CDI) were obtained from Fluka (Ronkonkoma, NY), and poly(vinyl chloride) (PVC) were from Polysciences (Warrington, PA). Tetrahydrofuran (THF) was purchased from Fisher (Fair Lawn, NJ) and tris-(hydroxymethyl)aminomethane (Tris) was obtained from sigma (St. Louis, MO), chitosan (750,000 Da) with 85% degree of deacetylation as determined according to (Kwon et al., 2002) was obtained from Aldrich and fatty acid amide softener (BELFASIN-2597 CONC-V) was also obtained from Aldrich (Saint Louis, MO). Anticoagulant solution CPDA-1 was obtained as a gift from the central laboratory of the armed forces (Cairo, Egypt). Each 100 ml of CPDA-1 contains: 3.1900 g dextrose, 2.6300 g sodium citrate dihydrate, 0.2990 g of anhydrous citric acid, 0.2220 g of monobasic sodium phosphate monohydrate, and 0.0275 g of adenine. Blood samples were collected from jugular vein of healthy sheep (Ministry of agricultural, Cairo, Egypt). All standard solutions and buffers were prepared with de-ionized distilled water.

2.2. Preparation of membranes, electrodes, and potentiometric setup

2.2.1. Chitosan membrane

Chitosan acetate with softener paste was prepared according to the literature procedures (Gouda & Keshk, 2010) by dissolving chitosan 4% (w/v) in acetic acid (2% v/v), and the mixture was strongly stirred by using homogenizer for 30 min in the presence of BELFASIN-2597 (2% w/v) as a softener because chitosan films are brittle and not suitable for use in the dry state. These properties of chitosan films are ameliorated by incorporating this softener (Chen, Yeh, & Chiang, 1996; Hasegawa, Isogai, Onabe, Usuda, & Atalla, 1992; Hoagland & Parris, 1998; Xu, Kim, Hanna, & Nag, 2005; Zhong & Xia, 2008). The formed paste was casted over Teflon plate