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

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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

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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

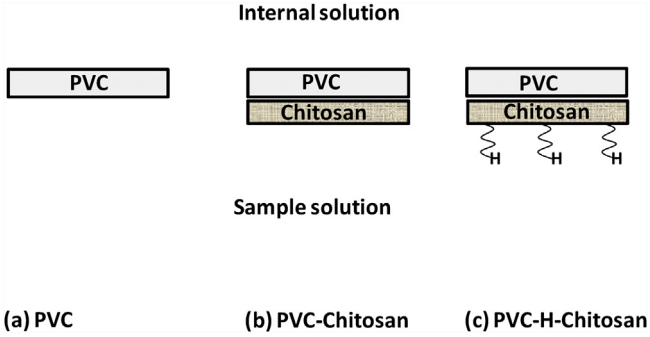

Fig. 1. Schematic diagram for the three sets of sodium selective membrane electrodes used in this study: (a) conventional PVC, (b) PVC-chitosan and (c) PVC-H-chitosan. The letter H indicates the immobilized heparin.

and placed in a dryer at 80 °C for 20 min to form a thin film after curing.

2.2.2. H-chitosan membrane

Heparin-modified chitosan membrane was prepared similar to heparin-modified cellulose triacetate using CDI activation procedures (Badr et al., 2005). First, chitosan membranes were immersed in cold deionized water, and then five increments of CDI were added within a 15-min period, to give 0.10 M final concentration of CDI. The activated membranes were immediately incubated overnight in 1.0% (w/v) heparin solution prepared in 1.00 M sodium carbonate, pH 10 to promote covalent heparin attachment. The activated membranes were removed from the heparin solution and rinsed sequentially with 0.10 M $NaHCO_3$ (pH 8.5), distilled water, acetate buffer (pH 4.0), and finally rinsed with distilled water.

2.2.3. Conventional sodium selective poly(vinyl chloride) membranes

Sodium selective membranes were prepared by dissolving 2.0 mg of sodium ionophore-X (corresponding to 0.70 wt%), 0.50 mg of the lipophilic salt potassium tetrakis(chlorophenyl)borate KTClPB (corresponding to 0.20 wt%), 200.0 mg of plasticizer *o*-nitrophenyl octylether (NPOE) (corresponding to 66.1 wt%), and 100.0 mg of poly vinyl chloride (corresponding to 33.0 wt%) in 2.00 ml of tetrahydrofuran (THF). This cocktail was poured into 25-mm internal diameter glass ring placed onto a glass plate, and the membrane was formed after evaporation of THF overnight.

2.2.4. Sodium selective membrane electrodes based on PVC, PVC-chitosan and PVC-H-chitosan

A schematic representation of the three sets of membrane electrodes used in this study is shown in Fig. 1. Small disks of about 7.00 mm diameter were cut from PVC, chitosan and H-chitosan master membranes. PVC membrane electrode was prepared by inserting the PVC membrane into the tip of Philips electrode body IS-561 body (Glasblaserei Möller, Zurich, Switzerland) to give the control PVC membrane electrodes. PVC-chitosan and PVC-H-chitosan were prepared first by inserting chitosan, H-chitosan, respectively, into the tip of the Philips electrode body, so that chitosan or H-chitosan faces the sample solution. Then, the PVC sensing film was inserted so that it faces the internal filling solution. The internal filling solution for all electrodes was 1.0 mM NaCl prepared in the measuring buffer solution. A double-junction Ag/AgCl electrode (Orion Model 90-02-00) with an Orion (90-02-02) internal filling solution was used as the reference electrode. The outer compartment of the reference electrode was filled with 1.00 M lithium acetate. All potentiometric measurements were performed in 0.05 M Tris–HCl, pH 7.4. Membrane potentials were monitored at room temperature using data acquisition system, eight-channel electrode-computer interface (Nico2000 Ltd., London, UK) controlled by Nico-2000 software. Combination glass electrode [Orion model (8-02)] was used for all pH measurements. The detection limit, linear range, and selectivity coefficients were determined according to the reported methods (Bakker, Pretsch, & Bühlmann, 2000; Guilbault et al., 1976) and the data presented in this paper are average of three ion-selective electrodes measurement.

2.2.5. Biocompatibility studies

Blood was obtained from the jugular vein of a healthy sheep at the ministry of agriculture (Cairo, Egypt). Each pint of blood was drawn into a blood collection bag (JMS, Singapore, PTE Ltd.) containing 70.00 ml of CPDA-1 anti-coagulant solution. Within 30 min after collection, blood was centrifuged at 240 × *g* for 15 min at 4 °C. The supernatant was platelet-rich plasma (PRP) with a concentration of approximately 105 cells/μl as determined with hemocytometer. The PRP was pre-incubated at 37 °C for 20 min. All membranes (PVC, chitosan, heparin-modified chitosan) were conditioned in phosphate-buffered saline (PBS, containing 10 mM phosphate, 138 mM sodium chloride, and 2.7 mM potassium chloride, pH 7.4) for 3 days, and were immersed in PRP for 2 h at 37 °C. They were then rinsed in PBS and fixed in a PBS solution containing 2% (w/v) glutaraldehyde for 2 h at room temperature. Membranes were dehydrated by immersion in serial dilutions of ethanol (30, 50, 70, 90, and 95% v/v), two immersions in absolute ethanol, and two immersions in hexamethyldisilazane. After solvent evaporation within desiccators, the membranes were coated with gold and examined using a GEOL-JEM 1200EX Scanning electron microscope (SEM). Micrographs and results presented below are representative of various membranes materials subjected to this procedure.

3. Results and discussion

In this study we investigated the effect of introducing chitosan or H-chitosan layer on the response characteristics of PVC-based sodium-selective membrane electrodes formulated with sodium ionophore-X.

Energy dispersive X-ray (EDX) was used to characterize the prepared chitosan and H-chitosan membranes. Immobilization of heparin to chitosan takes place through CDI-reactive functional groups on heparin. The conditions for heparin attachment result in 0.32 mg of attached heparin per mg of membrane as measured according to the literature procedures (Brooks, Allen, Feldhoff, & Bachas, 1996). Fig. 2 shows EDX spectra of prepared chitosan as a blank chitosan membrane and H-chitosan membrane. The data showed the presence of sulphur Kα peak at 2.3 keV in case of H-chitosan films (spectrum 2) which confirms the attachment of heparin on the outer surface of the chitosan film. This sulphur peak is absent in the EDX (spectrum 1) of blank chitosan membrane.

Potentiometric response characteristics of three types of sodium selective membrane electrodes (i.e., PVC, PVC-chitosan and PVC-H-chitosan) were studied in order to probe the effect of chitosan and H-chitosan layer on the potentiometric response behaviour of PVC based sodium selective membrane electrodes. PVC sensing films in the three types of electrodes were prepared using 33.0 wt% polyvinyl chloride, 0.7 wt% sodium ionophore-X, 0.2 wt% KTClPB, and 66.1 wt% *o*-NPOE. Potentiometric responses of the three membrane electrodes (PVC, PVC-chitosan PVC-H-chitosan) towards different cations are shown in Figs. 3–5. The response characteristics (e.g., selectivity, response time, linear range, etc.) of the three types of sodium selective membrane electrodes (PVC, PVC-chitosan, PVC-H-chitosan are summarized in Table 1. As shown in Fig. 3 sodium selective membrane electrode based on PVC (control membrane) exhibited sodium detection limit of 1.0×10^{-5} M and had a linear response range from 1.6×10^{-5} to 0.135 M.

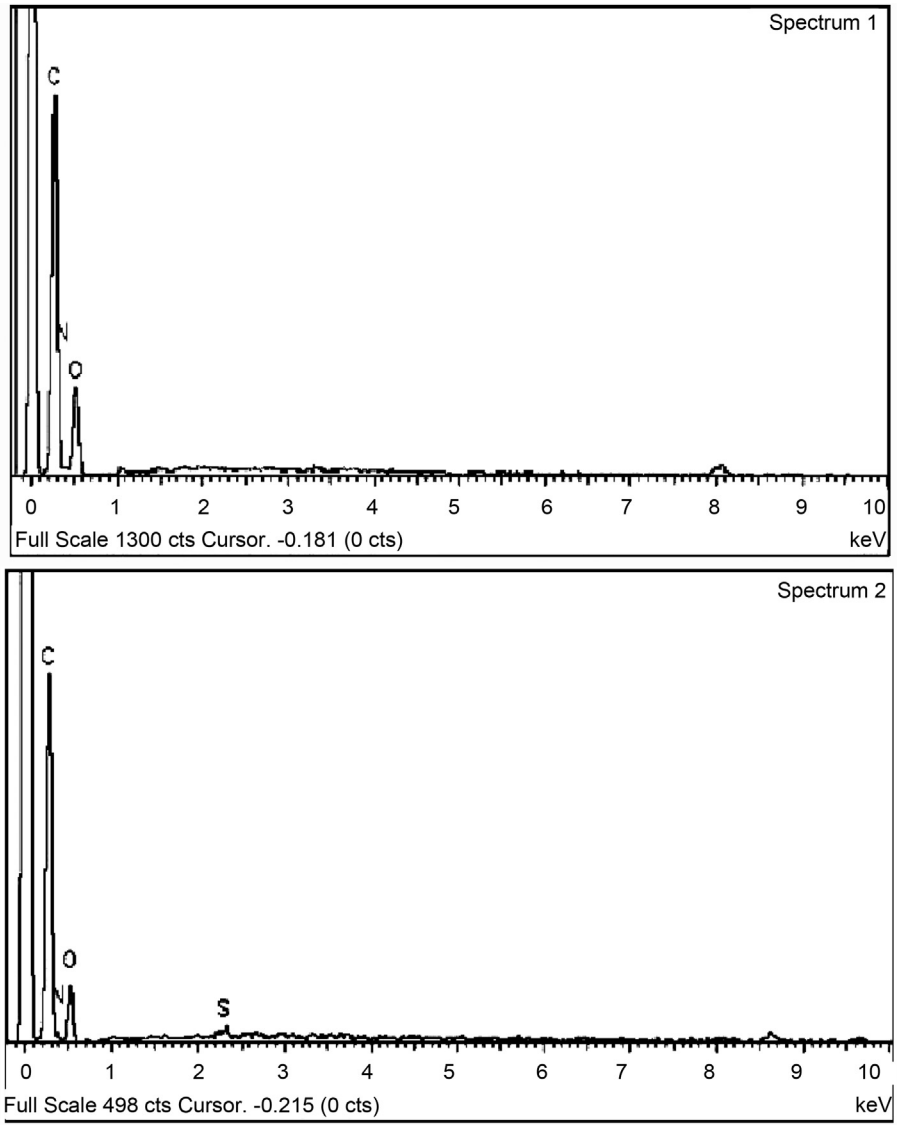

Fig. 2. EDX: (a) chitosan (b) H-chitosan.

Figs. 4 and 5 showed that the sodium detection limits for PVC-chitosan and PVC-H-chitosan membrane electrodes are slightly higher than PVC based sodium selective membrane electrodes. As indicated in Table 1, the detection limit is in the following order: PVC < PVC-chitosan < PVC-H-chitosan. It could be also noticed in Table 1 that lower limit of linear range is shifted to higher sodium ion concentrations (PVC-H-chitosan > PVC-chitosan > PVC), which is consistent with the shift in the detection limit. However, it is worth mentioning that the lower detection limits of PVC-chitosan and PVC-H-chitosan are much lower than the physiological concentration of sodium (135–145 mM), which should enable easily measurement of sodium in physiological fluids. It is possible that the diffusion layer thickness in case of PVC-chitosan or PVC-H-chitosan is larger than that in classical PVC membrane electrode, because of the smaller effect of stirring on the thin film of aqueous buffer solution lies between chitosan or H-chitosan and PVC.

Table 1
Summary of response characterization of sodium membrane electrode.

	Potentiometric selectivity coefficient[a] $\log K \pm SD$						Slope (mV/decade)	Detection limit (μM)	Linear range (M)
	Na^+	K^+	Li^+	NH_4^+	Ca^{2+}	Mg^{2+}			
PVC	0	-1.7 ± 0.1	$<-2.7 \pm 0.3$	$<-3.1 \pm 0.2$	-2.6 ± 0.2	$<-3.5 \pm 0.4$	55.6	10	1.6×10^{-5} to 0.135
PVC-chitosan	0	-1.5 ± 0.2	$<-2.4 \pm 0.2$	$<-2.3 \pm 0.2$	-3.1 ± 0.2	$<-3.1 \pm 0.4$	54.9	25	7.1×10^{-5} to 0.135
PVC-H-chitosan	0	-2.3 ± 0.2	$<-2.8 \pm 0.3$	$<-2.6 \pm 0.1$	-2.3 ± 0.2	$<-3.2 \pm 0.4$	57.0	40	7.9×10^{-5} to 0.135
Required selectivity coefficient[b]	0	<−0.6	<−0.1, <2.1[c]	<1.6	<−1.3	<−1.2	NA	NA	NA

NA: not applicable.

[a] Maximal selectivity limit (<) was used for ions that exhibited minimal reponse towards intefernet ions (Bakker et al., 2000),
[b] Required selectiviy coefficient for blood analysis (1% error; worst case) (Oesch et al., 1986),
[c] Therapeutic level of lithium ion.

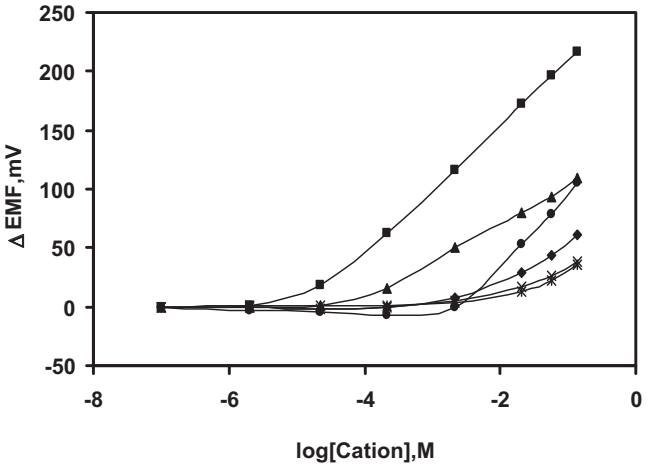

Fig. 3. Potentiometric responses of sodium selective PVC membrane electrode towards different ions measured in 50 mM Tris–HCl, pH 7.4. The cations tested are: (♦) lithium, (■) sodium, (▲) potassium, (×) magnesium, (*) ammonium, and (●) calcium.

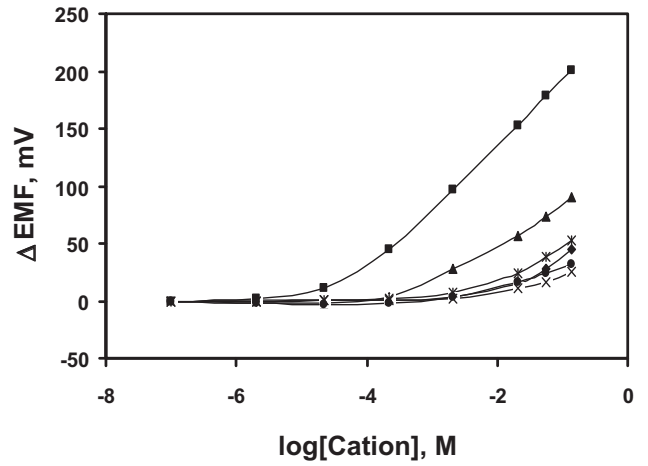

Fig. 4. Potentiometric responses of sodium selective PVC-chitosan membrane electrode towards different ions measured in 50 mM Tris–HCl, pH 7.4. For a key to the symbols associated with each calibration plot, see legend in Fig. 3.

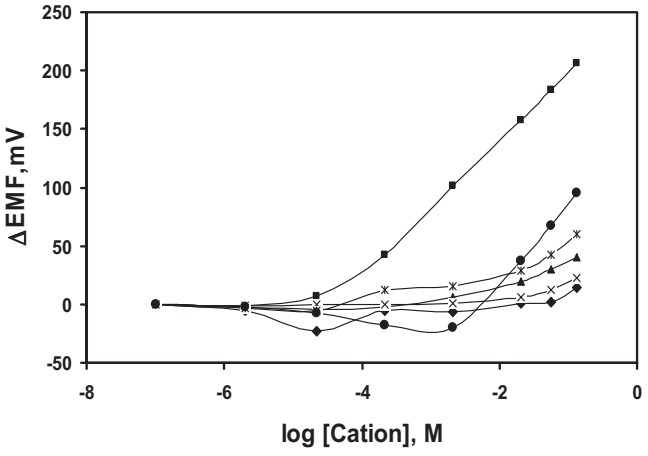

Fig. 5. Potentiometric responses of sodium selective PVC-H-chitosan membrane electrode towards different ions measured in 50 mM Tris–HCl, pH 7.4. For a key to the symbols associated with each calibration plot, see legend in Fig. 3.

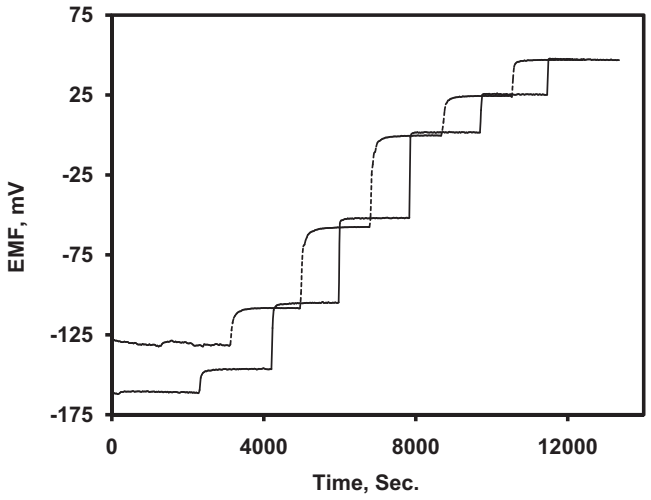

Fig. 6. Dynamic response time of sodium selective membrane electrodes towards increasing sodium ion concentrations: PVC (solid line), and PVC-H-chitosan (dashed line).

This increase in the diffusion layer thickness is expected to increase the concentration of sodium at the PVC sample solution interface and consequently shifts the lower detection limit to high sodium ion concentrations. Similar shift in the detection limit to high sodium concentrations was observed in case of heparin-modified cellulose triacetate compared to cellulose acetate based membrane electrodes formulated with sodium ionophore-X (Badr et al., 2005). Unlike the heparin-modified cellulose acetate and cellulose acetate based sodium selective membrane electrodes which exhibited sub-Nernstian response slopes towards sodium ions (Badr et al., 2005), the response slopes of the PVC-H-chitosan and PVC-chitosan membrane electrodes towards sodium were found to be near-Nernstian with slopes of 57 and 54.9 mV/decade, respectively. The observed response slopes for PVC-H-chitosan and PVC-chitosan are close to that of the control PVC membrane (55.6 mV/decade) indicating that chitosan and H-modified chitosan layers did not alter the interaction between sodium ions and the sensing PVC layer (see data depicted in Table 1). To further study the effect of introducing chitosan or H-chitosan layer on the response characteristics of sodium selective membrane electrodes based on PVC and formulated the sodium ionophore-X, the response times of PVC-H-chitosan towards sodium ion was compared to that of control PVC-based membrane. As can see in Fig. 6 the response time of PVC-H-chitosan (t_{90} = 2 min at 21 mM sodium concentration) towards sodium ion is longer than that of control PVC-based sodium-selective membrane electrodes (t_{90} = 0.5 min at 21 mM sodium concentration). The response time of PVC-chitosan towards sodium ions was found to be similar to PVC-H-chitosan based membrane electrodes (data not shown). The slight increase in the response time of PVC-chitosan and PVC-H-chitosan compared to control PVC is consistent with the higher detection limit observed for those membrane electrodes. In such cases, more time is required for sodium ions to penetrate the chitosan or H-chitosan layer and reach the PVC sensing film.

Selectivity of membrane electrodes is the most important character and it is important to probe the effect of modified or unmodified chitosan layer of the selectivity of PVC-based sodium selective membrane electrodes. Selectivity coefficients of all membrane electrodes (PVC, PVC-chitosan, PVC-H-chitosan) were calculated at 1.0 M concentrations and maximal selectivity coefficients are reported for interferent ions that give significantly lower than Nernstian slopes (Bakker et al., 2000). Selectivity data are reported in Table 1, and potentiometric responses of

all membrane electrodes towards different ions are represented in Figs. 3–5 as recommended (Bakker et al., 2000). Data presented in Table 1 indicated that the selectivity coefficients of PVC-chitosan and PVC-H-chitosan are comparable to the selectivity coefficients of control PVC-based sodium selective membrane electrodes. This finding further proves that the physical attachment of chitosan and H-chitosan layers to the PVC sensing film did not interfere with the ion-ionophore binding chemistry (i.e. presence of H-chitosan or chitosan did not alter the binding constants of ion-ionophore or single ion partition coefficient). It is worth mentioning that the selectivity coefficient of the three sets of membranes electrodes (PVC, PVC-chitosan and PVC-H-chitosan) meet the selectivity requirements for sodium analysis in physiological fluids with < 1% error (worst case) (Oesch, Ammann, & Simon, 1986).

Platelet adhesion to the polymeric membranes pictured using scanning electron microscopy (SEM) can be used to compare the thrombogenicity of different surfaces. Platelets play a key role in the coagulation cascade. Therefore, the adhesion and activation of platelets on a surface is an indicator of the potential

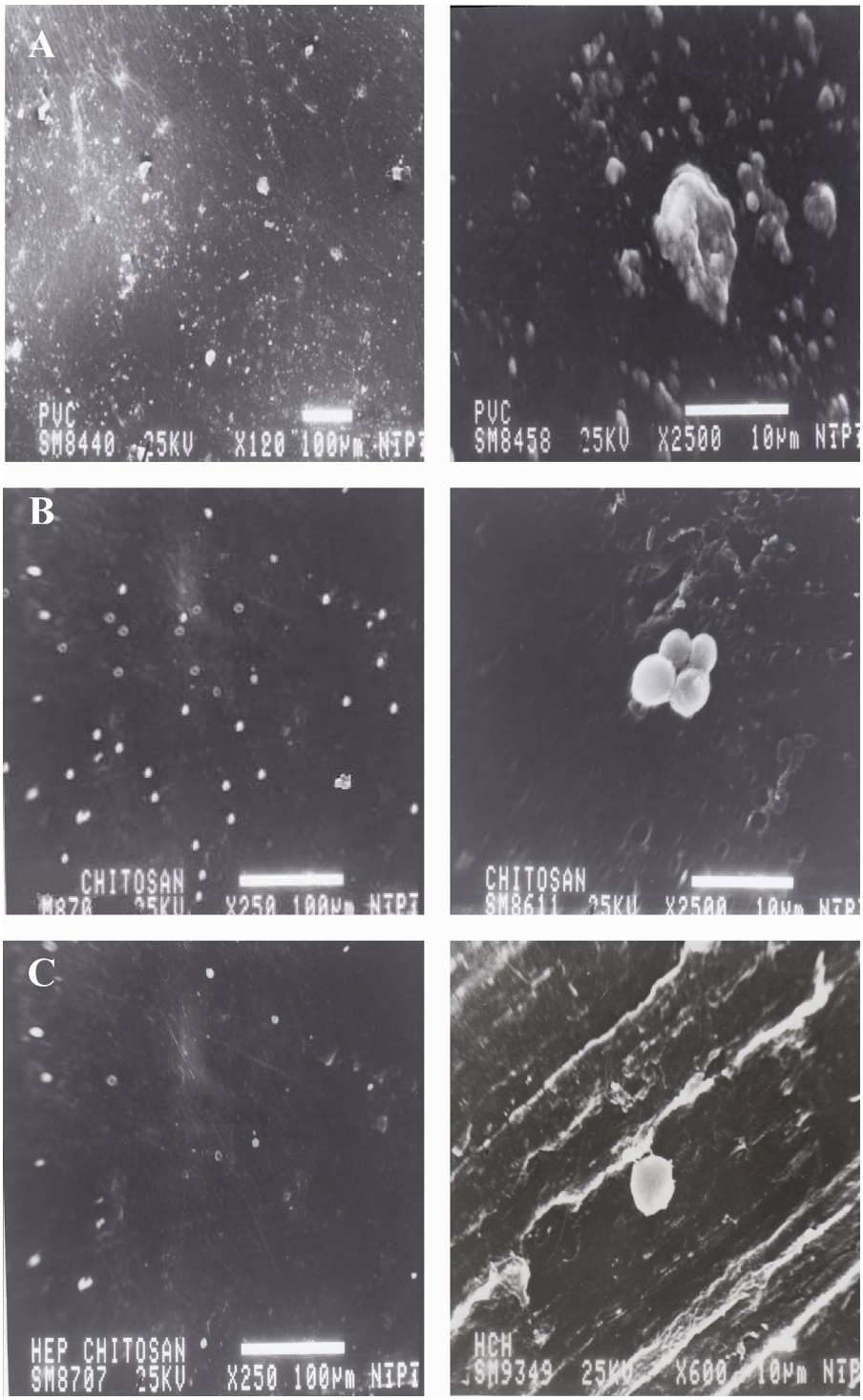

Fig. 7. Scanning electron micrographs of various membranes after 2-h contact with PRP: (A) PVC, (B) chitosan, and (C) H-chitosan. SEM to the right is a magnification of a selected section of left SEM.

thrombogenicity of this surface. Platelet adhesion is routinely used to investigate the interactions between materials and blood in vitro (Berrocal et al., 2000, 2001; Espadas-Torre & Meyerhoff, 1995; Espadas-Torre et al., 1997; Frost et al., 2003; Kim et al., 2011; Schenfisch et al., 2000). The relative thrombogenicity of different membranes (PVC, chitosan, and H-chitosan) were investigated using the platelet adhesion protocol. Results of the in vitro blood compatibility studies are presented via representative SEM micrographs shown in Fig. 7. The SEM pictures indicated that the various polymer materials examined differ significantly in their platelet adhesiveness under the same conditions. PVC membranes consistently had the largest number of deposited platelets and platelet aggregates, indicating a high thrombogenicity of this membrane. Densely packed platelet aggregates are shown in Fig. 7A for PVC membranes. Chitosan has amine-rich reactive functional groups with a pK_a value of about 6.3 (Rinaudo, Pavlov, & Desbrieres, 1999). When the pH is above the pK_a (e.g., measurement conditions, pH 7.4), the amino groups are deprotonated and chitosan becomes mostly uncharged. It is reported that platelet adhesion decreased with the neutralization of this charge (Sagnella & Mai-Ngam, 2005). Moreover, it is well-documented in the literature that blending polymers with surfactants exhibited improved blood compatibility (Amiji, 1995; Espadas-Torre & Meyerhoff, 1995). Therefore, blending chitosan with softener, which has surfactant properties, and measurement of the platelet adhesion at pH 7.4 is expected to improve chitosan biocompatibility. Indeed, as can be seen in Fig. 7B, chitosan membrane appeared to have much smaller number of platelets adhesion compared to the highly thrombogenic PVC. Further improvement in platelet adhesion is expected utilizing heparin grafted chitosan. As can be seen Fig. 7C, heparin-modified chitosan membrane seems to have minimal platelets adhesion. A few platelets were observed on H-chitosan. These platelets were isolated and appeared to have a spherical shape, meaning that they were not activated, although they had adsorbed onto the surface. A cell count from the micrographs of 5 PVC segments (of the same membrane) yielded an average of $13.0(\pm5.3)\times10^3$ cells/mm^2. A cell counts of $0.7(\pm0.3)\times10^3$ cells/mm^2 ($n=5$), and $0.15(\pm0.1)\times10^3$ cells/mm^2 ($n=5$) were observed for chitosan and H-chitosan membranes. Plasma exposure and SEM experiments were repeated in triplicate using pairs of three separately cast membranes of PVC and H-chitosan. The PVC membranes had $11.0(\pm4.5)\times10^3$ cells/mm^2 ($n=3$), whereas H-chitosan only had $0.13(\pm0.07)\times10^3$ cells/mm^2 ($n=3$). It is worth mentioning that no platelets were observed on PVC surface in case of PVC-H-chitosan or PVC-chitosan (SEM picture is not shown). Because platelets are central in the blood clotting cascade, preventing platelets from reaching PVC would effectively prevent coagulation on the surface of PVC and improve the blood compatibility of ISEs based on PVC.

4. Conclusions

Heparin modified chitosan was first utilized as novel material to improve the biocompatibility of ion-selective electrodes prepared with highly thrombogenic PVC. Three types of sodium selective membrane electrodes formulated with sodium ionophore-X were prepared: control PVC, PVC-chitosan, and PVC-H-chitosan. In all types of electrodes PVC film acted as the sensing layer. Chitosan and H-chitosan were physically attached to PVC sensing film. Chitosan and H-chitosan were utilized to improve the blood compatibility of PVC-based membrane electrodes by preventing platelets from reaching PVC surface. The response characteristics of chitosan-PVC and PVC-H-chitosan membrane electrodes (e.g., detection limit, linear range, response slope, and selectivity coefficients) were found to be comparable to that of the control PVC-based sodium electrodes. The overall performance of the modified membrane electrodes (PVC-chitosan and PVC-H-chitosan) was found to be comparable to that of the control PVC electrodes. This indicates that physical attachment of chitosan and H-chitosan did not alter the ion-ionophore interaction. SEM micrograph of in vitro platelet adhesion demonstrate that heparin-modified chitosan is substantially less thrombogenic than PVC, and therefore the use of H-chitosan membrane would enhance the PVC-based potentiometric membrane electrodes for clinical and physiological applications.

Acknowledgement

The authors are grateful to Ain Shams University Scientific Research Sector (Grant 08-010) for providing financial support to undertake this work.

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