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Notes

Functionalized surface for electrochemical sensing of electrochemically inactive alkali metal ion

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The binding of Na^+ ion to the ionophore 4-aminobenzo-crown-5 has been monitored by cyclic voltammetry using the redox couple $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$ as a mediator redox probe. The recognition properties of the ionophore covalently bound to 11-MUA self assembled monolayer on gold surface is found to be selective for Na^+ ion over Li^+ and K^+ ions. The self-assembled sensor interface is highly stable, reproducible and shows good sensitivity for detection of Na^+ ion. The host-guest recognition of the simple crown ether derivative makes the system highly promising for real-time applications for selective detection of electrochemically inactive alkali metals by electrochemical transducer.

Keywords: Electrochemical sensors, Sensors, Cyclic voltammetry, Host-guest complexation, Self assembled monolayers, Alkali metals, Sodium

Electrolytes such as sodium and potassium are physiologically important[1,2] and their imbalance is associated with blood pressure, kidney damage, improper cardiac function, nerve impulse transmission, water balance, atherosclerosis. Concentration levels of specific electrolytes in biological fluids need to be under constant monitoring for therapeutic management of associated diseases. Prevailing instrumental analytical methods for analyzing electrolyte levels in biological samples such as flame atomic absorption[3], ion chromatography[4], inductively coupled plasma mass spectrometry (ICP-MS)[5] and capillary electrophoresis[6] requires elaborate sample treatment and expensive instrumentation. Ion selective electrodes being comparatively simple devices offer an alternative to these instrumental techniques where output signal is generated by the potential differences of oxidation/reduction reactions associated with the analyte. Crown ether derivatives[7-12], cryptand derivatives[13], calix[4]crown derivatives[14-16] and tungstosilicic acid[17] based ionophores have been extensively used in construction of sodium ion selective electrodes. However, the time required for achieving the equilibrium across the membrane of an ISE is undesirably high and hence sensors offering real time response are much in demand. Optical sensors based on rhodamine ester with sodium selective commercial ionophore (ETH 157)[18], calixarenes[19,20] and crown ether[21-23] based ionophores are reported for sodium ion sensing. The pH dependent chromogenic activity poses some limitations to optical sensors. For electrochemical sodium ion sensor, self assembled monolayer of 2-[(6-mercaptohexyl)oxy] methyl-12-crown-4 on gold electrode[24] and multiwall carbon nanotubes functionalized with acyclo-oligomeric calixarene[25] have been used for fabrication of Na^+ ion sensor, both involve reduction in current signal to mark the binding event. A sub-micropipette probe[26] with PVC lining containing bis(12-crown-4) and microhole supported liquid/ gel interface[27] containing dibenzo-18-crown-6 have also been reported as Na^+ ion sensing platform. An updated review of the ionophores employed for constructions of sodium ion selective electrode and sensors based on host-guest complexation has been collected in Table S1 (Supplementary data).

Herein, we propose a sodium ions sensor based on immobilization of 4-aminobenzo-15 crown-5-(4-AB-15-C-5) as an ionophore on to a functionalized gold surface through self assembled monolayer.

Experimental

The electrochemical analyzer (model BASi EPSILON, Bioanalytical Systems, USA with software ver. 2.13.77), with Ag/AgCl electrode (sat. KCl) as reference electrode, platinum wire as counter electrode and gold electrode (dia. 1.76 mm) as working electrode was used. The gold electrode was polished sequentially using diamond lapping compound (1 μ; Kemet, Kent, UK) and by ∝-alumina (0.05 μ), rinsed thoroughly with deionized water, followed by ultrasonication in ethanol. Precision LCR meter (model 4284A, Agilent) and liquid test fixture (model 16542A Agilent) was used for conductance measurement. Ultrasonic bath (model 3120, Elma, Germany) for dip coating under ultrasonication and an inverted homogenizer (model RQ-127, Remi, New Delhi), improvised in our laboratory for spin coating were used at 8000 rpm.

3-Mercaptopropionic acid (MPA), 11-mercaptoundecanoic acid (MUA), N-hydroxysuccinimide (NHS), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), 4-aminobenzo-15-crown-5 (4-AB-15-C-5), potassium ferricyanide $K_3Fe(CN)_6$ and tetrabutylammonium bromide (TBAB) were procured from Sigma–Aldrich and the remaining chemicals were of AR grade from E. Merck (India), Mumbai, India. De-ionized water and HPLC grade solvents were used to prepare the solutions. Aqueous solution of potassium ferricyanide in 0.1 *M* TBAB as supporting electrolyte and chlorides salts of analytes (NaCl, KCl, LiCl) were used for sensing the metal ion by cyclic voltammetry. All solutions were freshly prepared and deoxygenated by passing a soft stream of argon for 15 min. Methanolic stock solutions of 0.1 *M* 4-aminobenzo-15-crown-5 and 0.1 *M* sodium thiocyanate were used for conductance studies.

To obtain the 1:1 complex, equimolar quantities of crown ether derivative, 4-AB-15-C-5 (100 µL, 0.1 *M*) and sodium thiocyanate (100 µL, 0.1 *M*) in 100 mL methanol were mixed with gentle stirring under a soft stream of nitrogen for 5-7 min.[28]. The resultant brown mass was reserved for conductometric studies.

The sensor was fabricated as follows: Different strategies were tested to allow self-assembly of adsorbent (MPA and MUA) monolayer on to the clean gold electrode viz. immersion for extended hours, spin coating and dip coating under ultrasonication. After monolayer formation, the electrode was taken out from the adsorbent solution, rinsed thoroughly with ethanol followed by water to remove any physiosorbed material. The next step was activation of monolayers by introducing NHS–EDC reagent (1:1 *v/v* aqueous 100 m*M* NHS and 400 m*M* EDC) followed by 100 m*M* 4-aminobenzo-15-crown-5/methanol. Then the surface was left undisturbed for the solvent to evaporate. The sensor electrode thus modified (Au/MUA/4-AB-15-C-5) is used for sensing alkali metal ion by monitoring the change in current signal for redox activity of mediator redox probe $Fe(CN)_6^{3-}$ / $Fe(CN)_6^{4-}$.

Results and discussion

The cation recognition properties of proposed ionophore, 4-aminobenzo-15-crown-5 (4-AB-15-C-5) towards sodium ion was first tested in solution by comparing the conductance (Fig. 1) of ionophore and alkali metal salts in free and complexed state. The specific conductivity of methanolic solution of NaSCN (0.2 m*M*), 4-aminobenzo-15-crown-5 (0.2 m*M*) and 1:1metal ion-ionophore complex at 27 °C was recorded to be 7×10^{-3}, 0.92×10^{-3} and 2.82×10^{-3} S m^{-1} respectively. Quenching of high mobility of sodium salt in complexed state with ionophore, 4-AB-15-C-5 shows successful host-guest complexation. Having confirmed the selective binding of 4-aminobenzo-15-crown-5 to the Na^+ ion in solution, the fact that electron donating amino substituent at *para* position, enhances the binding ability of crown ether[29], prompted us to attempt *in-situ* immobilization of ionophore, onto a electrochemical working electrode for selective detection of sodium ion in solution through a mediator redox probe.

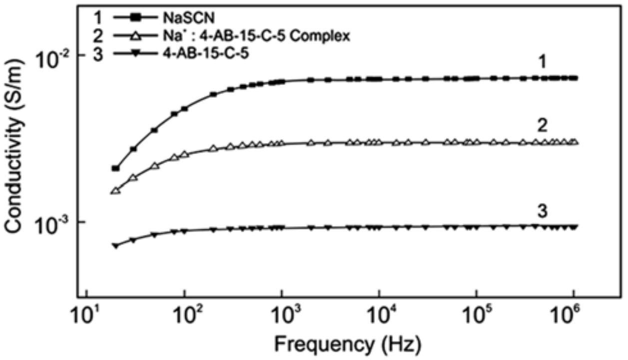


Fig. 1 — Conductivity of methanolic solution of 0.2 m*M* sodium thiocyanate, 0.2 m*M* 4-aminobenzo-15-crown-5 and 1:1 complex of Na:4-aminobenzo-15-crown-5 at 27 °C.

Potassium ferricyanide, a well studied redox ion ($Fe(CN)_6^{3-}$ / $Fe(CN)_6^{4-}$), reveals highly reproducible electrochemical characteristics at solid electrodes and therefore has been used as a redox probe for characterization of SAM formation[30-32]. The present study exploits ($Fe(CN)_6^{3-}$ / $Fe(CN)_6^{4-}$) redox probe as mediator redox probe which undergoes electrochemical reduction-oxidation at gold electrode through an ion-gate of ionophore for sensing Na^+ in solution.

For sensor development, to avoid non-specific binding, a suitable modification of sensor surface is desirable, therefore we envisaged immobilizing the proposed ionophore on to the gold surface through self assembly approach. For this purpose, the gold sensor electrode surface was immobilized with SAM of a short chain and a long chain thioic acid, viz., 3-mercaptopropionic acid (MPA) and 11-mercapto-undecanoic acid (MUA). The success of monolayer formation on gold surface was assessed by carrying out cyclic voltammetry (CV) of potassium ferricyanide, $K_3Fe(CN)_6$. The electrostatic repulsion between the depolarizer anion, $Fe(CN)_6^{3-}$ and negatively charged SAM of thioic acid impede the electron transfer to the sensor electrode. As shown in Fig. 2, for MPA immobilization, the cathodic current signal (I_{pc}) is

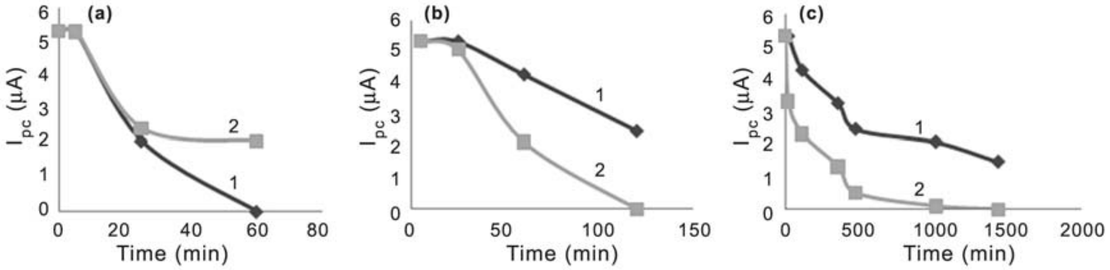
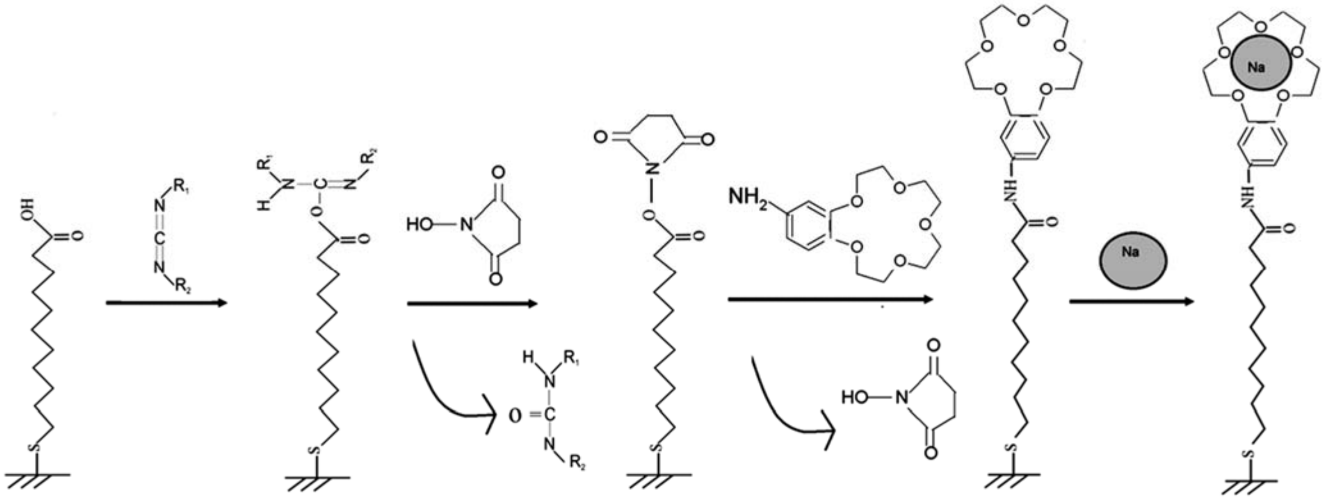


Fig. 2 — Depletion of cathodic current (at 0.100 V vs Ag/AgCl) for electrochemical reduction of 1 m*M* $Fe(CN)_6^{3-}$ / 0.1 *M* TBAB at different stages of SAM formation (2 m*M* MPA/ 2 m*M* MUA) over gold sensor electrode employing (a) spin coating; (b) dip coating under ultra-sonication, and, (c) immersion technique. 1, MPA; 2, MUA].



Immobilization of long chin self assembled monolayer of MUA, covalently bonded to 4-aminobenzo-15-crown-5 as selective ionophore for sodium ion onto the gold surface, where R_1 = 3-dimethylaminopropyl- and R_2 = ethyl

Scheme 1

impeded, whereas for the MUA immobilized electrode, the current signal gets completely suppressed. In the case of short chain (MPA) SAM modification, the electron transfer to the electrode is slower, however in the case of MUA with 11-carbon chain, the electron transfer is completely blocked[30]. As far as SAM formation technique is concern, if time permits overnight immersion technique (Fig. 2c) is well proven. However a stable SAM of 11-MUA could also be achieved in 1 h of spin coating (Fig. 2a) or 2 h of dip coating in an ultrasonic bath (Fig. 2b).

The self-assembled monolayer (SAM) of 11-mercaptoundecanoic acid acts as a matrix for covalent binding of ionophore through carbodiimide chemistry, followed by host-guest complexation with sodium ion, as shown schematically in Scheme 1. The carboxyl groups of monolayer extending out from the sensor surface was activated by EDC-NHS reagent incorporating N-hydroxy succinimide, through carbodimide activation and facilitating stable amide bond between MUA and ionophore 4-AB-15-C-5. The sensor surface (Au/MUA/4-AB-15-C-5) thus prepared was reserved for recognition of electrochemically inactive sodium ion by electrochemical assay.

Cyclic voltammetric scan was carried out for mediator redox probe, 1 m*M* $K_3Fe(CN)_6$/0.1 *M* TBAB at SAM coated gold electrode, functionalized with 100 m*M* 4-aminobenzo-15-crown-5, in the potential range 0.6 V to 0.0 V vs Ag/AgCl electrode, at scan rate of 100 mV s^{-1}. The voltammetric signal in the absence of ionophore bearing SAM (E_{pc}/I_{pc} as 210 mV/10.42 μA and E_{pa}/I_{pa} as 280 mV/ 12.57 μA; figure not shown) got completely blocked as shown in Fig. 3a. Now, on addition of 1 m*M* Na^+ ion solution to the mediator redox probe solution in contact with sensor electrode (Au/MUA/ 4-AB-15-C-5), a well characterized voltammetric current signal reappeared with its characteristic signal, E_{pc}/I_{pc} as 211 mV/2.74 μA and E_{pa}/I_{pa} as 274 mV/2.45 μA (Fig. 3b).

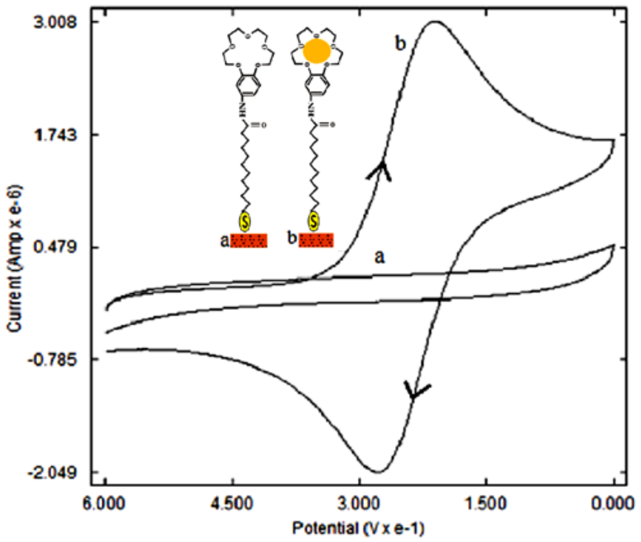


Fig. 3 — Cyclic voltammogram of mediator redox probe 1 m*M* Fe(CN)$_6^{3-}$/0.1 *M* TBAB at Au/MUA-4-AB-15-C-5 sensor electrode. [(a) in absence of Na$^+$ ion; (b) in presence of 1 m*M* Na$^+$ ion. Inset: variation of I_{pc} with Na$^+$ ion concentration for 3 m*M* Fe(CN)$_6^{3-}$ / 0.1 *M* TBAB. Scan rate: 100 mV s^{-1}].

The negatively charged species, Fe(CN)$_6^{3-}$, gets repelled by negatively charged MUA monolayer present on the electrode surface[30,31], and by the same analogy the SAM having crown ether head as sensor probe (negatively charged due to the lone pairs of electrons at oxygen of crown) also inhibits the electron transfer towards sensor electrode and the current signal gets impeded[30-32]. Electrochemical current signal of Fe(CN)$_6^{3-/4-}$ redox process can be blocked completely by tuning the concentration of ionophore at the electrode and that of mediator ion. When the metal cation is successful in binding to the ionophore of the sensor probe the negative charge on the crown is neutralized which facilitates the anion depolarizer Fe(CN)$_6^{3-}$ to reach the electrode surface and the electrochemical redox signal reappears indicating presence of Na$^+$ in the solution.

Considering the sodium ion concentration in physiological fluids, it was desirable to investigate the functioning of the proposed sensor for selective monitoring at higher levels of sodium ion in solution. By elevating the concentration of mediator ion to 3 m*M*, the range of detection 10-300 m*M* could be achieved. The inset of Fig. 3 reveals the effect of increasing concentration of analyte ion (Na$^+$) on current signal (I_{pc}) of mediator redox probe at higher concentration (3 m*M* K_3Fe(CN)$_6$ /0.1 *M* TBAB). The linear regression for sodium ion in concentration range 10-300 m*M* was $y = 0.0122x+3.987$ ($R = 0.9954$), shows that voltammetric current at peak potential of mediator redox probe is suitable for quantitative sensing of sodium ions in solution.

The cross reactivity of other electrolytes of potent interfering alkali metal ions of physiological significance (viz., K$^+$, Li$^+$), was checked, by repeating the cyclic voltammetric scan with the addition of 1 m*M* K$^+$ / Li$^+$ to the mediator ion with the proposed sensor electrode (Au/MUA/4-AB-15-C-5). The blocked voltammetric signal did not reappear on addition of K$^+$ or Li$^+$ ion, confirming that the proposed ionophore is highly specific to Na$^+$ ions. The Na$^+$ ion (ionic diameter, 1.90 Å) appear to fit in well as a guest in the cavity of 15-crown-5 (1.7-2.2 Å) based ionophore whereas ionic diameters of Li$^+$ (1.20 Å), and of K$^+$ (2.66 Å) do not support the host-guest binding with the proposed ionophore, making it highly specific for Na$^+$ ions.

In the present study a simple procedure is employed to obtain a highly ordered monolayer of the sensing element, 4-aminobenzo-15-crown-5 covalently bonded to self-assembled monolayer of 11-mercaptoundecanoic acid at gold sensor surface through carbodiimide activation for construction of sodium ion sensor. The binding of sodium ion to the ionophore has been monitored by cyclic voltammetry using redox couple Fe(CN)$_6^{3-}$/ e(CN)$_6^{4-}$ as mediator redox probe. The detection range of sodium ion could be tuned by adjusting the mediator redox probe concentration. The host-guest recognition of the simple crown ether derivative makes the systems highly promising for real time applications for selective detection of electrochemically inactive metals by electrochemical transducer.

Supplementary data

Supplementary data associated with this article are available in the electronic form at http://www.niscair.res.in/jinfo/ijca/IJCA_56A(09)934-938_SupplData.pdf.

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