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# New calix[4]arene derivatives as ionophores in polymeric membrane electrodes for Ag(I): Comparative selectivity studies and detection of DNA hybridization

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ABSTRACT

Four calix[4]arene derivatives containing various donor atoms and different topology (**L1**–**L4**) have been synthesized and used as neutral ionophores to fabricate silver ion selective electrodes (Ag-ISEs) which were characterized in terms of their potentiometric selectivities and complex formation constants. The ionophore **L2** having two nitrogen and two sulfur donors showed stronger interactions with $Ag^+$ and the highest selectivity coefficient towards $Ag^+$. The best membrane electrode was prepared from **L2** and used to fabricate silver ion selective microelectrodes (Ag-ISμEs) which could detect silver ions in 1000 μL samples with detection limit around 1 μM using sodium ion microelectrodes as a pseudo reference electrode. Such potentiometric measurement was then applied to detect DNA hybridization on a gold substrate, employing immobilized lipoic acid-modified pyrrolidinyl PNA (Lip-acpcPNA) as a probe. The hybridization between the neutral Lip-acpcPNA probe and DNA target led to a negatively charged surface that could bind positively charged silver nanoparticles ($AgNPs^+$) via electrostatic interactions. The hybridization signal was observed by dissolution of the electrostatically adsorbed $AgNPs^+$ with hydrogen peroxide. Excellent discrimination of complementary from single mismatched and non-complementary DNA targets was achieved under non-stringent conditions. The detection limit of DNA was 0.2 μM in 1000 μL samples.

## 1. Introduction

Potentiometry based on ion selective electrodes (ISEs) has been widely used for determining the activity of metal ions for several decades [1]. Since ISEs offer several advantages, especially low cost, simplicity and obvious compatibility with miniaturization, they are an excellent tool in analytical applications such as clinical measurements, industrial process control and environmental monitoring. Recently, there has been an increasing interest in using miniaturized ISEs for monitoring protein immunoassays [2] or DNA hybridization [3–5] using Ag, CdS-nanocrystal labels, enzyme tags or liposomes.

Peptide nucleic acid (PNA) is a DNA analog containing nucleobase-modified *N*-(2-aminoethyl)glycine subunits linked together by amide bonds. PNA can form duplexes with complementary DNA with high thermal stability owing to the absence of electrostatic repulsion between PNA and DNA strands. PNA also discriminates single-base mismatched DNA with high specificity [6]. Employing the unique uncharged properties of PNA backbone, two research groups [7,8] reported the use of PNA as a probe to detect non-labeled oligonucleotides specifically through electrostatic interactions between positively charged metallic nanoparticles and negatively charged backbone of DNA hybridized with the neutral PNA probe.

We aim to fabricate a polymer membrane silver ion selective microelectrode (Ag-ISμE) that can detect DNA hybridization with the neutral PNA probe using positively charged silver nanoparticles ($AgNPs^+$) as a potentiometric marker. A variety of ionophores containing π-electrons [9–11] or heteroatoms [12–17] as coordinate sites have been successfully used to construct Ag-ISEs for monitoring and determining silver ions. Nevertheless, Szigeti and co-workers showed that selectivity coefficients of membranes containing π-coordinating calix[4]arene ionophores were worse than those containing calix[4]arene incorporating sulfur donors, resulting in less discrimination of $Ag^+$ from other cations [18].

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Therefore, we designed and synthesized four calix[4]arene derivatives **L1** – **L4** containing various donor atoms and different topology, which were used as neutral ionophores to fabricate Ag-ISEs. The characteristics such as selectivity, lower detection limit and formation constant of the four ionophores synthesized towards $Ag^+$ in polymeric membranes were compared in order to choose the best ionophore from which to fabricate a microelectrode to be used in combination with PNA for monitoring DNA hybridization.

The principle of our potentiometric sensors for detection of DNA hybridization is illustrated in Fig. 1. Pyrrolidinyl PNA with a D-propyl-2-aminocyclopentanecarboxylic acid backbone (acpcPNA) [19–21] which shows excellent specificity for DNA recognition is used as a probe in this work. The acpcPNA is modified at the *N*-terminus with lipoic acid and the so-obtained Lip-acpcPNA is covalently immobilized on the surface of a gold substrate. The target DNA is hybridized with the electrostatically neutral Lip-acpcPNA resulting in a negatively charged surface due to the phosphate backbone of the target DNA. Addition of $AgNPs^+$ [22,23] results in adsorption of the $AgNPs^+$ on the gold substrate through electrostatic interactions. The adsorbed $AgNPs^+$ on the gold surface is then oxidized by $H_2O_2$ to release silver ions, which can be detected with our fabricated Ag-ISμE.

## 2. Experimental

### 2.1. Materials

*p*-*tert*-Butylcalix[4]arene-tetraacetic acid tetraethyl ester (Na(X)), potassium tetrakis[4–chlorophenyl]borate (KT*p*ClPB), bis(2-ethylhexyl)sebacate (DOS), *o*-nitrophenyl octyl ether (*o*-NPOE), high molecular weight poly(vinyl chloride) (PVC), and tetrahydrofuran (THF) were purchased in selectophore® or puriss quality from Fluka. Nitrate salts of cations were of analytical grade obtained from Merck, Fluka, Sigma–Aldrich, Reidel and Carlo Erba. 30% (v/v) Hydrogen peroxide ($H_2O_2$) was purchased from Fisher Scientific. All solutions were prepared with deionized water with the specific resistivity of 18.2 MΩ cm (Milli-Q water purification system; Bedford, MA, USA).

Chemicals for synthesis of $AgNPs^+$, i.e., silver nitrate ($AgNO_3$), cetyltrimethylammonium bromide (CTAB) and sodium borohydride ($NaBH_4$) were purchased from Merck.

All oligonucleotides were purchased from Biogenomed Co., Ltd. The sequences of complementary, single base mismatched and non-complementary DNA targets were 5′-GTCATAGCATCA-3′, 5′-GTCATCGCATCA-3′ and 5′-AAGGCCTATGTC-3′, respectively.

### 2.2. Preparation of membrane electrodes

The membrane contained 1 wt% ionophore, KT*p*ClPB (75 mol% relative to ionophore), 33 wt% PVC and 66 wt% *o*-NPOE. All components (220 mg in total) were dissolved in 2.5 mL of THF or MeOH/$CHCl_3$/THF (1:2:10 v/v) and poured into a glass ring (30 mm i.d.) fixed on a glass plate. Then, the solvent was allowed to evaporate at room temperature overnight. A transparent membrane of about 200 μm in thickness was obtained. The membrane was punched into circular films (7.5 mm i.d.) and glued with a PVC/THF slurry on the top of a PVC tube and connected to a micropipette tip as an electrode body. An internal filling solution containing 10 mM $AgNO_3$ was put into this PVC tube which connected to a micropipette tip and a AgCl-coated silver wire was placed into it. The prepared membrane electrode was conditioned in 10 mM $AgNO_3$ solution for overnight prior to use.

### 2.3. Selectivity measurements

The potentiometric selectivity coefficients were determined by the separate solution method (SSM) [24]. The membranes were conditioned overnight in the solution of interfering metal ions (nitrate salt). The response of the electrode was first measured against the interfering metal ions ($10^{-7}$–$10^{-2}$ M) by using 10 mM of the same interfering cations (chloride salt) as an inner filling solution. The electrode was then used to measure the response of silver nitrate solutions. Selectivity coefficients of each metal were performed in triplicate (using a new membrane for each replicate). The interfering ions in this study were $Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$, $Ni^{2+}$, $Cu^{2+}$, $Zn^{2+}$, $Cd^{2+}$, $Pb^{2+}$ and $Hg^{2+}$. To determine the selectivity coefficients of $Hg^{2+}$ and $Pb^{2+}$, the pH of solution was

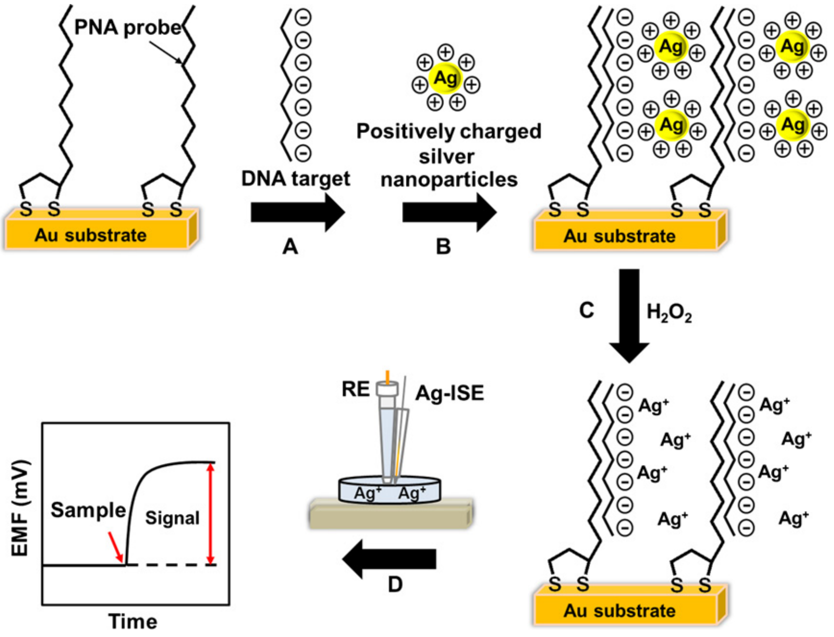

**Fig. 1.** Representation of $AgNPs^+$-based label-free potentiometric DNA detection.

adjusted to 2 and 4, respectively with $HNO_3$. The selectivity coefficients were calculated from the mean value of triplicate experiments with the standard deviation.

### 2.4. Preparation of sandwich membranes

In order to determine ion–ionophore complex formation constants in the solvent polymeric membrane, it requires membrane potential measurements on two-layer sandwich membranes [25–27], where only one side contains the ionophore. The membrane compositions and the formation constant are shown in Table 1.

The conventional standard size membranes, with and without ionophore, were conditioned in 5 mM $AgNO_3$ overnight. The sandwich membrane was prepared by attaching two individual membranes together and visibly checked for air bubbles before placing in the electrode body. The membrane with the ionophore was contacted with sample solution (5 mM $AgNO_3$) that is the same solution as the conditioning and inner filling solutions. All potentiometric measurements were carried out at ambient temperature in unstirred salt solution versus a Ag/AgCl reference electrode with 1 M LiOAc as a salt bridge electrolyte. The range of time required from attaching the membrane until the membrane potential measurement was less than 1 min. The potential value was continuously recorded for several hours.

Membrane potential values, $E_M$, were determined by subtracting the cell potential for membrane without ionophore from that of the sandwich membrane. The formation constants were calculated using Eq. (1).

$$\log\beta_{IL_n} = \left(\frac{E_M z_I F}{2.303RT}\right) - n\log\left(L_T - \frac{nR_T}{z_I}\right) \quad (1)$$

$L_T$ is the total concentration of the ionophore. $R_T$ is the concentration of lipophilic ion exchanger. $R$, $T$, $F$, $n$ and $z_I$ are the gas constant, the absolute temperature, the Faraday constant, the complex stoichiometry and the charge of the tested ion, respectively.

### 2.5. Preparation of Lip-acpcPNA

The 12-mer acpcPNA probe with a sequence of TGATGCTATGAC-Lys (Mtt) was synthesized from the respective *N*-Fmoc-protected monomers on a solid support (Tentagel, Rink amide linker) according to a previously published protocol [19–21]. After *N*-Fmoc and nucleobase side-chain deprotections, it was further modified at the *N*-terminus with an aminoethoxyethoxyacetyl (O) linker followed by DL-lipoic acid (HATU/DIEA activation). The Lip-acpcPNA was then cleaved from the solid support using trifluoroacetic acid (TFA) and purified by reverse phase HPLC. The sequence was confirmed by MALDI-TOF mass spectrometry (Lip-O-TGATGCTATGAC-Lys$NH_2$, Calcd.: $m/z=4540.0$, Found: $m/z=4539.7$) and by hybridization with its complementary DNA (d 5′-GTCATAGCATCA-3′) ($T_m=76.7\ ^\circ C$ at 1.0 μM PNA, 1.0 μM DNA in 10 mM sodium phosphate buffer pH 7.0 containing 100 mM NaCl).

### 2.6. Preparation of fabricated Ag-ISμE and EMF measurements for DNA biosensor

The membrane for the Ag-ISμE was prepared as reported previously [28]. The cocktail membrane containing **L2** (0.74 wt%, 10.31 mmol $kg^{-1}$), KT*p*ClPB (0.25 wt%, 5.03 mmol $kg^{-1}$), PVC (32.99 wt%) and *o*-NOPE (66.02 wt%) was prepared in an appropriate volume of THF after stirring 1 h. The Ag-ISμE was fabricated from the tip of 1000 μL micropipette (0.5 mm i.d.) dipped into membrane cocktail for 3 s. It was left to stand in vertical position to allow the solvent to evaporate. The membrane thickness is around 0.5 mm. The resulting microelectrodes were conditioned in 10 mM $AgNO_3$ solution for overnight prior to use. An internal filling solution containing 10 mM $AgNO_3$ was put into a micropipette tip and a AgCl-coated silver wire was placed into the solution.

Sodium ion selective microelectrode (Na-ISμE) prepared from Na(X) (0.99 wt%, 10.00 mmol $kg^{-1}$), KT*p*ClPB (0.25 wt%, 5.03 mmol $kg^{-1}$), PVC (32.85 wt%) and DOS (65.91 wt%) was used as a reference electrode. The components were dissolved in THF. The inner filling solution was 10 mM NaCl, and the conditioning solution was 10 mM $NaNO_3$.

Potentiometric measurements were performed in 1000 μL samples with a Na-ISμE as pseudo reference electrode in unstirred solutions with a 16-channel electrode monitor (Lawson Labs Inc., Malvern, PA 19355, USA) at room temperature. The activity coefficients of silver ions in sample solutions were calculated according to the Debye–Hückel approximation [29].

### 2.7. Preparation of $AgNPs^+$

The preparation of CTAB-coated silver nanoparticles was carried out using the previous published procedure with modifications [22,23]. The solution of CTAB (2 mL, 1 mM) in ethanol was added in aqueous solution of $AgNO_3$ (25 mL, 5 mM) and stirred for 10 min. Then, 1% v/v freshly prepared aqueous $NaBH_4$ was added under vigorous stirring. The mixture immediately turned yellow-green and left stirring for 1 h.

Fig. 2 shows the UV–vis spectrum of the synthesized CTAB-coated AgNPs with a strong surface plasmon band at 397 nm.

**Table 1**
Membrane compositions, response properties based on ionophores **L1**–**L4** and experimental membranes potentials and corresponding ionophore complex formation constants determined with segmented sandwich membranes for ionophores **L1**–**L4** in PVC membrane assuming 1:1 stoichiometry of their complexes with $Ag^+$.

| Ionophore | Membrane composition (wt%) | | | | Slope (mV/decade) | Linear range (M) | Detection limit (M) | Membrane potential $E_M$ (mV) | Formation constant log $\beta_{ILn}$ |
|---|---|---|---|---|---|---|---|---|---|
| | Ionophore, $L_T$ (mmol $kg^{-1}$) | KT*p*ClPB, $R_T$ (mmol $kg^{-1}$) | PVC | Plasticizer | | | | | |
| **L1** | 0.59<br>(10.27) | 0.37<br>(7.47) | 32.91 | *o*-NPOE, 66.13 | $43.1\pm0.6$ | $10^{-5}$ to $10^{-2}$ | $3.9\times10^{-6}$ | $126\pm6$ | $4.66\pm0.10$ |
| **L2** | 0.73<br>(10.10) | 0.37<br>(7.49) | 32.93 | *o*-NPOE, 65.97 | $58.8\pm0.6$ | $10^{-6}$ to $10^{-2}$ | $4.8\times10^{-7}$ | $162\pm3$ | $5.25\pm0.05$ |
| **L3** | 0.88<br>(9.96) | 0.37<br>(7.40) | 33.00 | *o*-NPOE, 65.75 | $56.3\pm0.8$ | $10^{-6}$ to $10^{-2}$ | $1.0\times10^{-6}$ | $162\pm5$ | $5.31\pm0.08$ |
| **L4** | 1.15<br>(10.24) | 0.37<br>(7.40) | 32.78 | *o*-NPOE, 65.70 | $51.0\pm0.9$ | $10^{-6}$ to $10^{-2}$ | $5.0\times10^{-6}$ | $134\pm9$ | $4.80\pm0.16$ |
| *o*-NPOE | – | 0.37<br>(7.46) | 33.11 | *o*-NPOE, 66.52 | $44.3\pm1.5$ | $10^{-5}$ to $10^{-2}$ | $4.9\times10^{-6}$ | – | – |

Transmission electron microscopy (TEM) has been used to characterize shape and size of the AgNPs$^+$. A TEM image in Fig. 3 clearly indicates that AgNPs$^+$ are spherical and have an average particle size of 20 nm. The particles have been found to be colloidally stable for at least two months.

### 2.8. Preparation of immobilized Lip-acpcPNA on gold substrate and DNA hybridization

The gold substrates obtained from Sigma–Aldrich were cut to $10 \times 5$ mm$^2$ pieces with uniform thickness (0.25 mm) by a shearing machine. They were cleaned with piranha solution (3:1 $H_2SO_4$: 30% (v/v) $H_2O_2$), thoroughly rinsed with Milli-Q water, absolute ethanol and dried under pure nitrogen gas. The Lip-acpcPNA solution (10 µM in 10 mM $NaNO_3$, 100 µL) was dropped on the gold substrate and the substrate was incubated at 4 °C for 40 h, followed by washing with Milli-Q water.

The immobilized Lip-acpcPNA on the gold substrate was treated with a target DNA solution in 10 mM $NaNO_3$ (0, 2, 5, 10, 15, 20, 40 and 80 µM, 100 µL) at room temperature for 3 h. The washing procedure was performed in three times to remove any unbound DNA. Then, a solution of AgNPs$^+$ (250 ppm, 50 µL) was deposited onto the gold substrate. After 2 h at room temperature, the gold substrate was washed three times with Milli-Q water.

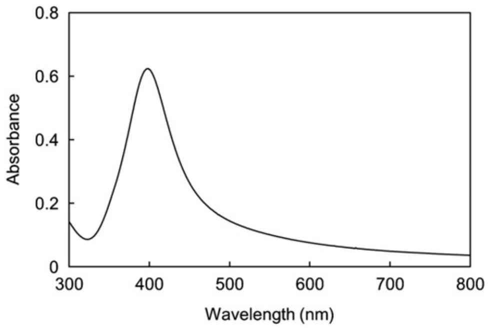

**Fig. 2.** The plasmon absorption band of diluted AgNPs$^+$ ($\lambda_{max}$=397 nm).

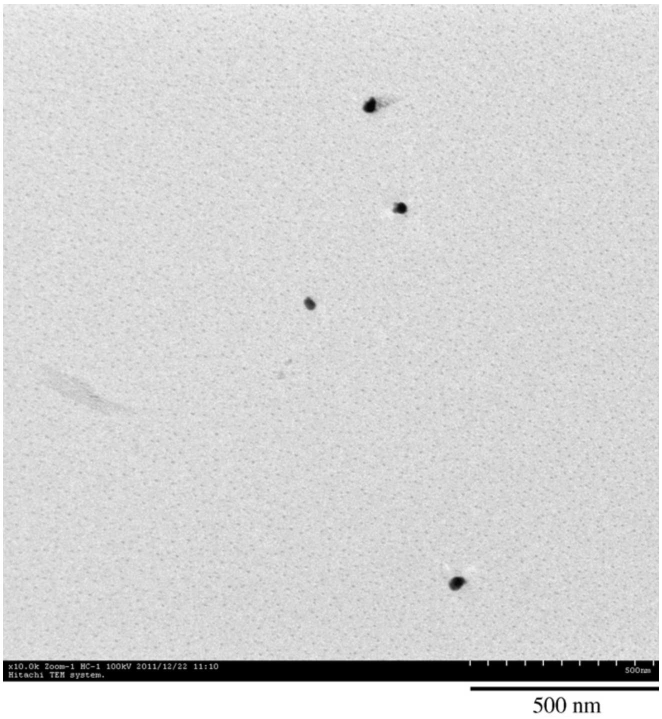

**Fig. 3.** TEM image of the synthesized AgNPs$^+$.

Hydrogen peroxide was used for dissolution of the AgNPs$^+$ to silver ions. A 0.2 M solution of $H_2O_2$ (100 µL) was added on the gold substrate and left for 45 min. The solution on the gold substrate was then transferred to an eppendorf tube and the volume adjusted to 1000 µL with 10 mM $NaNO_3$. The solution was stirred to decompose the remaining $H_2O_2$. The detection of silver ions in the sample was performed in the 1000 µL eppendorf tube by using a Na-ISµE as a reference electrode.

## 3. Results and discussion

### 3.1. Design and synthesis of ionophores

Calix[4]arene derivatives were designed and synthesized by attaching different functional groups to obtain different topology with various donor atoms for binding Ag$^+$. Each compound was modified at one or two opposite phenolic groups of calix[4]arene by benzothiazole and dipicolylamine derivatives yielding calix[4]arene derivatives, **L1** – **L4** (Fig. 4). **L1** and **L2** [28] contained soft nitrogen and sulfur as donor atoms and **L3** and **L4** [30] contained soft nitrogen and hard oxygen donor atoms. In preliminary studies, all synthesized ionophores incorporated in polymeric membranes response to Ag$^+$. Ionophore **L4** showed high selectivity towards Ag$^+$ as investigated by fluorescence, absorption, and $^1$H NMR spectroscopy [30]. Generally, Hg$^{2+}$ was a major interference for Ag-ISEs because soft metal ions, Ag$^+$, Hg$^{2+}$ and Pb$^{2+}$ preferred to coordinate to soft donor atoms such as sulfur and nitrogen according to Pearson [31].

### 3.2. Complex formation constants of L1 – L4 with Ag$^+$

The complex formation constants between Ag$^+$ and ionophores **L1**–**L4** were determined with segmented sandwich membranes, assuming a 1:1 ion–ionophore stoichiometry, and the results are shown in Table 1. The results showed that **L2** and **L3** possessed higher complex formation constants than **L1** and **L4**.

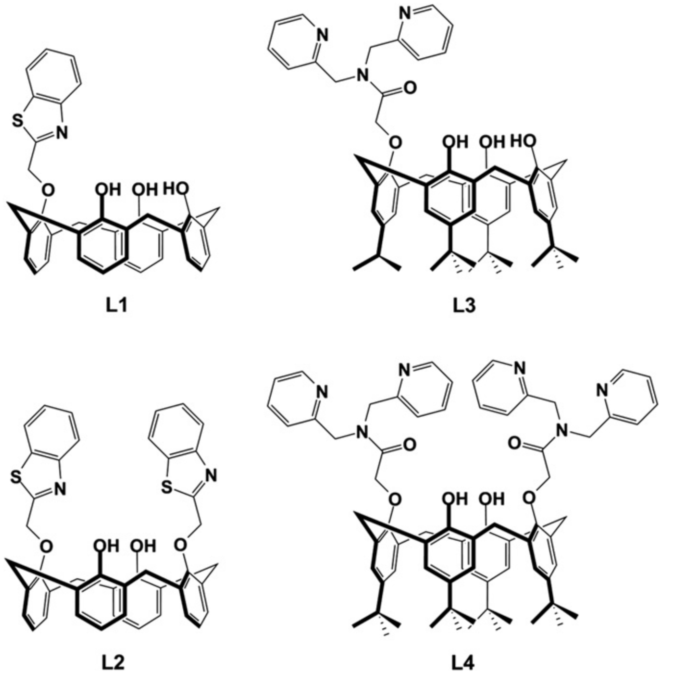

**Fig. 4.** Structures of four synthesized ionophores **L1**–**L4**.

Ionophores **L2** and **L3** formed strong and stable complexes with $Ag^+$ in the same order with logarithmic complex formation constants of $5.25 \pm 0.05$ and $5.31 \pm 0.08$ for **L2** and **L3**, respectively. The higher formation constant could result in a better selectivity towards the analyte ions due to the superior ability of the ionophore to discriminate between primary ions and interfering ions.

### 3.3. *Effect of functional groups and the number of substituents on the selectivity*

The ion selectivity of electrodes based on calix[4]arene singly modified with benzothiazole (**L1**) and dipicolylamine (**L3**) were investigated (Fig. 5). These ionophores possessed soft nitrogen donors capable of forming complexes with $Ag^+$. The ionophore **L3** containing three nitrogen and one oxygen donors from dipicolylamine and carbonyl, respectively exhibited more interference from $Hg^{2+}$. The introduction of heteroatoms of one sulfur and one nitrogen donors from benzothiazole **L1** resulted in better selectivity towards $Ag^+$ than **L3** as well as decreased interference from $Hg^{2+}$.

Two substituents on the opposite phenolic groups of calix[4]arene with two benzothiazole **L2** and two dipicolylamine **L4** were synthesized in order to investigate the effect of increasing number of donor groups on ion selectivity compared to the singly-substituted calix[4]arene ionophores **L1** and **L3**. The ionophores having two substituted phenolic groups consistently gave higher selectivity coefficients to $Ag^+/Hg^{2+}$: **L2** > **L1** and **L4** > **L3**. This suggested that more donor atoms of the disubstituted calix[4]arene could coordinate with $Ag^+$ more effectively. Furthermore, the increased lipophilicity of two substituted phenolic groups can prevent leaching of the ionophore from the membranes. Therefore, **L2** gave the best ion selectivity towards $Ag^+$ compared to other ionophores and exhibited a good discrimination with $> 10^4$ times higher than all tested cations except $Hg^{2+}$. The best selectivity towards $Ag^+$ of **L2** corresponded to its high complex formation value. This characteristic may be attributed to the two symmetrical benzothiazole groups on the calix[4]arene bearing two nitrogen and two sulfur donors which could rearrange in a suitable geometry for complexing $Ag^+$ better than other interfering ions. The results from complex formation and selectivity studies suggested that **L2** was the most appropriate ionophore to fabricate Ag-ISμEs.

### 3.4. *Fabrication of Ag-ISμE*

Our fabricated Ag-ISE has been successfully employed in speciation analysis of the AgNPs solution [28] and in glucose biosensor by using AgNPs as cation maker [32]. In addition, the fabricated Ag-ISE can be used in a wide range of solution pH from pH 2 to 8 with no significant change in the EMF value [28]. In this work, a Ag-ISμE is developed for monitoring of DNA hybridization. The calibration curve of $Ag^+$-microelectrode was performed in 1000 μL and used 10 mM $NaNO_3$ (pH 6.0) as background by using a $Na^+$-microelectrode as pseudo reference electrode as shown in Fig. 6. $Na^+$-microelectrode was chosen as a reference electrode and $Na^+$ was selected as the background electrolyte since this $Ag^+$-microelectrode showed a distinctive discrimination of the logarithmic selectivity coefficient as compared to $Na^+$ with $log\ K^{pot}_{Ag,Na} = -6.70$ [28]. The Ag-ISμE showed a low detection limit of $9.12 \times 10^{-7}$ M (~ 1 μM) in 1000 μL solution with near the theoretical Nernstian slope of 58.7 mV per decade (inset, Fig. 6).

### 3.5. *Detection of DNA hybridization*

Molecules carrying a thiol or a disulfide group can be immobilized by chemisorption onto a gold surface to form self-assembled monolayer (SAM). Covalent immobilization of lipoic

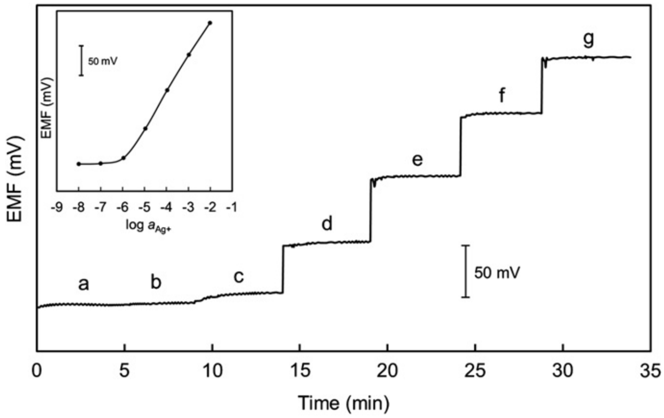

**Fig. 6.** Time trace line response of the Ag-ISμE to increasing level of $Ag^+$: (a) $10^{-8}$; (b) $10^{-7}$; (c) $10^{-6}$; (d) $10^{-5}$; (e) $10^{-4}$; (f) $10^{-3}$; (g) $10^{-2}$ M in 1000 μL of solution with 10 mM $NaNO_3$ as background with Na-ISμE as the pseudo reference electrode. Inset displays the corresponding calibration plot.

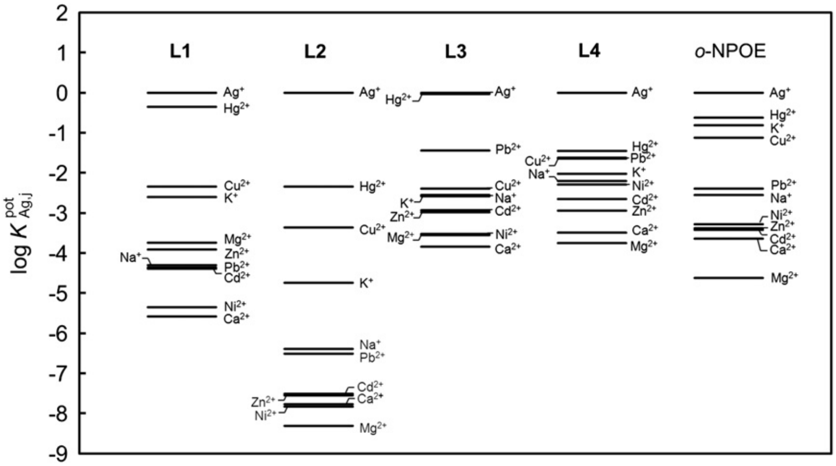

**Fig. 5.** Comparison of selectivity coefficients (log $K^{pot}_{Ag,j}$) of electrodes based on **L1**–**L4** and the blank membrane (without ionophores). The membrane compositions are 10 mmol $kg^{-1}$ ionophore and 7.5 mmol $kg^{-1}$ KT*p*ClPB in PVC plasticized with *o*-NPOE.

acid derivatives on gold surface provided a more stable SAM than that of the simple thiol via two S–Au bonds [33]. The disulfide group of Lip-acpcPNA was attached on the gold substrate by immersing in PNA solutions of various concentrations (1, 5 and 10 μM) to investigate the suitable concentration of the PNA probe that allows efficient detection of DNA and discrimination of complementary and mismatched DNA. The potentials of solutions containing $Ag^+$ obtained from a bare gold substrate, the substrate after Lip-acpcPNA immobilization, and after hybridization with the target DNA on gold substrate were compared after treatment with $AgNPs^+$ followed by $H_2O_2$ oxidation (Fig. 7). The hybridization signals with complementary DNA were obtained when the gold surface was treated with 10 μM of Lip-acpcPNA. Initially, non-specific adsorption between $AgNPs^+$ and the bare gold substrate was observed as shown by the non-zero ΔEMF values. The signals were lowered after immobilization with Lip-acpcPNA (at 10 μM), indicating that the probe can self-assemble onto gold substrate and that $AgNPs^+$ could not effectively bind to the electrostatically neutral PNA. After hybridization with DNA, the signal was increased compared to without DNA, suggesting that the electrostatic interactions between the negatively charged of DNA backbone and $AgNPs^+$ had taken place. At lower concentrations of Lip-acpcPNA, i.e. 1 and 5 μM, no difference in EMF values were observed before and after hybridization with target DNA. This suggests that there was not enough immobilized PNA to hybridize effectively with the target DNA. Therefore, 10 μM of Lip-acpcPNA was selected for further experiments.

To determine the specificity of the DNA detection, the Lip-acpcPNA immobilized on gold substrate was hybridized with fully complementary, single base mismatched and non-complementary DNA under the same hybridization condition (10 mM $NaNO_3$, pH 6.0). The results illustrated in Fig. 8 showed that the hybridization with fully complementary DNA produced the highest signal, $144 \pm 3$ mV. Hybridization with single base mismatched and non-complementary DNA gave very low signals which were indistinguishable from the control signal, indicating that there were no hybridization between single base mismatched and non-complementary DNA to Lip-acpcPNA immobilized on the gold substrate. The time trace lines in Fig. 8 clearly showed that this Lip-acpcPNA probe could discriminate fully complementary DNA from non-complementary DNA and single base mismatched DNA under non-stringent conditions.

The Lip-acpcPNA immobilized on gold substrate (at 10 μM concentration) was next hybridized with complementary DNA at different concentrations (2, 5, 10, 15, 20, 40 and 80 μM). Fig. 9 showed that EMF values of hybridization depended on the increasing in concentration of the target DNA. Higher concentrations of the target DNA resulted in more negative charges from phosphate backbone of DNA and gave larger detectable signals of $Ag^+$. The lower detection limit was 0.2 μM of the target DNA in 1000 μL sample with a linear range from 0.2 to 2.0 μM target DNA correlating with concentration of $Ag^+$ in micromolars (μM) was obtained as shown in Fig. 9.

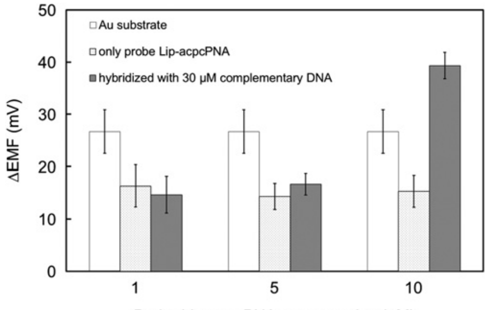

**Fig. 7.** Effect of concentration of probe Lip-acpcPNA on the response (ΔEMF values obtained from the subtraction from the background solution).

The results indicate that the proposed system can detect DNA hybridization by following signals of $Ag^+$ which obtained from dissolution of positively charged CTAB-coated AgNPs interacting with negatively charged DNA. In addition, this detection system is inexpensive and requires fewer steps for detecting the hybridization between Lip-acpcPNA probe and the DNA target as compared to other systems using electrostatic interactions between positively charged nanoparticles and negatively charged backbone of DNA hybridized with the neutral PNA probe [7,8].

## 4. Conclusion

The potentiometric studies of electrodes from compounds **L1**–**L4** showed that they could be used as ionophores in Ag-ISEs. The ionophore **L2** containing two nitrogen and two sulfur donor atoms showed optimum selectivity towards $Ag^+$ compared to other ionophores. The complex formation constant of **L2** was relatively high corresponding to its best selectivity towards $Ag^+$. Furthermore, we have demonstrated that the simple potentiometric method based on Ag-ISμE using **L2** as an ionophore in combination with pyrrolidinyl peptide nucleic acid probe could be used to detect DNA hybridization from the signal of silver ions, which derived from the electrostatic interactions between the

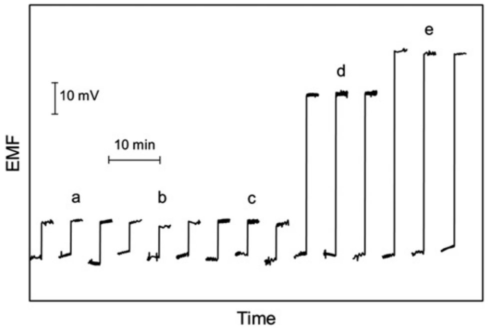

**Fig. 8.** Potentiometric hybridization response: (a) control solution (10 mM $NaNO_3$, zero target), (b) 40 μM non-complementary DNA, (c) 40 μM single base mismatched DNA, (d) 20 μM target DNA, and (e) 40 μM target DNA (as complementary targets) after DNA hybridization. Potentiometric measurements were performed in 1000 μL samples with Na-ISμE as the pseudo reference electrode and 10 mM $NaNO_3$ as a background solution.

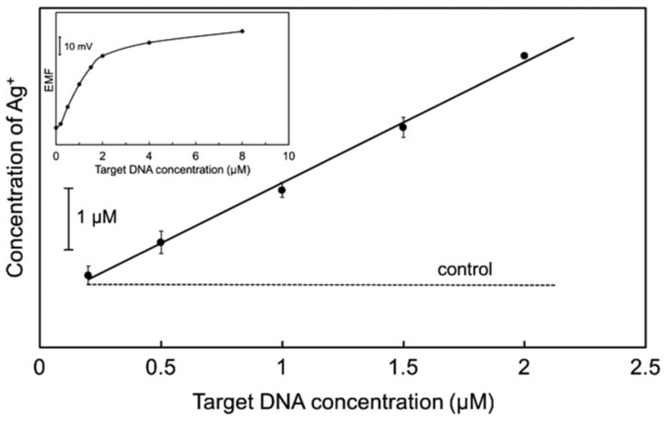

**Fig. 9.** Calibration plot for the potentiometric monitoring of $AgNPs^+$-based label-free DNA hybridization in 1000 μL eppendorf tube (error bars: SD, $N=3$). The dashed line corresponds to control signal (no target DNA).

AgNPs$^{+}$ and the negatively charged target DNA hybridized to the neutral Lip-acpcPNA probe immobilized on the gold substrate.

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## Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.11.046.

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