

Full Paper

Determination of Lead in Oral Squamous Cell Carcinoma Tissue by New Potentiometric Sensor based on [2-{4-(Diphenylphosphanyl) Phen-2-Yl} Pyrimidin-4-Yl]-3-(Triethoxysilyl) Prop-1-Yl Urea

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Abstract- Inorganic lead compounds have recently been classified as human carcinogens by the International Agency for Research on Cancer (IARC). In this light and given the capability of ion-selective electrodes for potentiometric measurements of heavy metal ions, a novel lead ion membrane electrode was prepared based on a novel synthesized compound and was used for the determination of lead content in oral squamous cell carcinoma tissues. The selectivity of the electrode response to lead ions was good in the presence of many commonly occurring interfering species such as alkali, alkaline earth, transition, and other heavy metal ions, as further reflected by conductance studies. The optimal membrane composition was 7 % wt. of the ion carrier, 30 % wt. of PVC powder, 60 % wt. of NPOE, and 3% wt. KTCPB. The response of the sensor was linear in the concentration window of 1.0×10^{-7} to 1.0×10^{-1} M with a detection limit of 8.5×10^{-8} M, and the slope of the calibration plot was determined to be 29.4 ± 0.3 mV per decade of concentration. The electrode response was pH-independent in the wide range of 2.2–8.9, and it offered merits of quick response, further to its low detection limit, selectivity profile, and broad linear response range. The sensor showed a fairly fast response of ~30 s and could be reliably used for over 4 months. The membrane electrode was used in the determination of Pb^{2+} concentration in healthy and cancerous oral tissues.

Keywords- Lead; Squamous cell carcinoma; Potentiometric determination; Sensor; Oral cancer

1. INTRODUCTION

Heavy metals are long-lasting toxic pollutants of soil and the atmosphere which tend to bioaccumulate in human organs. Many heavy metal ions (e.g. lead, thallium, cadmium, and antimony ions) have widespread industrial applications [1]. Arsenic, cadmium, chromium, nickel, lead, and barium are substances linked to molecular toxicity in previous literature [2]. Lead is a carcinogen that interrupts the DNA repair, the tumor-regulating genes, and the structure and sequence of chromosomes since it leads to the release of reactive oxygen species (ROS), and shifts Zn^{2+} from specific regulatory proteins, disrupting transcription [3]. In a recent assessment of the carcinogenic behavior of inorganic Pb^{2+} compounds by IARC, the evidence on the carcinogenicity of the compounds on animals was determined to be sufficient, while the proof of causing cancer in humans was concluded not enough. Consequently, these compounds have been classified as possible carcinogens to humans [4]. Pb^{2+} is very toxic to living organisms since it tends to accumulate in inner organs, and chronic contact with its compounds can damage the central nervous system [5,6].

Cancer of head and neck cancer is among the most significant cancers throughout the world, and the 3rd common cancer in developing countries [7]. The most prevalent types of malignancy, arising from the mucosal epithelium of the oral cavity, pharynx, and larynx are head and neck squamous cell carcinomas (HNSCCs). These cancers are usually associated with tobacco and alcohol consumption. Exposure to chemical and physical agents is among the risk factors for this type of malignancy [8]. Numerous epidemiological studies have linked the heavy metal to the development of several types of cancer in people [9].

So, the analysis and control of the lead ion concentration even at low concentrations is extremely important [10]. However, the complex matrix of the samples and the low amounts of the species turns the analyses difficult and necessitates the application of highly sensitive and sensitive techniques, further the need for pre-concentration [11]. Flame-furnace atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS), and inductively coupled plasma atomic emission spectrometry (ICP-AES) [12-14] are now common techniques used for the determination of traces of heavy metals. In the case of real samples, FAAS lacks sufficient sensitivity for the determination of trace and ultra-trace amounts of heavy metal ions; which necessitates pre-concentration [15].

Ion-selective electrodes (ISEs) are analytical tools that translate ionic concentration (activity) to an electrical signal, which is commonly electrical potential. These instruments are usually coupled with reference electrodes to measure the potentials. These sensors have been proven to be applicable in the in-situ analysis of traces of heavy metals, given their excellent selectivity profiles and low detection limits [15-17]. Various chelating, intercalating, and composite materials have been evaluated for the design of ISEs. The instruments are also capable of quick analyses with high sensitivity and have also been used in online detection

setups or compact analyzers based on microelectronics to be used in continuous and real-time analyses [18,19].

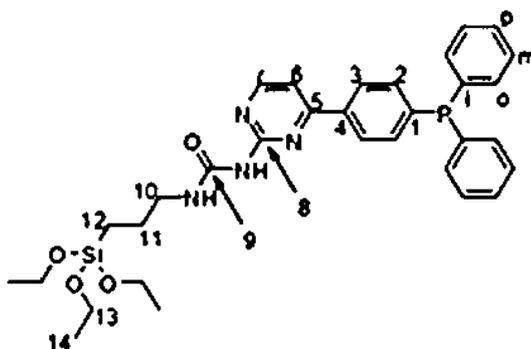


Figure 1. Chemical structure of [2-{4-(Diphenylphosphanyl)phen-2-yl}pyrimidin-4-yl]-3-(triethoxysilyl)prop-1-yl urea(L)

In This study, [2-{4-(Diphenylphosphanyl) phen-2-yl} pyrimidin-4-yl]-3-(triethoxysilyl) prop-1-yl urea(L), (Figure 1) was used as an ionophore, in a PVC membrane further containing nitro phenyl octyl ether (NPOE) as a plasticizer, and an ionic additive (potassium tetrakis(p-chlorophenyl) borate (KTCPB)). The resulting liquid membrane Lead selective electrode had a low limit of detection, short response time, and good selectivity for Pb^{2+} against various ions. Then prepared ISE was applied to the determination of lead concentration in cancerous tissue affected by squamous cell carcinoma.

2. EXPERIMENTAL

2.1. Chemicals and reagents

The chemicals (i.e. nitro phenyl octyl ether (NPOE), dibutyl phthalate (DBP), benzyl acetate (BA), sodium tetraphenyl borate (STPB), oleic acid (OA), tetrahydrofuron (THF) and high relative molecular weight PVC were all of reagent grade purity and were obtained from Fluka Co, and used without any treatments. Solutions of the cations were all prepared using their nitrate salts with the highest purity available (Merk Co.) without any treatments other than vacuum drying over P_2O_5 . All water used in the experiments was triply distilled deionized. The remaining chemicals were prepared either in house or obtained from the above-mentioned chemical companies.

2.2. Synthesis of the ligand

4-(3-Dimethylamino-1-oxoprop-2-en-yl) phenyl] diphenylphosphine (1c)

To prepare phosphine 1c 593 g~3.07 mol of (*E*)-3-(*N,N*-dimethylamino)-1-(4'-fluorophenyl) prop-2-en-1-one, 789 mL~3.08 mol of diphenyl(trimethylsilyl)phosphine (1a)

and 94.80 g ~ 624.09 mmol of CsF were reacted 600 ml of dehydrated DMF under stirring at 80 °C for 1 hour 60 min to obtain a yield of 1036 g (94%) of the yellow solid.

4-(4-(2-Amino) pyrimidinyl) phenyl] diphenylphosphine (2a)

2a was prepared via reacting 7.50 g ~ 20.87 mmol of 4-(3-Dimethylamino-1-oxoprop-2-en-yl) phenyl] diphenylphosphine (1c); 3.25 g ~ 30.00 mmol of $Gd_2(SO_4)_3$ in 100 mL of ethanol. Next 1.68 g ~ 30.00 mmol of potassium hydroxide was added to the mixture, before refluxing for 5 h. After eliminating the solvent under vacuum, the solid was dispersed in dichloromethane and filtered, before an eventual crystallization in ethanol with a yield of 6.3 g (85%).

[2-{4-(Diphenylphosphanyl)phen-2-yl}pyrimidin-4-yl]-3-(triethoxysilyl)prop-1-yl urea (27)

27 was synthesized via adding 2.50 g ~ 10.11 mmol of [(3-triethoxysilyl)prop-1-yl]isocyanate were to 1.77 g ~ 4.98 mmol) of [4-(4-(2-Amino) pyrimidinyl) phenyl] diphenylphosphine (2a). The temperature of the resulting mixture was raised to 170 °C for 60 minutes. After this step the mixture was cooled to ambient temperature and the residue, recrystallized using methanol, was washed using a 1:1 mixture of diethyl ether and hexane. The yield of the produced yellow powder was 2.28 g (76%) (Figure 2).

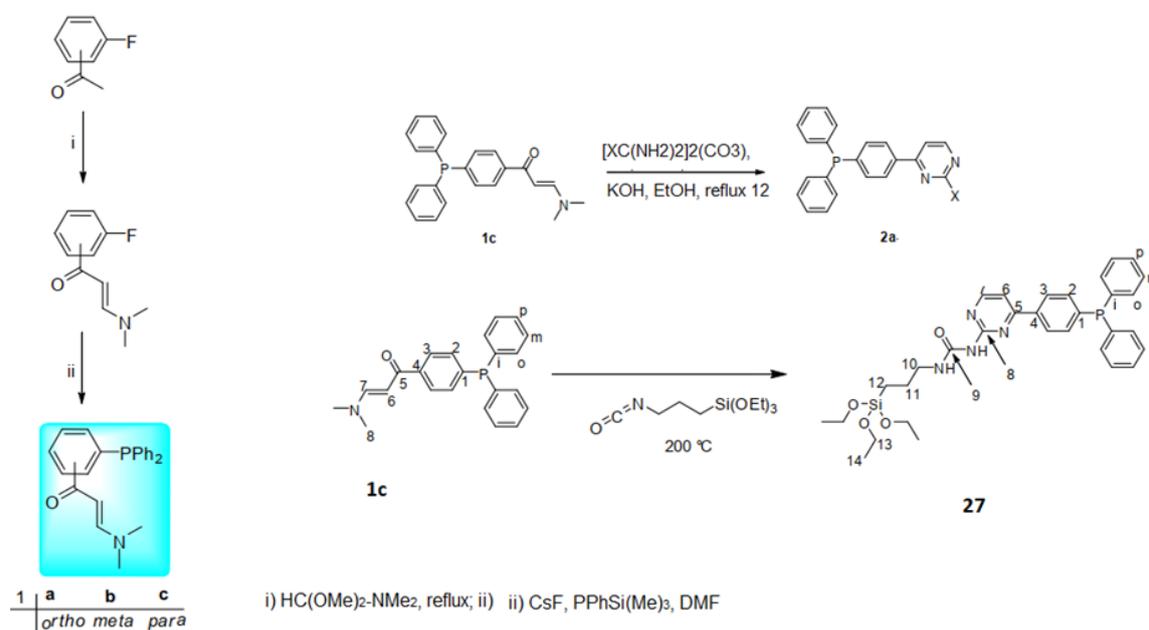


Figure 2. Schematic steps of L synthesis

2.3. Construction of the electrode

The optimal PVC membrane was prepared through admixing 30 mg of PVC powder, 60 mg of NPOE, 3 mg of KTCPB, and 7 mg of the ionophore L in 4 mL of tetrahydrofuran (THF),

and transferring the resulting viscose solution to a glass dish, to allow for the THF to evaporate yielding an oily solution. Into this solution was inserted a Pyrex tube (3–5 mm o.d.) for 5 s. As a result, a transparent membrane with an approximate thickness of 0.3 mm formed at the tip of the tube, which was allowed to lose its remaining THF content by resting in ambient conditions for around 1 day. Then, some 1.0×10^{-3} M of $\text{Pb}(\text{NO}_3)_2$ solution was filled in the tube to act as the internal filling solution, before the outer surface of the electrode was contacted with a 1.0×10^{-2} M $\text{Pb}(\text{NO}_3)_2$ solution for 1 day for conditioning, prior to application [20,21]. An Ag/AgCl reference electrode was inserted into the inner filling solution to serve as the internal reference electrode.

2.4. The emf measurements

Potential readings were performed using the following cell assembly:

Ag–AgCl|3 M KCl| internal solution, 1.0×10^{-3} M $\text{Pb}(\text{NO}_3)_2$ | PVC membrane |test solution |Hg – Hg_2Cl_2 , KCl (satd).

The potential measurements were performed using a Corning ion analyzer 250 pH/mV meter at 25.0 °C. Calculation of the activities was performed based on the Debye–Hückel procedure [22].

2.5. squamous cell carcinoma tissue preparation

Formalin-fixed, paraffin-embedded malignant and non-malignant tissues obtained from the archive of Qazvin oral pathology department were used. To create these blocks, cancerous tissue, and non-cancerous tissue that was biopsied from the patient's mouth for therapeutic and diagnostic purposes spent 24 hours in 10% formalin to be fixed. The fixed tissue was then cut into pieces and put inside a histotechnical device. After dehydrating the sample, it was embedded in paraffin.

3. RESULTS AND DISCUSSION

3.1. Primary evaluations

The first step involved evaluating the strength and selectivity of the interactions of the ion-carrier (L) and various cations in acetonitrile at 25 ± 0.05 °C, [20,21] Plots of the molar conductance against $[\text{L}]/[\text{Mn}^+]$ were indicative of the formation of 1:1 complexes.

The values of the complexation constants (K_f) were calculated by fitting the molar conductance/mole ratio data using appropriate equations. [20,21] The data revealed the Pb^{2+} -L complex is stronger than the other complexes including those of alkali, alkaline earth, transition, and heavy metals, which means L can act as an ionophore in a Pb^{2+} ion-selective membrane sensor.

3.2. Membrane Ingredients

It has been well established that the performance of an ionophore-based membrane electrode is a function of the composition of the nature and amounts of the membrane ingredients [23-34], and hence various membrane compositions were prepared and tested to obtain the optimal results. A summary of the data is given in Table 1.

Table 1. Optimization of membrane ingredients

No.	Composition(%)						slope(mV/decade)
	PVC	Plasticizer	Ligand	KTCPB	STPB	OA	
1	30	70, BA	0	-	-	-	~0
2	35	60, BA	5	-	-	-	15.9 ± 0.6
3	33	60, BA	7	-	-	-	17.3 ± 0.2
4	31	60, BA	9	-	-	-	12.3 ± 0.5
5	33	60,DBP	7	-	-	-	19.6 ± 0.6
6	33	60,NPOE	7	-	-	-	22.7 ± 0.7
7	38	55,NPOE	7	-	-	-	19.6 ± 0.9
8	28	65,NPOE	7	-	-	-	21.4 ± 0.5
9	31	60,NPOE	7	-	2	-	25.5 ± 0.5
10	31	60,NPOE	7	2	-	-	27.9 ± 0.9
11	28	60,NPOE	7	-	-	5	22.1 ± 0.4
12	30	60,NPOE	7	3	-	-	29.4 ± 0.3
13	29	60,NPOE	7	4	-	-	26.7 ± 0.6

The effect of the amount of the ionophore was devalued through evaluating the behavior of compositions 1-4, which clearly indicate that 7% wt. of the leads to the best sensor response while exceeding this amount diminished the sensor's response slope due probably to membrane saturation and homogeneity. [35]

Another factor influencing the response of an ISE is the polarity of the membrane, which is mainly determined by the dielectric constant of the [35-37] plasticizer as the main ingredient of the membrane electrode. In the case of the present work the effects of using BA, DBP, and NPOE as the membrane solvents were evaluated, and as seen in the results (Table 1), the most polar solvent with the maximum dielectric constant (i.e. NPOE) led to the best sensitivity of response profiles. It is noteworthy that not only its dielectric constant, but the ability of a solvent to facilitate of ionophore mobility and its effect on the conformation of the ionophore influence the sensor response. [36, 38]. In terms of the amount of the plasticizer, experimental data indicated that the presence of 60% of NPOE led to optimal response behavior, while as in the case of the ionophore, exceeding this amount diminished the slope of the calibration plot.

A further established fact is that incorporating lipophilic ionic additives can greatly modify the of a membrane-based ISE [39-42] since the additives not only modify the response and selectivity [40] but they can further catalyze the ion exchange kinetics of the membrane-sample interface.[43] In this case potassium tetrakis(KTCPB) and NaTPB, were evaluated as additives (Table 1). The results indicate that when no additive was used the PVC membrane containing the ionophore showed very low sensitivity (nos. 1-8, slopes <22.7 mV decade⁻¹), yet adding 3% wt. of KTCPB (no. 12), considerably enhanced the response slope to 29.4 mV decade⁻¹, which could be attributed to the improved dielectric constant of the membrane. The experimental data clearly indicate that membrane 12 (with a respective composition of PVC/NPOE/KTCPB/L 30:60:3:7) produced the best response.

3.3. Effect of Internal Solution

As has been proven before [34] the composition of the internal solution can influence the response, if the internal diffusion potential of the membrane is large. This factor was evaluated using different internal solution (1.0×10^{-2} and 1.0×10^{-3} M Pb²⁺. The results showed that this did not significantly influence the potential response of the sensor, except for altering the intercept of the calibration plot (Figure 3). In this work as 1.0×10^{-3} M inner reference solution was used in all experiments.

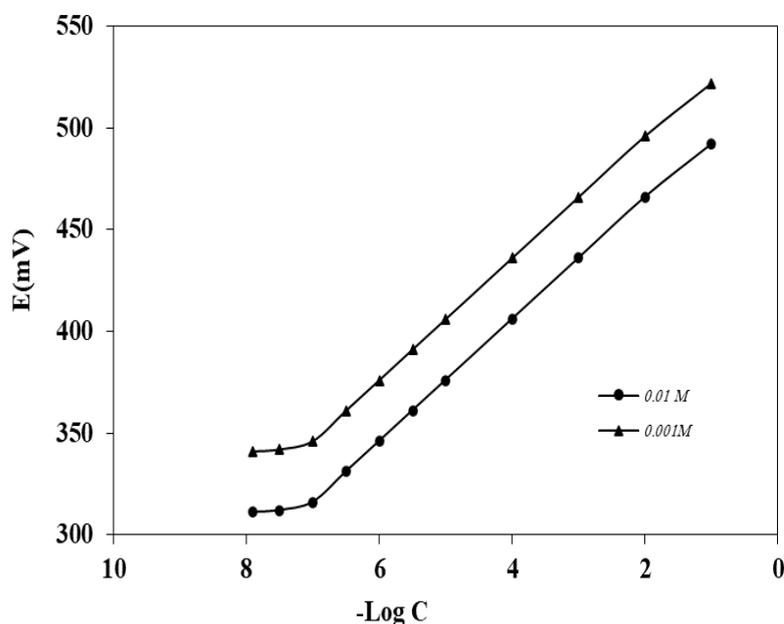


Figure 3. Effect of Internal Solution

3.4. Response behavior of the sensor

The response of the sensor was evaluated according to IUPAC guidelines [45], and it was observed that the emf response (Figure 4) has a Nernstian behavior (with a slope of slope

$29.4 \pm 0.3 \text{ mV decade}^{-1}$) in a wide concentration window of 1.9×10^{-7} to $1.0 \times 10^{-1} \text{ M}$ with a detection limit as low as $8.5 \times 10^{-8} \text{ M}$, determined via intercepting extrapolated linear section of the calibration curve.

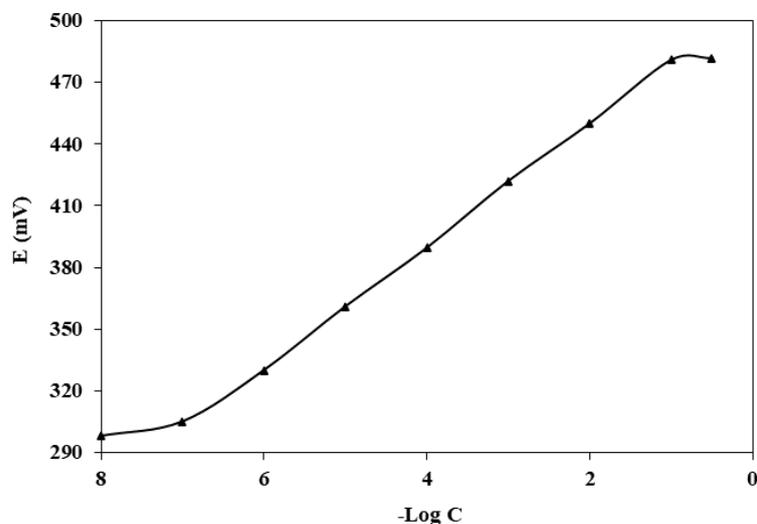


Figure 4. Calibration curve of Pb^{2+} Ion selective electrode Linearity range 1×10^{-7} - 10^{-1} M

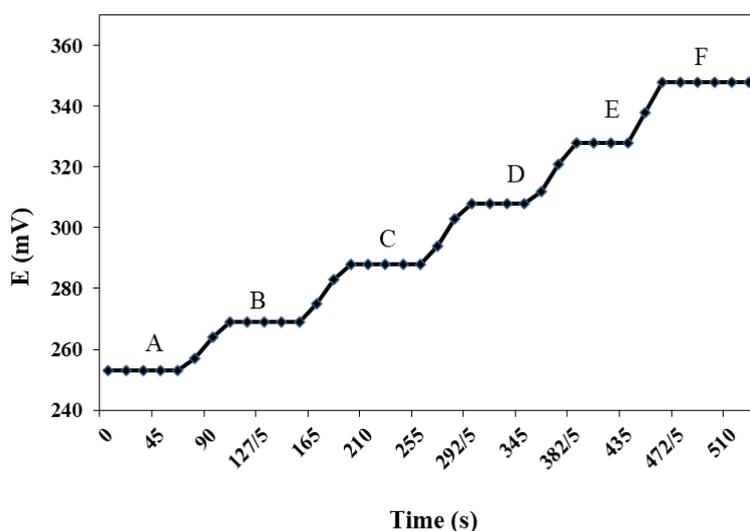


Figure 5. Dynamic response time of the proposed sensor for step changes in the concentration of Pb^{2+} (M): A) 1.0×10^{-6} , B) 1.0×10^{-5} , C) 1.0×10^{-4} , D) 1.0×10^{-3} , E) 1.0×10^{-2} , F) 1.0×10^{-1} .

IUPAC defines practical response [46] as, “the length of time which elapses between the instant at which an ion-selective electrode and a reference electrode are brought into contact with a sample solution (or the instant at which the concentration of the ion of interest in a solution in contact with an ion-selective electrode and a reference electrode is changed) and the first instant at which the potential of the cell becomes equal to its steady-state value within 1 mV”.

The response time of the sensor, which can be defined as the time needed for the sensor to reach ± 1 mV of the corresponding equilibrium value upon experiencing a 10-fold concentration change, was determined (Figure 5). The figure clearly shows that the response time of the sensor was less than 30 s in all of the tested concentrations.

An evaluation of the lifetime of the electrode was tested in a 5-month period, during this time, the electrode was used on a daily basis for 1 hour per day. The results showed that the sensor is able to work well for nearly 4 months without significant changes in its performance.

3.5. Effect of pH

To evaluate the effect of the pH on the response, the pH of the test solution was altered in the range of 2-10 while recording the potential response. The changes were made using concentrated acid and base solutions to avoid considerable concentration changes. The results (Figure 6) indicate that the response of the electrode was pH independent in the range of 2.2 to 8.9. Below and over this range the response was considerably influenced by pH indicating the response of the sensor to H^+ concentration at pH values below 2.2 and the formation of soluble lead hydroxy species at above 8.9.

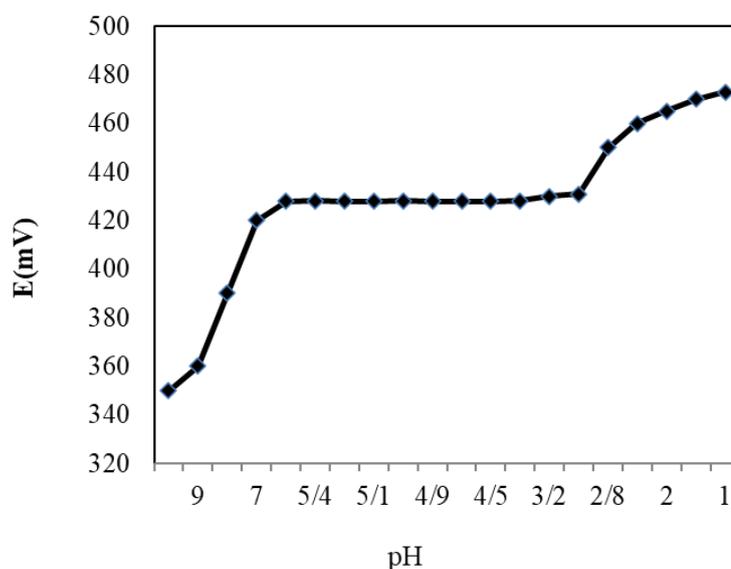


Figure 6. Influences of pH of the test solution on the potential response of electrode in the presence of 1.0×10^{-3} M Pb^{2+}

3.6. Selectivity

The effects of interfering ions on the response of a sensor are expressed in terms of the selectivity coefficients. In this research, the selectivity coefficients were determined through the separate solution method (SSM) suggested by IUPAC (Table 2 and Figure 7). The results

indicate that the electrode is very selective to lead ions in comparison to the tested cations since the SSM selectivity coefficients were 10^{-3} or smaller (Table 2).

Table 2. Selectivity coefficients ($K_{A,B}$) of Pb^{2+} ion-selective electrode for different cations

<i>ion</i>	$K^{pot}_{A,B}$	<i>ion</i>	$K^{pot}_{A,B}$
Zn²⁺	2.9×10^{-2}	Ca²⁺	4.4×10^{-3}
Co²⁺	9.1×10^{-3}	Fe²⁺	3.8×10^{-3}
Mn²⁺	8.5×10^{-3}	Ce³⁺	3.7×10^{-3}
Cu²⁺	7.7×10^{-3}	Na⁺	3.7×10^{-3}
Tl⁺	5.9×10^{-3}	Al³⁺	$< 10^{-4}$
Ba²⁺	5.2×10^{-3}	Mg²⁺	$< 10^{-4}$
Sr²⁺	5.0×10^{-3}	Cd²⁺	$< 10^{-4}$
K⁺	4.6×10^{-3}	Ag⁺	$< 10^{-4}$

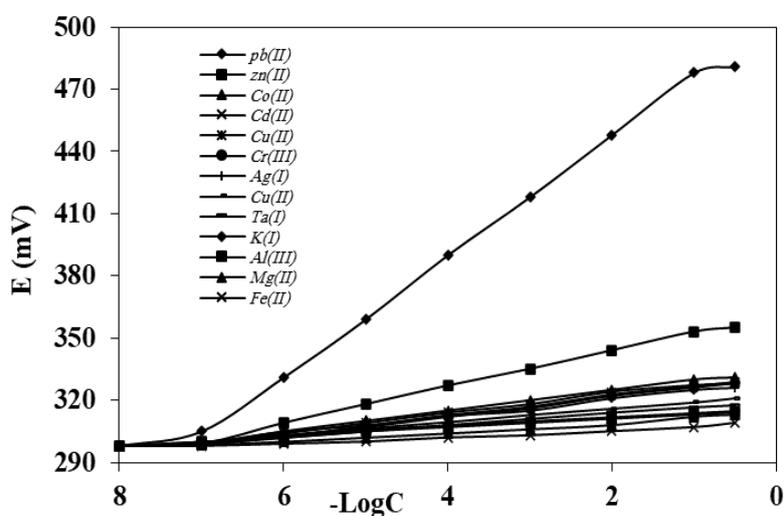


Figure 7. The potential responses of new electrodes to various cations.

3.7. Analytical application

To investigate the possible relationship between the presence of lead heavy metal and oral cancer, two healthy and cancerous tissue samples were selected, and the amount of lead they contained was measured by the prepared electrode. To check the accuracy of the measurement, the samples were also analyzed using ICP-MS. The results (Table 3) indicate that the concentration of lead in cancerous tissue is almost 20 times higher than in healthy tissue, which can confirm the possible role of heavy metals in the etiology of oral cancer.

Table 3. Measuring the amount of Lead by ICP-MS and Pb selective electrode methods in healthy and cancerous oral tissues

	Lead content (ppb)	
	ICP-MS	Pb selective electrode
Healthy sample	436 ± 5	428 ± 7
Cancer sample	9310 ± 3	9415 ± 9

4. CONCLUSION

Carcinogenic compounds of lead led to the use of a potentiometric sensor for heavy metal analysis. A lead ion selective electrode was made of a newly synthesized compound and it was employed for the determination of lead content in some oral squamous cell carcinoma tissues. The selectivity of the sensor to lead ions with respect to many commonly interfering species pH-independent in the wide range of 2.2–8.9, its fast response, and its low detection limit, were remarkable features of the newly designed lead selective electrode. The sensor showed a fairly fast response of ~30 s and could be reliably used for over 4 months. The sensor was finally used in the determination of the Pb²⁺ content of the healthy and cancerous oral tissues.

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